Erythrocyte omega-3 index, ambient fine particle exposure, and brain aging

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Neurology® 2020;95:e995-e1007. doi:10.1212/WNL.0000000000010074

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Abstract

Objective

To examine whether long-chain omega-3 polyunsaturated fatty acid (LCn3PUFA) levels modify the potential neurotoxic effects of particle matter with diameters <2.5 μm ($PM_{2.5}$) exposure on normal-appearing brain volumes among dementia-free elderly women.

Methods

A total of 1,315 women (age 65–80 years) free of dementia were enrolled in an observational study between 1996 and 1999 and underwent structural brain MRI in 2005 to 2006. According to prospectively collected and geocoded participant addresses, we used a spatiotemporal model to estimate the 3-year average $PM_{2.5}$ exposure before the MRI. We examined the joint associations of baseline LCn3PUFAs in red blood cells (RBCs) and $PM_{2.5}$ exposure with brain volumes in generalized linear models.

Results

After adjustment for potential confounders, participants with higher levels of RBC LCn3PUFA had significantly greater volumes of white matter and hippocampus. For each interquartile increment (2.02%) in omega-3 index, the average volume was 5.03 cm^3 ($p < 0.01$) greater in the white matter and 0.08 cm^3 ($p = 0.03$) greater in the hippocampus. The associations with RBC docosahexaenoic acid and eicosapentaenoic acid levels were similar. Higher LCn3PUFA attenuated the inverse associations between $PM_{2.5}$ exposure and white matter volumes in the total brain and multimodal association areas (frontal, parietal, and temporal; all p for interaction <0.05), while the associations with other brain regions were not modified. Consistent results were found for dietary intakes of LCn3PUFAs and nonfried fish.

Conclusions

Findings from this prospective cohort study among elderly women suggest that the benefits of LCn3PUFAs on brain aging may include the protection against potential adverse effects of air pollution on white matter volumes.

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Glossary

AQS = Air Quality System; **BME** = bayesian maximum entropy; **BMI** = body mass index; **DHA** = docosahexaenoic acid; **EPA** = eicosapentaenoic acid; **LCn3PUFA** = long-chain omega-3 polyunsaturated fatty acid; **NSES** = neighborhood socioeconomic status; **$PM_{2.5}$** = particle matter with diameters <2.5 μm; **RBC** = red blood cell; **SVID** = small vessel ischemic disease; **WHI** = Women's Health Initiative; **WHIMS-MRI** = Women's Health Initiative Memory Study–Magnetic Resonance Imaging; **WMV** = white matter volume.

Increasing evidence has shown that exposure to ambient fine particle matter with diameters <2.5 μm ($PM_{2.5}$) is a novel environmental risk factor for cognitive decline among the elderly.[1] Previous studies examining the association between $PM_{2.5}$ exposure and brain size have reported smaller brain volumes, especially in normal-appearing white matter, among cognitively intact elderly people living in locations with higher levels of long-term $PM_{2.5}$ exposure.[2,3]

Long-chain omega-3 polyunsaturated fatty acids (LCn3PUFAs) are important components of synaptic membranes and play a critical role in maintaining brain structure and function during aging.[4] Through multiple modes of protective actions,[5–8] higher LCn3PUFA levels were associated with greater total brain volume,[9] greater global gray matter[10] and hippocampal volumes,[9] and increased integrity of white matter.[11–14] Prompted by the promise of these neurotrophic effects, many toxicologists have reported that LCn3PUFAs reduce the brain damage caused by exposures to various environmental neurotoxins, including lead,[15] organic solvents,[16] and methylmercury.[17] However, no previous studies have examined whether LCn3PUFAs offer similar protection against the potential neurotoxic effects of $PM_{2.5}$ exposure.

Therefore, we investigated whether red blood cell (RBC) LCn3PUFA levels (RBC docosahexaenoic acid [DHA] + eicosapentaenoic acid [EPA], or the omega-3 index) modify the association between $PM_{2.5}$ exposure and brain structure using data from the Women's Health Initiative Memory Study–Magnetic Resonance Imaging (WHIMS-MRI).

Methods

Study design and population

WHIMS was an ancillary study to the Women's Health Initiative (WHI) clinical trials of postmenopausal hormone therapy. In 1996 to 1999 (henceforth referred to as baseline), a total of 7,427 women 65 to 80 years of age who were free of dementia and community dwelling were recruited from 39 of the WHI clinical centers and 10 satellite sites. The detailed design and methods of the WHI clinical trials and WHIMS studies have been published elsewhere.[18] A subsample of 1,403 were recruited across 14 WHIMS centers in the contiguous United States to participate in the WHIMS-MRI study in 2005 to 2006.[19] This study included a total of 1,315 participants after the exclusion of 88 participants without valid omega-3 index measurements. In the secondary analyses of dietary LCn3PUFA and fish intakes, we excluded 2 with incomplete dietary data and 30 who reported an implausible total energy intake (<600 or >5,000 kcal/d) from 1,403 participants in WHIMS-MRI, leaving 1,371 participants in the analyses.

Standard protocol approvals, registrations, and patient consents

Approval was received from the ethics standards committee on human experimentation for all experiments with human participants. Written informed consent was obtained from all study participants (consent for research).

Omega-3 index measurements

We analyzed baseline erythrocyte membrane fatty acid composition by using gas chromatography with flame ionization detection and expressed it as a weight percent of total identified fatty acids.[20] The omega-3 index was defined as the sum of membrane DHA and EPA.[21] The intra-assay coefficient of variation for omega-3 index was 1.6% and 0.8% for the low and high controls, respectively; the interassay coefficient of variation was 3.8% and 1.7%.[9] During the aliquoting phase, the RBC samples were stored incorrectly at −20°C for a period of ≈2 weeks, causing oxidative degradation of some of the long chain omega-6 and omega-3 polyunsaturated fatty acid before measurement. To estimate the original LCn3PUFA levels, a multiple imputation strategy based on new laboratory analyses was undertaken.[20] This technique is well suited to correct bias.[20]

We used the standard statistical methods recommended in the WHI to analyze the RBC data. Specifically, we used the SAS procedure PROC MIANALYZE (SAS Institute, Inc, Cary, NC), which implements the Rubin technique and calculates confidence intervals for the overall inference by using the covariance matrix and parameter estimates from linear models.[20]

Assessment of LCn3PUFA intake and fish consumption

We assessed LCn3PUFA intake and fish consumption at baseline screening using a semiquantitative food frequency questionnaire modified from the original National Cancer Institute and Block food frequency questionnaire.[22] Because frying, especially deep-fat frying, may substantially alter the fatty acid content of a fish meal,[23] fish consumption was divided into fried and nonfried fish groups. Nonfried fish was the sum of nonfried shellfish, canned tuna, tuna salad, tuna casserole, and broiled or baked white and dark fish. Nutrient intakes were estimated from a database derived from the

University of Minnesota's Nutrition Coordinating Center (Minnesota Nutrition Data System for Research, Minneapolis). In this study, we defined LCn3PUFA intake as the sum of DHA and EPA intakes from diet. Data on supplemental use of fish oil were collected, but the frequency of use and dosage were not available.

MRI scanning and data processing

This study followed standardized scan acquisition and processing protocols, developed by the WHIMS-MRI Quality Control Center in compliance with the American College of Radiology MRI Quality Control Program, in all centers.[24] In brief, standard T1-weighted, T2-weighted, proton density–weighted, and fluid-attenuated inversion recovery scans were acquired with 1.5T scanners. Regional volumetric measurements of gray matter, white matter, and CSF were subsequently obtained by the use of a validated, automated computer-based template warping method.[25] We summed the numbers of voxels in gray matter, white matter, and CSF to calculate volumes of each labeled brain region. Intracranial volume was estimated as the total cerebral hemispheric volumes, including ventricular CSF and the CSF within the sulcal spaces. To segment small vessel ischemic diseases (SVIDs), a brain lesion segmentation algorithm was applied to T1, T2, and fluid-attenuated inversion recovery images.[26,27] By combining the tissue segmentation and lesion segmentation algorithm, we classified every voxel as normal (not SVID affected) or abnormal (SVID affected), allowing calculation of normal-appearing brain volumes and SVID volumes in each region. Volumes of gray and white matter reported in the present study referred to normal-appearing brain tissue only. At the association cortices, including frontal, parietal, occipital, and temporal lobes, we focused on frontal, parietal, and temporal lobes that are critical to memory and complex cognitive processing.

Estimation of $PM_{2.5}$ exposure

WHIMS-MRI participant addresses, collected prospectively at each clinic visit and updated at least biannually, were geocoded following a standardized protocol.[28] Using the bayesian maximum entropy (BME)–based spatiotemporal modeling method,[29,30] we modeled the daily ambient concentration of $PM_{2.5}$ across the nation from 1999 to 2005 to 2006, when the MRI scans were performed. We could not estimate $PM_{2.5}$ exposure before 1999 due to the limited monitoring data. By integrating nationwide monitoring data from the US Environmental Protection Agency Air Quality System (AQS) and the output of chemical transport models, this BME method characterizes spatiotemporal interdependence of environmental data to estimate mean trends and covariance of the air pollution fields over space and time. To assess the estimation accuracy of the BME model, we performed a 10-fold estimation analysis, with the AQS monitoring stations evenly divided in 10 distinct sets. For each fold, we implemented the BME estimation to obtain the daily estimates using only the data from the remaining 90% of the monitoring stations. The empirical data showed that the resulting BME estimates of daily $PM_{2.5}$ exposures correlated well with the AQS-recorded concentrations (with average Pearson $r^2 = 0.70$).[30] This statistically validated BME model was applied to each geocoded participant address to generate a yearly time series of $PM_{2.5}$ exposure and then combined with participant address histories, including relocations, to calculate the 3-year moving average $PM_{2.5}$ as an indicator of long-term exposure.

Other covariates

Participants provided information on demographics, socioeconomic status, lifestyle factors, medial history, and clinical characteristics through self-administered questionnaires at baseline. The information included age, race/ethnicity, US region, education attainment, family income, employment status, smoking status, alcohol consumption, body mass index (BMI), physical activity, prior depression, random assignment to hormone therapy, and medical histories of hypertension, diabetes mellitus, hypercholesterolemia, and cardiovascular diseases. BMI (kilograms per meter squared) was calculated as weight divided by height squared. The presence of prior depressive disorders was examined with the Shortened Center for Epidemiologic Studies Depression scale using the Diagnostic Interview Schedule. A value >0.06 was defined as having depression, as defined by the Burnam screening algorithm.[31] We defined hypertension by any self-reported use of antihypertensive medication or elevated blood pressure (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg)[32]; diabetes mellitus by a physician's diagnosis and oral medications or insulin therapy via self-report[33]; and hypercholesterolemia by any self-reported use of antihypercholesterolemic medication.[2] History of cardiovascular diseases included self-reported previous coronary heart disease (myocardial infarction, coronary angioplasty, or coronary artery bypass graft), stroke, or TIA.[2] To measure neighborhood socioeconomic status (NSES) at baseline, WHI calculated the NSES score.[34] This index was composed of (1) median household income; (2) median value of owner-occupied housing units; (3) percent of households with interest, dividends, or rent income; (4) percent of adults >25 years of age with a high school degree; (5) percent of adult >25 years of age with a college degree; and (6) percent of civilian population >16 years of age with professional, managerial, or executive occupations.

Statistical analyses

We summarized baseline characteristics and brain volumes of participants using mean values with SDs for continuous variables and proportions for categorical variables. Analysis of variance and χ^2 test were used to assess differences across quartiles of the omega-3 index. We used multivariable-adjusted generalized linear regression models to examine the associations between erythrocyte membrane and dietary levels of LCn3PUFAs and nonfried fish consumption in relation to brain volumes, adjusting for intracranial volume, total energy intake (in the analyses of dietary intakes), age (65–69, 70–74, or ≥75 years), race/ethnicity (non-Hispanic white, black, Hispanic/Latino, or others), US regions (Northeast, South, Midwest, or West), education attainment (less than high school, high school graduate or equivalents, or college graduate

or higher degree), family income (<$10,000, $10,000–$34,900, $35,000–$74,900, ≥75,000 [US dollars], or do not know), smoking status (never, former, or current smokers), alcohol consumption (never, former, current drinkers <1 drink per day, or current drinkers ≥1 drink per day), BMI (<25.0, 25.0–29.9, or ≥30.0 kg/m^2), moderate or strenuous activity ≥20 min/day (none, some activity, 2–4 episodes per week, or >4 episodes per week), random assignment to hormone therapy (estrogen alone or estrogen + progesterone), and medical histories (hypertension, diabetes mellitus, hypercholesterolemia, and cardiovascular diseases; yes or no).

The associations between $PM_{2.5}$ exposure and brain volumes were assessed in generalized linear regression model, stratified by erythrocyte membrane and dietary levels of LCn3PUFAs, DHA, and EPA and by nonfried fish consumption. We presented the linear regression coefficients per interquartile increase (3.22 μg/m^3) in the continuous variable of $PM_{2.5}$ exposure. We tested interactions using the continuous variable of $PM_{2.5}$ exposure and the dichotomous (less than median vs median or greater) nutritional variables (erythrocyte membrane and dietary levels of LCn3PUFAs, nonfried fish consumption). In a sensitivity analysis, the Benjamini-Hochberg procedure was used to control type 1 error across the multiple interaction tests.[35]

To test the robustness of the findings, several sensitivity analyses were performed. First, we also adjusted for the modified Mini-Mental State Examination score at baseline or excluded participants who had developed dementia or cognitive decline before MRI scans to evaluate the possibility of biases resulting from self-selection among participants with better brain health at baseline. Second, to explore whether SVID could affect the observed associations, we in addition adjusted the models for SVID volume in the corresponding brain region. Third, because stroke and TIA may alter brain MRI measures independently of $PM_{2.5}$ exposure or omega-3 index levels, we excluded elderly women who experienced these incident events before the MRI scans. Similarly, common rheumatologic/demyelinating diseases such as multiple sclerosis, major depression, and bipolar disorder may affect brain structure[36–38]; thus, participants who reported these diseases at baseline were excluded. Forth, because socioeconomic status may be an important confounder for the association between air pollution and brain aging, we further adjusted for NSES score when examining the effect modifications. Fifth, we further adjusted for supplemental use of fish oil in the analyses of dietary LCn3PUFA intake. Finally, because intracranial volume is the sum of the total hemispheric volume and 2 compartments of CSF, 2 methods are accepted to control for intracranial volume: (1) include intracranial volume as a covariate in models or (2) divide hemispheric volume by intracranial volume and use this variable as the outcome. We used the first method in our main analyses and the second one as a sensitivity analysis. All analyses were performed with SAS version 9.4. A 2-sided value of $p \leq 0.05$ was considered statistically significant.

Data availability

The data-sharing plan of WHIMS-MRI study is consistent with the policy of National Heart, Lung, and Blood Institute, NIH, and US Department of Health and Human Services. Any data not published within this article are available in WHIMS-MRI data repository. Anonymized data may be shared by request from any qualified investigator in compliance with the regulations of National Heart, Lung, and Blood Institute, NIH, and US Department of Health and Human Services.

Results

In the study population, 91% of participants were non-Hispanic whites with an average age of 70 years at baseline. Participants with higher levels of the omega-3 index were less likely to be non-Hispanic white. More detailed baseline characteristics of participants are summarized in table 1. Participants with higher levels of the omega-3 index had higher intakes of LCn3PUFAs, DHA, EPA, and nonfried fish. In the univariate model, participants in the highest omega-3 index quartile, compared to those with lower omega-3 index, had the largest normal-appearing white matter volume (WMV), and this pattern was observed in the WMV of the frontal lobe, parietal lobe, temporal lobe, and corpus callosum (table 2). Participants with higher omega-3 index also had larger hippocampal volumes (table 2). However, no statistically significant differences were observed across omega-3 index quartiles in the volumes of cortical gray matter, basal ganglia, or ventricular size (table 2).

Results of multiple linear regression models are summarized in tables 3 and 4. Participants with higher omega-3 index levels had significantly greater volumes in the total association brain, white matter, and hippocampus after adjustment for potential confounders, similar to a previous report in the WHIMS-MRI cohort.[9] In addition, a higher omega-3 index was consistently associated with greater volumes of regional WMV, including the frontal lobe, parietal lobe, temporal lobe, and corpus callosum. The associations with erythrocyte membrane DHA and EPA levels were similar. The observed associations were not changed materially in any sensitivity analysis, including the one with additional adjustment for SVID. However, no association with cortical gray matter of association brain areas, basal ganglia, or ventricular volumes was observed for the omega-3 index in the multiple linear regression.

Elderly women living in locations with higher ambient $PM_{2.5}$ exposure had significantly smaller WMV (data not shown), as shown in a previous report that also found no apparent exposure effects on cortical gray matter and hippocampal volumes.[2] The putatively neurotoxic effect of $PM_{2.5}$ on WMV was attenuated by the omega-3 index (figure 1). Among women with higher omega-3 index levels (above the median level 5.01%), the observed inverse association between $PM_{2.5}$ exposure and

Table 1 Baseline characteristics of the study population among quartiles of RBC omega-3 index (n = 1,315)[a,b]

	Quartiles of the omega-3 index (%)					
	Q1 (<4.12)	Q2 (4.12–5.01)	Q3 (5.01–6.14)	Q4 (≥6.14)	Total	*p* Value
Omega-3 index, %	3.43 ± 0.13	4.57 ± 0.19	5.53 ± 0.23	7.45 ± 0.28	5.25 ± 0.21	—
Demographics						
Age, %						0.430
65–69 y	27.3	24.2	23.7	24.7	50.2	
70–74 y	21.3	26.5	26.8	25.4	35.7	
≥75 y	25.7	24.1	25.0	25.2	14.1	
Race/ethnicity, %						<0.001
Non-Hispanic white	26.2	25.8	25.1	22.8	90.8	
Black	8.6	12.7	28.6	50.0	4.6	
Hispanic white	31.2	26.4	18.2	24.2	1.6	
Other or missing	7.5	19.1	19.8	53.6	3.0	
US region, %						<0.001
Northeast	17.2	18.9	24.7	39.2	23.8	
South	21.6	28.2	28.8	21.4	15.3	
Midwest	34.6	29.6	23.3	12.5	34.0	
West	21.5	22.9	25.3	30.3	26.9	
Socioeconomic status						
Education, %						0.075
Less than high school	25.4	28.1	24.5	22.1	4.4	
High school graduate or equivalents	31.0	28.7	23.1	17.2	23.7	
College graduate or higher degree	23.0	23.6	25.6	27.7	71.9	
Family income, %						<0.001
<$10,000	33.7	25.0	22.3	18.9	3.7	
$10,000–$34,999	29.9	27.4	25.2	17.5	48.1	
$35,000–$74,999	20.4	24.0	24.6	31.0	35.5	
≥$75,000	14.8	17.3	24.3	43.6	9.5	
Do not know	21.1	23.3	32.8	22.8	3.3	
Employment, %						0.797
Currently employed	27.9	23.3	25.4	23.4	18.2	
Currently not employed	27.3	25.3	19.9	27.6	10.7	
Retired	23.8	25.5	25.7	25.1	71.1	
Lifestyle factors						
Smoking status, %						0.059
Never	26.3	26.5	24.5	22.7	58.4	
Former	21.4	22.9	27.1	28.7	37.5	
Current	39.6	23.9	12.3	24.2	4.1	
Alcohol consumption, %						0.075

Continued

Table 1 Baseline characteristics of the study population among quartiles of RBC omega-3 index (n = 1,315)[a,b] *(continued)*

	Quartiles of the omega-3 index (%)					
	Q1 (<4.12)	Q2 (4.12–5.01)	Q3 (5.01–6.14)	Q4 (≥6.14)	Total	*p* Value
Never	30.8	25.5	22.3	21.5	12.8	
Former	28.1	26.0	26.7	19.2	16.4	
Current <1 drink/d	24.5	24.9	25.1	25.5	59.3	
Current ≥1 drink/d	16.1	23.0	26.3	34.6	11.5	
BMI, %						0.035
<25.0 kg/m^2	22.8	21.0	24.3	31.9	30.7	
25.0–29.9 kg/m^2	24.7	26.2	26.1	23.0	37.9	
≥30.0 kg/m^2	27.4	27.3	24.6	20.8	31.4	
Moderate or strenuous activities ≥20 min/d, %						0.047
No activity	28.7	25.8	23.7	21.8	56.3	
Some activity	23.6	27.0	22.7	26.7	5.3	
2–4 episodes/wk	20.5	24.0	27.7	27.8	20.4	
≥4 episodes/wk	18.6	23.2	26.7	31.5	18.1	
Clinical characteristics						
Depression (yes), %	26.6	26.2	20.8	26.4	8.0	0.999
Random assignment to HT, %						0.585
Estrogen-alone intervention	24.6	26.7	25.6	23.1	18.2	
Estrogen-alone control	28.1	26.3	24.9	20.7	18.6	
Estrogen + progesterone intervention	25.4	25.3	25.0	24.3	31.1	
Estrogen + progesterone control	22.9	23.1	24.8	29.3	32.1	
Hypertension (yes), %	25.2	24.7	23.9	26.2	36.2	0.778
Diabetes mellitus (yes), %	26.2	25.8	25.1	22.9	3.2	0.995
Hypercholesterolemia (yes), %	21.7	22.6	27.1	28.5	16.1	0.444
Cardiovascular disease (yes), %	25.5	25.6	24.0	24.9	14.0	0.823
Intakes of LCn3PUFAs and fish						
LCn3PUFAs, mg/d	59.93 ± 3.46	81.87 ± 5.03	108.66 ± 7.08	178.10 ± 8.31	107.20 ± 2.93	<0.001
DHA, mg/d	39.37 ± 2.20	53.76 ± 3.32	71.65 ± 4.73	121.27 ± 5.83	71.55 ± 2.01	<0.001
EPA, mg/d	20.55 ± 1.32	28.11 ± 1.79	37.01 ± 2.45	56.83 ± 2.64	35.65 ± 0.96	<0.001
Nonfried fish, servings/d	0.05 ± 0.005	0.08 ± 0.007	0.11 ± 0.01	0.20 ± 0.01	0.11 ± 0.004	<0.001
$PM_{2.5}$ exposure						
3-y moving average $PM_{2.5}$ exposure, μg/m^3	11.66 ± 0.17	11.56 ± 0.15	11.66 ± 0.16	12.30 ± 0.15	11.80 ± 0.07	0.005

Abbreviations: BMI = body mass index; HT = hormone therapy; LCn3PUFA = long chain omega-3 polyunsaturated fatty acids; $PM_{2.5}$ = particle matter with diameters <2.5 μm; Q = quartile; RBC = red blood cell.
[a] Results are presented by means ± SDs or proportions (row proportions across quartiles of the omega-3 index and column proportions for the total sample).
[b] *p* Values are for any differences across quartiles of the omega-3 index using analysis of variance or χ^2 test, as appropriate.

WMV were non-remarkable and statistically non-significant in the total brain and the multi-modal association areas. In contrast, the inverse associations were stronger and remained statistically significant in women with lower levels of omega-3 index. Similar modifications were observed for levels of both DHA and EPA. Tests for interaction were generally consistent with or without accounting for multiple comparison, and the findings remained in all sensitivity analyses (data not shown).

Table 2 Distribution of brain volumes in relation to quartiles of RBC omega-3 index[a,b]

	Quartiles of the omega-3 index				
MRI-measured brain volume	Q1 (<4.12%), cm^3	Q2 (4.12%–5.01%), cm^3	Q3 (5.01%–6.14%), cm^3	Q4 (≥6.14%), cm^3	*p* Value
Total brain volume	1,087.15 ± 6.22	1,093.37 ± 7.84	1,090.63 ± 8.99	1,087.02 ± 6.77	0.907
Normal brain volume	1,078.34 ± 6.12	1,084.23 ± 7.64	1,080.96 ± 9.00	1,078.38 ± 6.81	0.979
Association brain	717.39 ± 4.22	723.03 ± 4.55	721.65 ± 5.73	720.95 ± 4.86	0.362
Total white matter	402.68 ± 3.23	407.41 ± 3.64	407.20 ± 4.41	410.15 ± 3.44	0.038
Frontal lobe	156.85 ± 1.40	158.67 ± 1.52	158.52 ± 1.83	160.44 ± 1.47	0.033
Parietal lobe	85.76 ± 0.83	86.47 ± 0.89	86.59 ± 1.17	87.89 ± 0.90	0.042
Temporal lobe	93.16 ± 0.80	94.30 ± 0.93	94.37 ± 0.92	94.96 ± 0.78	0.026
Corpus callosum	8.86 ± 0.08	8.96 ± 0.09	9.01 ± 0.10	9.06 ± 0.08	0.023
Total gray matter	394.27 ± 2.90	396.06 ± 2.87	395.55 ± 2.93	392.55 ± 3.17	0.684
Frontal lobe	118.64 ± 1.02	119.10 ± 1.09	118.54 ± 0.99	116.74 ± 1.14	0.179
Parietal lobe	62.64 ± 0.59	62.61 ± 0.64	62.15 ± 0.65	61.19 ± 0.69	0.063
Temporal lobe	82.05 ± 0.67	82.25 ± 0.63	82.04 ± 0.74	81.17 ± 0.75	0.348
Hippocampus	5.61 ± 0.07	5.69 ± 0.07	5.79 ± 0.07	5.79 ± 0.06	0.016
Basal ganglia	34.71 ± 0.22	34.97 ± 0.20	35.05 ± 0.22	34.94 ± 0.22	0.395
Ventricle	36.93 ± 1.16	36.46 ± 1.15	36.36 ± 1.13	37.60 ± 1.10	0.640

Abbreviations: Q = quartile; RBC = red blood cell.
[a] Brain volumes (not divided by intracranial volume) are presented as means ± SDs.
[b] *p* Values are for any differences across quartiles of the omega-3 index using analysis of variance test with the adjustment for intracranial volume.

The modifications by the omega-3 index were limited to the normal-appearing association WMV, with no statistically significant differences observed in the corpus callosum, cortical gray matter, hippocampus, or ventricle volume.

In the secondary analyses, we examined whether dietary intakes of LCn3PUFAs and nonfried fish had similar associations with WMV (table 5) and modified the observed association of $PM_{2.5}$ on WMV (figure 2). Participants with

Table 3 Multiple linear regression of global brain volumes against RBC omega-3 index[a,b]

	Total brain	Normal brain	Association brain	Total white matter	Total gray matter	Hippocampus	Basal ganglia	Ventricle
Omega-3 index, %								
Adjusted β ± SE	1.70 ± 4.09	0.33 ± 0.47	3.90 ± 1.64	5.03 ± 1.61	−0.49 ± 1.52	0.08 ± 0.04	0.04 ± 0.13	0.09 ± 0.61
***p* Value**	0.678	0.476	0.018	0.002	0.747	0.028	0.751	0.885
RBC DHA, %								
Adjusted β ± SE	0.92 ± 4.32	0.31 ± 0.48	3.74 ± 1.72	4.69 ± 1.66	−0.33 ± 1.61	0.09 ± 0.04	0.03 ± 0.13	−0.06 ± 0.65
***p* Value**	0.832	0.514	0.031	0.005	0.838	0.024	0.839	0.921
RBC EPA, %								
Adjusted β ± SE	3.22 ± 3.15	0.23 ± 0.39	2.59 ± 1.18	3.74 ± 1.29	−0.74 ± 1.17	0.03 ± 0.03	0.06 ± 0.10	0.50 ± 0.48
***p* Value**	0.307	0.550	0.028	0.004	0.528	0.346	0.579	0.306

Abbreviations: DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; RBC = red blood cell.
[a] All models were constructed using multiple linear regression model with adjustment for intracranial volume, age, race/ethnicity, US regions, education attainment, family income, smoking status, alcohol consumption, body mass index, moderate or strenuous activity ≥20 min/d, random assignment to hormone therapy, and medical histories (hypertension, diabetes mellitus, hypercholesterolemia, and cardiovascular diseases).
[b] The associations are expressed as the linear regression coefficients ± standard errors per interquartile increment in the continuous variables of the omega-3 index, DHA and EPA. The interquartile ranges of the omega-3 index, DHA, and EPA were 2.02%, 1.80%, and 0.37%, respectively.

Table 4 Multiple linear regression of brain WMVs against RBC omega-3 index[a,b]

	Association WMV	Frontal lobe WMV	Parietal lobe WMV	Temporal lobe WMV	Corpus callosum
Omega-3 index, %					
Adjusted β ± SE	5.07 ± 1.59	2.16 ± 0.74	1.19 ± 0.45	1.35 ± 0.39	0.12 ± 0.05
***p* Value**	0.002	0.004	0.009	<0.001	0.012
RBC DHA, %					
Adjusted β ± SE	4.71 ± 1.64	2.02 ± 0.75	1.14 ± 0.48	1.25 ± 0.40	0.11 ± 0.05
***p* Value**	0.004	0.008	0.018	0.002	0.019
RBC EPA, %					
Adjusted β ± SE	3.79 ± 1.28	1.59 ± 0.60	0.79 ± 0.35	1.03 ± 0.33	0.07 ± 0.04
***p* Value**	0.003	0.008	0.025	0.002	0.039

Abbreviations: DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; RBC = red blood cell; WMV = white matter volume.
[a] All models were constructed using multiple linear regression model with adjustment for intracranial volume, age, race/ethnicity, US regions, education attainment, family income, smoking status, alcohol consumption, body mass index, moderate or strenuous activity ≥20 minutes, random assignment to hormone therapy, and medical histories (hypertension, diabetes mellitus, hypercholesterolemia, and cardiovascular diseases).
[b] The associations are expressed as the linear regression coefficients ± standard errors per interquartile increment in the continuous variables of the omega-3 index, DHA and EPA. The interquartile ranges of the omega-3 index, DHA, and EPA were 2.02%, 1.80%, and 0.37%, respectively.

higher dietary intake of LCn3PUFAs (per interquartile increment 104.25 mg/d) had significantly greater WMV after adjustment for potential confounders. Higher nonfried fish consumption (per interquartile increment of 0.14 servings per day) was also associated with a greater WMV in the temporal lobe. Similar to the observed effect modification using LCn3PUFA biomarker data, the potential neurotoxic effect of $PM_{2.5}$ exposure on WMV also was modified by the dietary intakes of LCn3PUFAs and nonfried fish. Higher levels (above the median level) of LCn3PUFA, DHA, EPA, or nonfried fish intake significantly attenuated the inverse associations between $PM_{2.5}$ and WMV in the total brain and the multimodal association areas, with much stronger adverse effects of $PM_{2.5}$ observed in elderly women with lower levels of intakes. The effect modifications persisted in all the sensitivity analyses (data not shown). The intakes of LCn3PUFAs and nonfried fish did not modify the associations between $PM_{2.5}$ and the volumes of the corpus callosum, cortical gray matter, hippocampus, or ventricle.

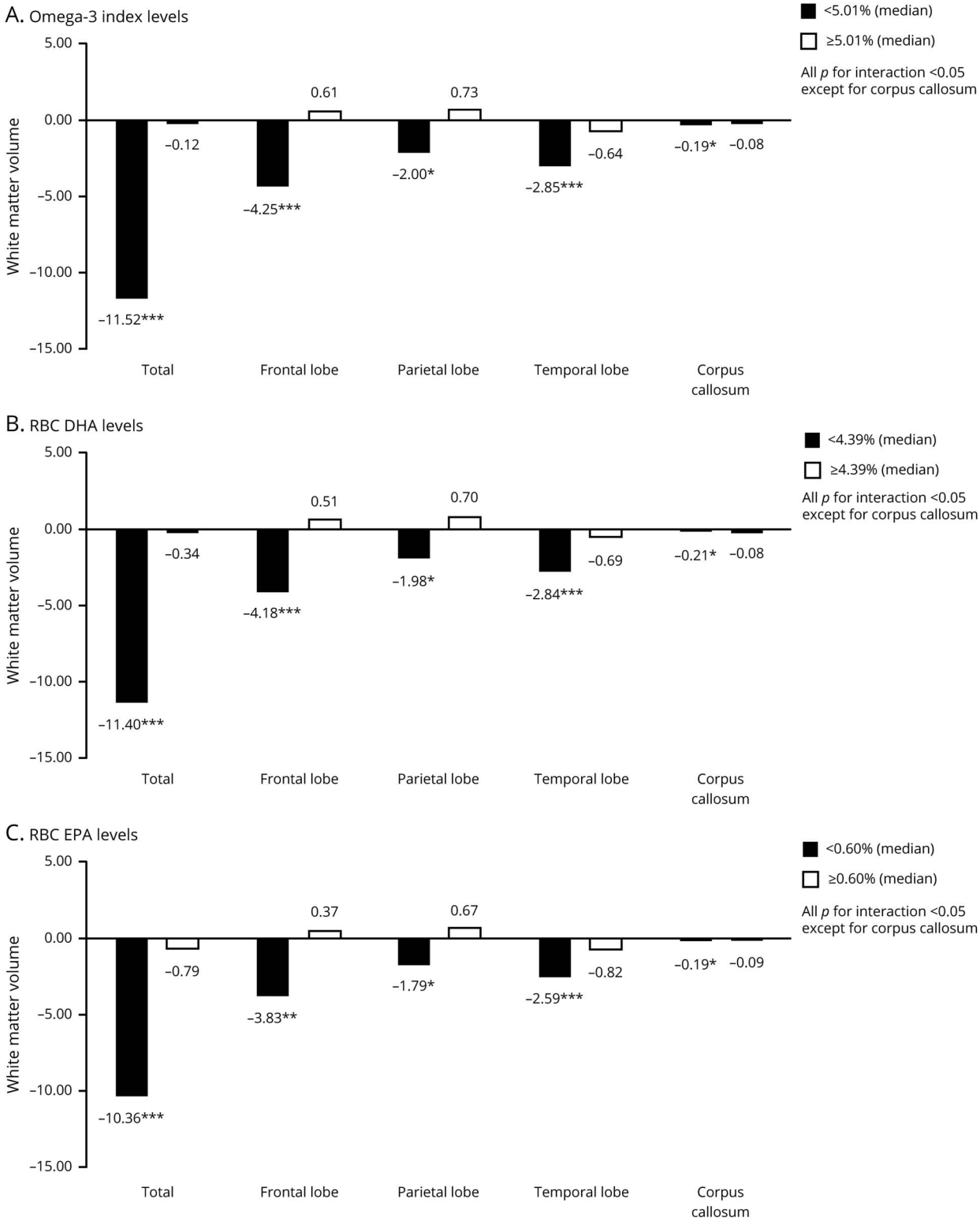
Discussion

In this prospective cohort study, we found that higher erythrocyte EPA + DHA levels (i.e., the omega-3 index) attenuated the inverse association between $PM_{2.5}$ exposure and WMV measured by brain MRI in elderly women. Similar patterns of modification by dietary intakes of LCn3PUFAs and nonfried fish were observed as expected owing to the documented validity of the omega-3 index as an intake biomarker.

Our study adds to the growing evidence that LCn3PUFAs may contribute to the healthy aging of white matter. The benefits of LCn3PUFAs on brain atrophy have been demonstrated in epidemiologic studies. For example, studies of dementia-free elderly participants found that nutrient intake patterns related to higher blood LCn3PUFA levels were associated with lower white matter hyperintensity volumes.[11–14,39] Similarly, a higher intake of nonfried fish was associated with fewer white matter abnormalities in a large prospective study of healthy elderly participants.[13] In our sensitivity analyses, the associations between the omega-3 index and normal-appearing WMV were independent of SVID volume (data not shown), and very similar associations were found in elderly women who remained cognitively intact without dementia or stroke. These interesting findings suggest that the neurotrophic effects of LCn3PUFAs observed in this study might take place in the preclinical stage before overt neurodegeneration or cerebrovascular disease. LCn3PUFAs have been reported to directly protect oligodendrocytes, the cells responsible for the production and maintenance of myelin, against excitatory cell death.[6] They also induce M2 (alternatively activated macrophages) polarization in cultured microglia,[5] which can resolve local inflammation and promote remyelination in the white matter, thereby facilitating white matter repair.[40] EPA has also been found to stimulate the expression of specific myelin proteins through decreased levels of cAMP-response element-binding protein phosphorylation.[41]

An increasing number of studies have suggested that white matter architecture may represent a novel target of airborne particle–induced neurotoxicity. Evidence from both laboratory animals[42] and humans[2,43] suggests that long-term exposure to ambient air particles may result in smaller WMVs, which may be attributable to their effects on myelin loss or chronic microglial activation. In mouse models, decreased myelin basic protein and increased Iba1 immunostaining, a marker for microglial activation, were induced by exposure to ambient fine

Figure 1 Associations between $PM_{2.5}$ exposure and brain normal-appearing WMVs stratified by RBC omega-3 index



All models (A–C) were constructed by using linear regression model with adjustment for intracranial volume, age, race/ethnicity, US regions, education attainment, family income, employment, smoking status, alcohol consumption, body mass index, moderate or strenuous activity ≥20 min/d, prior depression, random assignment to hormone therapy, and medical histories (hypertension, diabetes mellitus, hypercholesterolemia, and cardiovascular diseases). Associations are expressed as the linear regression coefficients per interquartile (3.22 μg/m^3) increment in the continuous variable of 3-year moving average particle matter with diameters <2.5 μm ($PM_{2.5}$) exposure before the MRI examination. For example, in panel A, for each interquartile increase in $PM_{2.5}$ exposure, the average white matter volume (WMV) was 11.52 cm^3 smaller among participants with lower omega-3 index (less than median level) and 0.12 cm^3 smaller among participants with higher omega-3 index (median level or greater). DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; RBC = red blood cell. Significance of *p* values: **p* < 0.05, ***p* < 0.001, ****p* < 0.0001.

Table 5 Multiple linear regression of white matter brain volumes against intakes of LCn3PUFAs and nonfried fish[a,b]

	Total white matter	Association WMV	Frontal lobe WMV	Parietal lobe WMV	Temporal lobe WMV	Corpus callosum
LCn3PUFAs intake, mg/d						
Adjusted β ± SE	2.38 ± 1.14	2.43 ± 1.13	1.07 ± 0.52	0.55 ± 0.32	0.74 ± 0.29	0.07 ± 0.03
***p* Value**	0.038	0.031	0.041	0.087	0.010	0.034
DHA intake, mg/d						
Adjusted β ± SE	2.67 ± 1.06	2.74 ± 1.04	1.20 ± 0.48	0.64 ± 0.30	0.77 ± 0.26	0.06 ± 0.03
***p* Value**	0.011	0.009	0.013	0.032	0.003	0.033
EPA intake, mg/d						
Adjusted β ± SE	1.26 ± 1.23	1.27 ± 1.22	0.57 ± 0.56	0.25 ± 0.35	0.52 ± 0.31	0.07 ± 0.03
***p* Value**	0.308	0.297	0.315	0.478	0.088	0.049
Nonfried fish intake, servings/d						
Adjusted β ± SE	1.72 ± 1.11	1.75 ± 1.09	0.77 ± 0.51	0.35 ± 0.31	0.59 ± 0.28	0.06 ± 0.03
***p* Value**	0.122	0.111	0.132	0.262	0.034	0.056

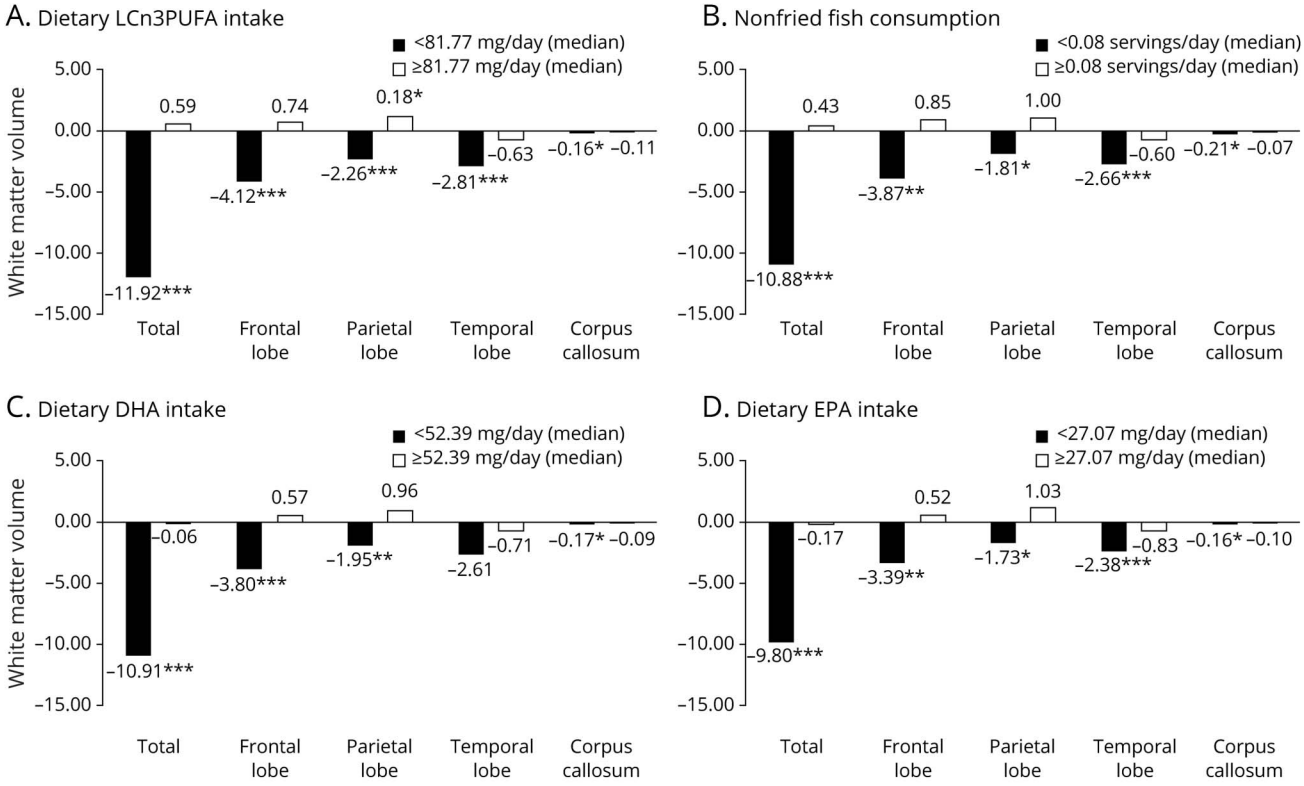
Abbreviations: DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LCn3PUFA = long chain omega-3 polyunsaturated fatty acids; WMV = white matter volume.
[a] All models were constructed using linear regression model with adjustment for intracranial volume, total energy intake, age, race/ethnicity, US regions, education attainment, family income, smoking status, alcohol consumption, body mass index, moderate or strenuous activity ≥20 min/day, random assignment to hormone therapy, and medical histories (hypertension, diabetes mellitus, hypercholesterolemia, and cardiovascular diseases).
[b] The association is expressed as the linear regression coefficients ± standard errors per interquartile increment in the continuous variables of LCn3PUFA and nonfried fish intakes. The interquartile ranges of total LCn3PUFA, DHA, EPA, and nonfried fish intakes are 104.25 mg/d, 66.08 mg/d, 37.33 mg/d, and 0.14 servings per day, respectively.

particle matter.[44] Because high levels of LCn3PUFAs may alleviate myelin damage and the subsequent white matter abnormalities, LCn3PUFAs may counteract the potential neurotoxicity of $PM_{2.5}$ on white matter. In this study on older women, there was no appreciable adverse $PM_{2.5}$ effect on WMV among participants with higher omega-3 index levels. We also found that the negative association between $PM_{2.5}$ and WMV was similarly attenuated by higher RBC DHA and EPA levels and by greater dietary intakes of LCn3PUFAs and nonfried fish consumption. Future laboratory studies may elucidate the underlying mechanisms (e.g., anti-inflammatory effects[45]), and clinical trials with LCn3PUFA supplementation may demonstrate the potential influences of LCn3PUFA intake as one of the critical strategies for preventing $PM_{2.5}$-induced neurotoxicity.

Some limitations of the present study need to be acknowledged. First, the multiple imputation method used to correct for the oxidative degradation of RBC fatty acids, although well suited for this purpose, increased the total variability in the imputed data and would therefore cause the reported associations and effect modifications to be underestimated.[20] Therefore, the true association and effect modification of LCn3PUFA status with WMV would be even stronger than what was seen in this study. Second, the present study included only elderly women; thus, our findings could not be generalized to men and younger women. However, the neurotrophic effects of LCn3PUFAs do not appear to be different by sex or age according to the literature.[46] Third, the present study focused on $PM_{2.5}$ as a regional pollutant, and we did not characterize the sources of $PM_{2.5}$. Fourth, the spatiotemporal models allowed estimates of only late-life exposure to $PM_{2.5}$ after 1999. Because air pollution levels have declined over the last few decades, exposure to $PM_{2.5}$ during midlife or earlier life may impart a greater risk for accelerated aging of white matter, which should be investigated in future studies. Currently, to the best of our knowledge, no existing cohort has $PM_{2.5}$ data available before 1999. Finally, according to a previous report in WHIMS-MRI, women who voluntarily provided MRI scans might vary by demographic and clinical characteristics, similar to other observational studies.[19] Thus, we could not completely rule out the possibility of survival bias.

Our study had major strengths. First, analyses of LCn3PUFA status were based on RBC membrane fatty acid composition and dietary intake, both of which showed consistent evidence of effect modification. These complementary measures have different strengths, limitations, and sources of errors. The use of both biomarkers and dietary estimates provides a comprehensive evaluation of the effect modification of interest, supporting the neurologic benefits of LCn3PUFAs on brain volumetric measures. Second, the long-term $PM_{2.5}$ exposure was estimated with longitudinal geocoded participant addresses reflecting changes in address over time.

Figure 2 Associations between $PM_{2.5}$ exposure and brain normal-appearing WMVs stratified by intakes of LCn3PUFAs and nonfried fish



All p for interaction <0.05 except for corpus callosum

All models (A–D) were constructed using linear regression models with the adjustment for intracranial volume, age, race/ethnicity, US regions, education attainment, family income, employment, smoking status, alcohol consumption, body mass index, moderate or strenuous activity ≥20 min/d, prior depression, random assignment to hormone therapy, and medical histories (hypertension, diabetes mellitus, hypercholesterolemia, and cardiovascular diseases). Associations are expressed as the linear regression coefficients per interquartile (3.22 μg/m^3) increment in the continuous variable of particle matter with diameters <2.5 μm ($PM_{2.5}$) exposure before the MRI examination. For example, in panel A, for each interquartile increase in $PM_{2.5}$ exposure, the average white matter volume (WMV) was 11.92 cm^3 smaller among participants with lower long chain omega-3 polyunsaturated fatty acid (LCn3PUFA) intake (less than median level) and 0.59 cm^3 greater among participants with higher LCn3PUFA intake (median level or greater). DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid. Significance of p values (main and interaction): *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$.

Findings from this prospective cohort study among elderly women suggest that higher LCn3PUFA intake (and thus blood levels) could help preserve WMV with aging and protect against the potential neurotoxic effects of $PM_{2.5}$ exposure.

Acknowledgment

The authors are grateful for the dedicated efforts of all investigators and staff at the WHI and WHIMS clinical centers as well as the WHI and WHIMS clinical coordinating center.

Study funding

The WHI program is funded by the National Heart, Lung, and Blood Institute, NIH, US Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. WHIMS was funded by Wyeth Pharmaceuticals, Inc, St. Davids, PA, and Wake Forest University. This study was supported by the NIH R01AG033078 and RF1AG054068. Drs. J.-C. Chen and Wang were in part supported by R01ES025888. Drs. C. Chen, Xun, and He were partially supported by NIH RF1AG056111.

Disclosure

C. Chen and P. Xun are supported by NIH grant (RF1AG056111). J. Kaufman, K. Hayden, M. Espeland, E. Whitsel, M. Serre, W. Vizuete, T. Orchard, and W. Harris report no disclosures. X. Wang is supported by NIH grant (R01ES025888). H. Chui reports no disclosure. J. Chen is supported by NIH grants (R01ES025888, P01AG055367, P30AG066530, and P30ES007048). K. He is supported by NIH grant (RF1AG056111). Go to Neurology.org/N for full disclosures.

Publication history

Received by *Neurology* June 5, 2019. Accepted in final form February 20, 2020.

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Joel D. Kaufman, MD	University of Washington, Seattle	Revised the manuscript for intellectual content
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William S. Harris, PhD	University of South Dakota and OmegaQuant Analytics LLC, Sioux Falls	Revised the manuscript for intellectual content
Xinhui Wang, PhD	University of Southern California, Los Angeles	Analyzed the data; revised the manuscript for intellectual content
Helena C. Chui, MD	University of Southern California, Los Angeles	Revised the manuscript for intellectual content
Jiu-Chiuan Chen, MD, ScD	University of Southern California, Los Angeles	Design and conceptualized study; major role in the acquisition of data; revised the manuscript for intellectual content
Ka He, MD, ScD	Columbia University Irving Medical Center, New York, NY	Designed and conceptualized study; major role in the acquisition of data; revised the manuscript for intellectual content

References

1. Heusinkveld HJ, Wahle T, Campbell A, et al. Neurodegenerative and neurological disorders by small inhaled particles. Neurotoxicology 2016;56:94–106.
2. Chen JC, Wang X, Wellenius GA, et al. Ambient air pollution and neurotoxicity on brain structure: evidence from Women's Health Initiative Memory Study. Ann Neurol 2015;78:466–476.
3. Wilker EH, Preis SR, Beiser AS, et al. Long-term exposure to fine particulate matter, residential proximity to major roads and measures of brain structure. Stroke 2015;46:1161–1166.
4. Cutuli D. Functional and structural benefits induced by omega-3 polyunsaturated fatty acids during aging. Curr Neuropharmacol 2017;15:534–542.
5. Chen S, Zhang H, Pu H, et al. n-3 PUFA supplementation benefits microglial responses to myelin pathology. Sci Rep 2014;4:7458.
6. Pu H, Guo Y, Zhang W, et al. Omega-3 polyunsaturated fatty acid supplementation improves neurologic recovery and attenuates white matter injury after experimental traumatic brain injury. J Cereb Blood Flow Metab 2013;33:1474–1484.
7. Eckert GP, Lipka U, Muller WE. Omega-3 fatty acids in neurodegenerative diseases: focus on mitochondria. Prostaglandins Leukot Essent Fatty Acids 2013;88:105–114.
8. Lee LK, Shahar S, Rajab N, Yusoff NA, Jamal RA, Then SM. The role of long chain omega-3 polyunsaturated fatty acids in reducing lipid peroxidation among elderly patients with mild cognitive impairment: a case-control study. J Nutr Biochem 2013; 24:803–808.
9. Pottala JV, Yaffe K, Robinson JG, Espeland MA, Wallace R, Harris WS. Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI study. Neurology 2014;82:435–442.
10. Conklin SM, Gianaros PJ, Brown SM, et al. Long-chain omega-3 fatty acid intake is associated positively with corticolimbic gray matter volume in healthy adults. Neurosci Lett 2007;421:209–212.
11. Witte AV, Kerti L, Hermannstadter HM, et al. Long-chain omega-3 fatty acids improve brain function and structure in older adults. Cereb Cortex 2014;24:3059–3068.
12. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. Neurology 2012;78:241–249.
13. Virtanen JK, Siscovick DS, Longstreth WT Jr, Kuller LH, Mozaffarian D. Fish consumption and risk of subclinical brain abnormalities on MRI in older adults. Neurology 2008;71:439–446.
14. Bowman GL, Dodge HH, Mattek N, et al. Plasma omega-3 PUFA and white matter mediated executive decline in older adults. Front Aging Neurosci 2013;5:92.
15. Singh PK, Nath R, Ahmad MK, Rawat A, Babu S, Dixit RK. Attenuation of lead neurotoxicity by supplementation of polyunsaturated fatty acid in Wistar rats. Nutr Neurosci 2016;19:396–405.
16. Meydan S, Altas M, Nacar A, et al. The protective effects of omega-3 fatty acid against toluene-induced neurotoxicity in prefrontal cortex of rats. Hum Exp Toxicol 2012;31: 1179–1185.
17. Kaur P, Schulz K, Aschner M, Syversen T. Role of docosahexaenoic acid in modulating methylmercury-induced neurotoxicity. Toxicol Sci 2007;100:423–432.
18. Shumaker SA, Reboussin BA, Espeland MA, et al. The Women's Health Initiative Memory Study (WHIMS): a trial of the effect of estrogen therapy in preventing and slowing the progression of dementia. Control Clin Trials 1998;19:604–621.
19. Jaramillo SA, Felton D, Andrews L, et al. Enrollment in a brain magnetic resonance study: results from the Women's Health Initiative Memory Study Magnetic Resonance Imaging Study (WHIMS-MRI). Acad Radiol 2007;14:603–612.
20. Pottala JV, Espeland MA, Polreis J, Robinson J, Harris WS. Correcting the effects of -20 degrees C storage and aliquot size on erythrocyte fatty acid content in the Women's Health Initiative. Lipids 2012;47:835–846.
21. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 2004;39:212–220.
22. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. Ann Epidemiol 1999;9:178–187.
23. Candela M, Astiasarán I, Bello J. Deep-fat frying modifies high-fat fish lipid fraction. J Agric Food Chem 1998;46:2783–2786.
24. Coker LH, Hogan PE, Bryan NR, et al. Postmenopausal hormone therapy and subclinical cerebrovascular disease: the WHIMS-MRI Study. Neurology 2009;72: 125–134.
25. Shen D, Davatzikos C. HAMMER: hierarchical attribute matching mechanism for elastic registration. IEEE Trans Med Imaging 2002;21:1421–1439.
26. Lao Z, Shen D, Liu D, et al. Computer-assisted segmentation of white matter lesions in 3D MR images using support vector machine. Acad Radiol 2008;15:300–313.
27. Launer LJ, Miller ME, Williamson JD, et al. Effects of intensive glucose lowering on brain structure and function in people with type 2 diabetes (ACCORD MIND): a randomised open-label substudy. Lancet Neurol 2011;10:969–977.
28. Whitsel EA, Quibrera PM, Smith RL, et al. Accuracy of commercial geocoding: assessment and implications. Epidemiol Perspect Innov 2006;3:8.
29. Reyes JM, Xu Y, Vizuete W, Serre ML. Regionalized PM2.5 Community Multiscale Air Quality model performance evaluation across a continuous spatiotemporal domain. Atmos Environ (1994) 2017;148:258–265.
30. Cacciottolo M, Wang X, Driscoll I, et al. Particulate air pollutants, APOE alleles and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. Transl Psychiatry 2017;7:e1022.
31. Burnam MA, Wells KB, Leake B, Landsverk J. Development of a brief screening instrument for detecting depressive disorders. Med Care 1988;26:775–789.
32. Honda T, Eliot MN, Eaton CB, et al. Long-term exposure to residential ambient fine and coarse particulate matter and incident hypertension in post-menopausal women. Environ Int 2017;105:79–85.
33. Margolis KL, Lihong Q, Brzyski R, et al. Validity of diabetes self-reports in the Women's Health Initiative: comparison with medication inventories and fasting glucose measurements. Clin Trials 2008;5:240–247.
34. Chi GC, Hajat A, Bird CE, et al. Individual and neighborhood socioeconomic status and the association between air pollution and cardiovascular disease. Environ Health Perspect 2016;124:1840–1847.
35. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat Med 1990;9:811–818.
36. Theodoridou A, Settas L. Demyelination in rheumatic diseases. J Neurol Neurosurg Psychiatry 2006;77:290–295.
37. Videbech P. MRI findings in patients with affective disorder: a meta-analysis. Acta Psychiatr Scand 1997;96:157–168.
38. Kempton MJ, Geddes JR, Ettinger U, Williams SC, Grasby PM. Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. Arch Gen Psychiatry 2008;65:1017–1032.

39. Tan ZS, Harris WS, Beiser AS, et al. Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging. Neurology 2012;78:658–664.
40. Miron VE, Boyd A, Zhao JW, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat Neurosci 2013;16:1211–1218.
41. Salvati S, Natali F, Attorri L, et al. Eicosapentaenoic acid stimulates the expression of myelin proteins in rat brain. J Neurosci Res 2008;86:776–784.
42. Allen JL, Liu X, Weston D, et al. Developmental exposure to concentrated ambient ultrafine particulate matter air pollution in mice results in persistent and sex-dependent behavioral neurotoxicity and glial activation. Toxicol Sci 2014;140:160–178.
43. Peterson BS, Rauh VA, Bansal R, et al. Effects of prenatal exposure to air pollutants (polycyclic aromatic hydrocarbons) on the development of brain white matter, cognition, and behavior in later childhood. JAMA Psychiatry 2015;72:531–540.
44. Woodward NC, Pakbin P, Saffari A, et al. Traffic-related air pollution impact on mouse brain accelerates myelin and neuritic aging changes with specificity for CA1 neurons. Neurobiol Aging 2017;53:48–58.
45. Calder PC. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. Biochim Biophys Acta 2015;1851:469–484.
46. Bos DJ, van Montfort SJ, Oranje B, Durston S, Smeets PA. Effects of omega-3 polyunsaturated fatty acids on human brain morphology and function: what is the evidence? Eur Neuropsychopharmacol 2016;26:546–561.