

Original Investigation

Association of Seafood Consumption, Brain Mercury Level, and *APOE* $\epsilon 4$ Status With Brain Neuropathology in Older Adults

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IMPORTANCE Seafood consumption is promoted for its many health benefits even though its contamination by mercury, a known neurotoxin, is a growing concern.

OBJECTIVE To determine whether seafood consumption is correlated with increased brain mercury levels and also whether seafood consumption or brain mercury levels are correlated with brain neuropathologies.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional analyses of deceased participants in the Memory and Aging Project clinical neuropathological cohort study, 2004-2013. Participants resided in Chicago retirement communities and subsidized housing. The study included 286 autopsied brains of 554 deceased participants (51.6%). The mean (SD) age at death was 89.9 (6.1) years, 67% (193) were women, and the mean (SD) educational attainment was 14.6 (2.7) years.

EXPOSURES Seafood intake was first measured by a food frequency questionnaire at a mean of 4.5 years before death.

MAIN OUTCOMES AND MEASURES Dementia-related pathologies assessed were Alzheimer disease, Lewy bodies, and the number of macroinfarcts and microinfarcts. Dietary consumption of seafood and n-3 fatty acids was annually assessed by a food frequency questionnaire in the years before death. Tissue concentrations of mercury and selenium were measured using instrumental neutron activation analyses.

RESULTS Among the 286 autopsied brains of 544 participants, brain mercury levels were positively correlated with the number of seafood meals consumed per week ($p = 0.16$; $P = .02$). In models adjusted for age, sex, education, and total energy intake, seafood consumption (≥ 1 meal[s]/week) was significantly correlated with less Alzheimer disease pathology including lower density of neuritic plaques ($\beta = -0.69$ score units [95% CI, -1.34 to -0.04]), less severe and widespread neurofibrillary tangles ($\beta = -0.77$ score units [95% CI, -1.52 to -0.02]), and lower neuropathologically defined Alzheimer disease ($\beta = -0.53$ score units [95% CI, -0.96 to -0.10]) but only among apolipoprotein E (*APOE* $\epsilon 4$) carriers. Higher intake levels of α -linolenic acid (18:3 n-3) were correlated with lower odds of cerebral macroinfarctions (odds ratio for tertiles 3 vs 1, 0.51 [95% CI, 0.27 to 0.94]). Fish oil supplementation had no statistically significant correlation with any neuropathologic marker. Higher brain concentrations of mercury were not significantly correlated with increased levels of brain neuropathology.

CONCLUSIONS AND RELEVANCE In cross-sectional analyses, moderate seafood consumption was correlated with lesser Alzheimer disease neuropathology. Although seafood consumption was also correlated with higher brain levels of mercury, these levels were not correlated with brain neuropathology.

JAMA. 2016;315(5):489-497. doi:10.1001/jama.2015.19451

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Numerous studies have found protective associations between seafood consumption and dementia.¹⁻⁷ Little is known about the relationship between seafood consumption and brain neuropathology. Seafood is high in the long-chain n-3 fatty acid docosahexaenoic acid (DHA), which has established properties for normal neuronal function.^{8,9} However, seafood is also a source of mercury, a neurotoxin that impairs neurocognitive development.¹⁰ Mercury toxicity is reduced by selenium, an essential nutrient present in seafood that has high binding affinity to mercury.¹¹ In this study, we examined the associations of dementia neuropathologies with dietary n-3 fatty acids and with brain levels of mercury and selenium.

Methods

Study Population

The analytic sample comprised autopsied cases of deceased participants in the Rush Memory and Aging Project (MAP) who died between November 2004 and November 2013 and who completed a dietary assessment before death. MAP is an ongoing clinical-neuropathological cohort study of older adults that began in 1997 and includes Chicago residents of retirement communities and subsidized housing.¹² At enrollment, participants were dementia free and agreed to undergo annual clinical neurological evaluations and brain autopsy at death. Annual dietary assessments of MAP participants began in February 2004. Written informed consent was obtained from all study participants, and the study was approved by the institutional review board of Rush University.

Brain Neuropathology

The methods for brain autopsies and pathologic evaluations are described in detail elsewhere.¹³ Briefly, slabs from 1 cerebral hemisphere were placed in a -80°C freezer and used for metal analyses. Slabs from the contralateral hemisphere were fixed in 4% paraformaldehyde, and then dissected tissue samples from brain regions were embedded in paraffin blocks, cut into 6-micron sections, and mounted onto slides. Alzheimer disease neuropathologies, including diffuse and neuritic amyloid plaques and neurofibrillary tangles, were identified using modified Bielschowsky silver-stained 6-micron sections in multiple cortical regions. Raw counts (greatest density in 1-mm² area) of the neuritic and diffuse plaques and tangles were standardized in each region and averaged across regions to create 3 summary scores that were then averaged for a global measure of Alzheimer disease pathology. We also analyzed 2 semiquantitative measures of Alzheimer disease pathology: CERAD (Consortium to Establish a Registry for Alzheimer Disease; [for neuritic plaque density])¹⁴ and Braak neurofibrillary tangle stage.¹⁵ CERAD scores ranged from 1 (no neuritic plaques) to 4 (frequent neuritic plaques). Braak staging scores ranged from 0 (no tangles in any region) to 6 (frequent tangles across multiple regions). Another variable described the level of Alzheimer disease pathology based on National Institute on Aging (NIA) and the Ronald and Nancy Reagan Research Institute of the

Alzheimer's Association criteria¹⁶ with scores ranging from 1 (low Alzheimer disease pathology) to 4 (high Alzheimer disease pathology). A board-certified neuropathologist, blinded to participant ages and clinical data, determined the neuropathology diagnoses.

Counts of chronic macroscopic cerebral infarctions and microinfarcts were made as previously described.¹⁷ Macroinfarcts were identified through visual inspection and were histologically confirmed. Multiple regions were examined for microinfarcts using hematoxylin and eosin stain.

Lewy body staging was assessed in 6 regions through staining with antibodies to α -synuclein.^{13,18}

Mercury and Selenium Analyses

Brain metal concentrations were measured in 2 cortical regions (inferior temporal and midfrontal) involved in Alzheimer disease neuropathology and the cerebellum (a region unaffected by Alzheimer disease neuropathology) to facilitate the interpretation of study findings. Brain tissue was cut into 100-g samples using a ceramic blade (to avoid metal contamination) and sent to the University of Missouri Research Reactor to be assessed for selenium and mercury by instrumental neutron activation analysis.¹⁹ Samples were loaded into cleaned, high-purity quartz vials, lyophilized, and then irradiated for 50 hours in a neutron flux of 6.5×10^{13} n/cm²-s and allowed to decay for 20 to 40 days before being counted using a high-purity germanium detector system. Measured concentrations of the metals in reference materials were analyzed for consistency with accepted standards and agreed well with certified values. For analyses, we first transformed ($\log_{10}$) the highly skewed metal concentrations and then computed *z* scores of these values for each brain region. Mean *z* scores for each region were then averaged across the 2 cortical regions and across all 3 regions.

Dietary Consumption

Dietary intake was assessed by a semiquantitative food frequency questionnaire that was validated for use in older Chicago residents.²⁰ The questionnaire included 4 seafood items (tuna sandwich; fish sticks, cakes, or sandwich; fresh fish as a main dish; and shrimp, lobster, or crab) that were used to compute weekly seafood consumption. Daily intakes of long-chain n-3 fatty acids (eicosapentaenoic acid [EPA, 20:5 n-3] + DHA [22:6 n-3]) and α -linolenic acid (18:3 n-3) were obtained by multiplying the nutrient content of all food items by frequency of consumption and summing over all items. Nutrient intakes were energy adjusted by the regression residual method.²¹ The questionnaire also included a query about intake of fish oil supplements. Dietary intake levels were averaged over all valid food frequency questionnaires obtained during the years before death. Evaluators of neuropathological measures and brain concentrations of metals were blinded to dietary intake data.

Statistical Methods

We used linear regression models to investigate the associations of the dietary variables and brain metal concentrations with Alzheimer disease neuropathology (general Alzheimer

disease pathology [log transformed] and CERAD, Braak, and NIA and Reagan Institute scores). We examined the diet/metal associations with Lewy bodies, macroinfarcts, and microinfarcts in logistic regression models with outcomes modeled present or absent because of the highly skewed distributions. Model assumptions were investigated graphically and statistically, and findings were confirmed through reanalyses using proportional hazards models. Analyses were conducted using SAS version 9.3. Models were adjusted for established predictors of neuropathology including age at death, sex, and education, and for the seafood model only, adjustment was made for total energy intake (kcal/d). Statistical interactions were tested in adjusted models with a multiplicative term between the diet/metal variable and the potential effect modifier. The level of statistical significance for all tests, including those for interactions, was 2-sided ($P \leq .05$). We investigated both linear and nonlinear correlations of the diet and metal exposures to neuropathologic outcomes. Consistent with previous studies,^{1,3,5,7,22} seafood consumption was not linearly correlated with the Alzheimer disease outcomes, and only the findings with seafood modeled as an indicator variable (≥ 1 meal[s] per week vs less) are presented. To make the data presentation succinct, we present only the linear model coefficients for analyses stratified by apolipoprotein E (*APOE* $\epsilon 4$). We examined the associations of brain metal concentrations to neuropathologic outcomes with the z score metal concentrations averaged over the 2 cortical regions and also the cortical and cerebellum regions. Because the findings were similar, we present averaged concentrations from the 3 brain regions.

Results

The study was conducted among 286 MAP participants who completed a dietary assessment, died between November 2004 and 2013 ($n = 554$), and had a brain autopsy performed ($n = 447$); thus representing 51.6% of all deceased MAP participants. The mean (SD) postmortem time interval was 8.3 (7.4) hours. Of the analyzed sample, 43.7% (125) completed 1 food frequency questionnaire, 20.3% (58) completed 2, and 36% (103) completed 3 or more. The correlation between the reported number of seafood meals consumed at the first and last obtained food frequency questionnaires was the Spearman rank-order correlation coefficient ($\rho = 0.57$; $P < .001$). The mean time interval between administration of the first questionnaire and death was 4.5 years, and there were 2.4 years between the last questionnaire and death. Participants were coded as fish oil supplement users if they reported taking the supplements in at least 1 dietary assessment ($n = 49$); most ($n = 31$) reported use at only 1 visit.

The analytic sample had a mean (SD) age of 89.9 (6.1) years at death, was largely composed of women (67%; $n=193$), had mean (SD) educational attainment of 14.6 (2.7) years, and 22.7% (65) were positive for the *APOE* $\epsilon 4$ allele. These characteristics were comparable with those of the total number of deceased participants (89.2 [6.2] years at death,

68% were women, 14.2 [3.0] years of education, and 23.6% were *APOE* $\epsilon 4$ positive). These and other characteristics were similar over the tertiles of dietary n-3 fatty acid intake levels with the exception that participants in the highest tertile of intake were more likely to be women and those in the lowest tertile more likely to be *APOE* $\epsilon 4$ positive (Table 1). Participants in the lowest n-3 fatty acid tertile also had somewhat higher levels of neuropathology.

The subsample of 203 autopsied cases for which brain concentrations of metals were analyzed had similar characteristics to the larger sample (Table 1). Brains of participants in the highest tertile of mercury concentrations were more likely to be older at death, to be women, and to have lower levels of global Alzheimer disease neuropathology and higher brain selenium concentrations.

Mean (SD) levels for brain mercury concentrations were 0.26 (0.87) $\mu\text{g/g}$ in the midfrontal region, 0.25 (0.78) $\mu\text{g/g}$ in the inferior temporal region, and 0.87 (1.80) $\mu\text{g/g}$ in the cerebellum. The levels were correlated across regions (Spearman ρ , 0.65 to 0.76 [$P < .001$]). Brain selenium concentrations were comparable across the brain regions (Table 2). Brain cortical mercury levels were positively correlated with cortical selenium levels (ρ , 0.35 [$P < .001$]) and with the number of seafood meals consumed per week (ρ , 0.16 [$P = .02$]).

Brain Neuropathology and Dietary n-3 Fatty Acids

We first analyzed the data in the entire sample of 286 deceased participants. Dietary intake of α -linolenic acid was correlated with decreased odds of cerebral macroinfarcts and microinfarcts in models adjusted for age at death, sex, and education but was not correlated with other neuropathological markers (Table 3). Dietary intake of long-chain n-3 fatty acids had a nonlinear correlation with cerebral macroinfarcts in the adjusted model but was not correlated with microinfarcts or other neuropathologic markers. Seafood consumption and fish oil supplement use were not correlated with any brain neuropathology in the total sample.

Based on observed statistical interactions between *APOE* $\epsilon 4$ genotype and seafood or n-3 fatty acid intakes on cognitive decline in previous studies,²³⁻³⁰ we investigated this potential effect modification on brain neuropathology. We observed significant interactions with *APOE* $\epsilon 4$ status and dietary intakes of seafood and long-chain n-3 fatty acids on every measure of Alzheimer disease neuropathology (all $P \leq .04$) except for n-3 fatty acids and neurofibrillary tangle severity (eTable 1 in the Supplement). Tests for interaction effects between *APOE* $\epsilon 4$ and either α -linolenic acid or fish oil supplement use on the neuropathologic outcomes did not reach the significance threshold ($P > .25$), with the exception of macroinfarcts (P for interaction = .05) for which fish oil supplement use was correlated with lower risk in *APOE* $\epsilon 4$ carriers.

Further analyses examined correlations between the dietary variables and brain neuropathology stratified by *APOE* $\epsilon 4$ status (Table 4). Individuals who were *APOE* $\epsilon 4$ positive and consumed at least 1 seafood meal per week or had higher intakes of long-chain n-3 fatty acids had less

Table 1. Characteristics of Deceased MAP Participants by Tertile of Dietary Long-Chain n-3 Fatty Acids (DHA + EPA) (n = 286) and by Tertile of Brain Mercury Concentrations (n = 203), 2005-2013

	Dietary n-3 Fatty Acid Level by Tertile, g/d			Brain Mercury Level by Tertile, µg/d		
	1	2	3	1	2	3
No. of participants	116	82	88	67	69	67
Tertile						
Mean (SD)	0.04 (0.02) ^a	0.09 (0.02) ^a	0.24 (0.13) ^a	0.02 (0.01) ^b	0.09 (0.09) ^b	1.28 (1.62) ^b
Median (interquartile range)	0.04 (0-0.06) ^a	0.09 (0.06-0.12) ^a	0.19 (0.12-0.92) ^a	0.02 (0.01-0.03) ^b	0.06 (0.03-0.20) ^b	0.56 (0.20-6.88) ^b
Characteristics						
Age at death, mean (SD), y	89.2 (6.3)	90.9 (6.6)	90.0 (5.2)	88.2 (6.7)	89.1 (5.2)	90.5 (5.7)
Women, No. (%)	77 (66.4)	51 (62.2)	65 (73.9)	44 (65.7)	42 (60.9)	47 (70.1)
APOE ε4, No. (%)	34 (29.3)	14 (17.1)	17 (19.3)	16 (24.6)	12 (17.4)	18 (28.1)
Education, mean (SD), y	14.3 (2.8)	14.8 (2.5)	14.7 (2.7)	14.6 (2.4)	15.0 (2.9)	14.3 (2.5)
Dietary Consumption						
Seafood consumption, mean (SD), meals/wk	1.3 (1.1)	2.0 (0.9)	2.7 (1.3)	1.8 (1.1)	2.0 (1.4)	2.3 (1.3)
Fish oil supplement use, No. (%)	14 (12)	20 (24)	15 (17)	11 (16)	13 (19)	8 (12)
α-Linolenic acid (18:3 n-3), mean (SD), g/d	1.14 (0.43)	1.16 (0.40)	1.10 (0.39)	1.12 (0.41)	1.13 (0.43)	1.14 (0.42)
Total energy intake, mean (SD), calories/d	1837 (611)	1891 (572)	1739 (505)	1837 (536)	1757 (547)	1807 (603)
Brain Measures						
Global Alzheimer disease neuropathology, mean (SD), score	0.77 (0.58)	0.57 (0.50)	0.69 (0.55)	0.73 (0.54)	0.64 (0.60)	0.59 (0.47)
Neuritic plaque density, mean (SD), z ^c	2.92 (1.05)	2.56 (1.18)	2.81 (1.18)	3.01 (1.06)	2.56 (1.23)	2.71 (1.09)
Neurofibrillary tangle severity, mean (SD), score ^d	3.68 (1.25)	3.41 (1.06)	3.61 (1.24)	3.54 (1.25)	3.48 (1.29)	3.54 (1.28)
Alzheimer disease diagnostic, mean (SD), score ^e	2.84 (0.72)	2.61 (0.60)	2.81 (0.74)	2.85 (0.70)	2.70 (0.79)	2.70 (0.70)
Brain macroinfarcts, No. (%)	52 (46)	24 (30)	29 (33)	29 (43)	28 (41)	20 (30)
Brain microinfarcts, No. (%)	36 (31)	21 (26)	21 (24)	28 (42)	14 (20)	15 (23)
Lewy bodies, No. (%)	27 (23)	22 (27)	13 (15)	14 (21)	11 (16)	13 (20)
Selenium, mean (SD), µg/g	1.43 (0.66)	1.38 (0.73)	1.36 (0.58)	1.14 (0.16)	1.20 (0.23)	1.85 (0.96)

Abbreviations: APOE 4, apolipoprotein E; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MAP, Memory and Aging Project.

^a Units of measure are reported as grams/day for dietary n-3 fatty acid levels.

^b Units of measure are reported as micrograms/day for brain mercury levels.

^c Data are reported as a z-score of square root counts. Consortium to Establish a Registry of Alzheimer Disease score range: 1 (no neuritic plaques) to 4 (frequent neuritic plaques).

^d Braak staging score range: 0 (no tangles in any region) to 6 (frequent tangles across multiple regions).

^e National Institute on Aging and the Ronald and Nancy Reagan Research Institute of the Alzheimer's Association score range: 1 (low Alzheimer disease pathology) to 4 (high Alzheimer disease pathology).

Table 2. Brain Concentrations of Total Mercury and Selenium by Brain Region for 203 Autopsied Brains of Deceased MAP Participants, 2005-2013

	Brain Region				P Value
	Inferior Temporal	Midfrontal	Cerebellum	All Regions	
Mercury, µg/g					
Mean (SD)	0.25 (0.78)	0.26 (0.87)	0.87 (1.80)	0.46 (1.09)	
Median (interquartile [1-3] range)	0.03 (0.01-0.06)	0.03 (0.02-0.07)	0.07 (0.03-0.67)	0.65 (0.02-0.28)	
Correlation, Spearman ρ				0.65-0.76	<.001
Selenium, µg/g					
Mean (SD)	1.18 (0.48)	1.24 (0.47)	1.77 (1.22)	1.40 (0.65)	
Median (interquartile [1-3] range)	1.16 (0.95-1.29)	1.18 (1.06-1.32)	1.36 (1.25-1.62)	1.23 (1.09-1.36)	
Correlation, Spearman ρ				0.42-0.49	<.001

Abbreviation: MAP, Memory and Aging Project.

Table 3. Relations of Cerebral Infarcts and Lewy Body Neuropathology to Dietary Intakes of Seafood, Long-Chain n-3 Fatty Acids (DHA + EPA), and α -Linolenic Acid Based on Adjusted Logistic Regression Models Among 286 Deceased MAP Participants, 2004-2013^a

Model	Odds Ratio (95% CI)		
	Macroinfarcts (n = 282)	Microinfarcts (n = 282)	Lewy Bodies (n = 283) ^b
Seafood (≥ 1 meal/wk vs <1 meal/wk)	0.99 (0.52-1.88)	0.98 (0.49-1.96)	0.70 (0.34-1.45)
DHA + EPA Food Sources			
Tertile			
1	1 [Reference]	1 [Reference]	1 [Reference]
2	0.50 (0.27-0.92)	0.74 (0.39-1.42)	1.15 (0.59-2.23)
3	0.60 (0.33-1.08)	0.67 (0.35-1.26)	0.54 (0.26-1.14)
P value for linear trend ^c	.12	.23	.09
α-Linolenic 18:3 n-3			
Tertile			
1	1 [Reference]	1 [Reference]	1 [Reference]
2	0.65 (0.36-1.18)	0.82 (0.44-1.54)	1.40 (0.69-2.86)
3	0.51 (0.27-0.94)	0.49 (0.25-0.96)	1.42 (0.69-2.90)
P value for linear trend ^c	.03	.04	.35

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MAP, Memory and Aging Project.

^a Models were adjusted for age at death, sex, and education and total energy intake (seafood model only).

^b Lewy body staging range: 0 (no disease), 1 (nigra-predominant disease), 2 (limbic-type disease), 3 (neocortical disease).

^c Linear trend variable in which values within each tertile were scored at the median intake level for that tertile.

Table 4. Relations of Alzheimer Disease Neuropathology to Dietary Intakes of Seafood, Long-Chain n-3 Fatty Acids (DHA + EPA), and α -Linolenic Acid Based on Adjusted Linear Regression Models in Analyses Stratified By APOE $\epsilon 4$ Status Among 286 Deceased MAP Participants, 2004-2013^a

Model	Global Alzheimer Disease Pathology	Neuritic Plaque Density ^b	Neurofibrillary Tangle Severity ^c	Alzheimer Disease Diagnostic Score ^d
Seafood (≥ 1 meal/wk vs <1 meal/wk)				
APOE $\epsilon 4$ -positive, No.	65	65	65	65
β (95% CI)	-0.77 (-1.53 to -0.01)	-0.69 (-1.34 to -0.04)	-0.77 (-1.52 to -0.02)	-0.53 (-0.96 to -0.10)
APOE $\epsilon 4$ -negative, No.	221	218	218	218
β (95% CI)	0.13 (-0.30 to 0.55)	0.26 (-0.14 to 0.65)	0.20 (-0.19 to 0.59)	0.20 (-0.04 to 0.43)
DHA + EPA Food Sources				
APOE $\epsilon 4$ -positive, No.	65	65	65	65
β (95% CI)	-4.80 (-8.60 to -0.99)	-5.04 (-8.20 to -1.88)	-2.36 (-6.25 to 1.53)	-2.46 (-4.65 to -0.28)
APOE $\epsilon 4$ -negative, No.	221	218	218	218
β (95% CI)	0.33 (-2.24 to 2.91)	1.24 (-1.17 to 3.65)	0.20 (-2.16 to 2.56)	0.81 (-0.63 to 2.25)
α-Linolenic 18:3 n-3				
APOE $\epsilon 4$ -positive, No.	65	65	65	65
β (95% CI)	-0.22 (-2.06 to 1.62)	0.00 (-1.57 to 1.58)	-0.18 (-1.99 to 1.64)	0.39 (-0.66 to 1.43)
APOE $\epsilon 4$ -negative, No.	221	218	218	218
β (95% CI)	-0.07 (-1.05 to 0.91)	-0.43 (-1.35 to 0.49)	-0.03 (-0.93 to 0.88)	-0.37 (-0.93 to 0.18)

Abbreviations: APOE $\epsilon 4$, apolipoprotein E; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MAP, Memory and Aging Project.

^a Models were adjusted for age at death, sex, education, and total energy intake (seafood model only).

^b Consortium to Establish a Registry of Alzheimer Disease score range: 1 (no neuritic plaques) to 4 (frequent neuritic plaques).

^c Braak staging score range: 0 (no tangles in any region) to 6 (frequent tangles across multiple regions).

^d National Institute on Aging and the Ronald and Nancy Reagan Research Institute of the Alzheimer's Association score range: 1 (low Alzheimer disease pathology) to 4 (high Alzheimer disease pathology).

Alzheimer disease neuropathology compared with those who consumed lower amounts. For example, among the APOE $\epsilon 4$ carriers who consumed seafood weekly, the likelihood score for Alzheimer disease was lower by 0.53 score units (Table 4). By contrast, there was no correlation between seafood consumption and neuropathology among those who were APOE $\epsilon 4$ negative. There were no material

differences in the estimated effects with further adjustment for vegetable consumption except that the correlation between seafood consumption and neurofibrillary tangle severity was no longer statistically significant (β , -0.76 score units [95% CI, -1.54 to 0.03]).

We reanalyzed the adjusted models after excluding all dietary assessments that were obtained at the time of an

Table 5. Effect Estimates for Brain Mercury and Selenium Levels With Alzheimer Disease Neuropathology Based on Adjusted Linear Regression Models (Alzheimer Disease Neuropathology) and Logistic Regression (Vascular and Lewy Body Neuropathology) Among 203 Deceased MAP Participants, 2005-2013^a

	Mercury Level by Tertile			P Value for Trend ^b	Selenium Level by Tertile			P Value for Trend ^b
	1	2	3		1	2	3	
Tissue Concentration								
Mean, µg/g	0.02	0.09	1.28		1.03	1.23	1.94	
Median (interquartile [1-3] range), µg/g	0.02 (0.01 to 0.02)	0.06 (0.04 to 0.11)	0.56 (0.23 to 1.48)		1.04 (1.00 to 1.09)	1.23 (1.18 to 1.27)	1.51 (1.36 to 2.31)	
Multiple Regression Models of Alzheimer Disease Neuropathology, β (95% CI)								
Global Alzheimer disease pathology	0.0 [Reference]	-0.31 (-0.72 to 0.11)	-0.40 (-0.82 to 0.02)	.07	0.0 [Reference]	0.02 (-0.39 to 0.43)	0.24 (-0.19 to 0.67)	.29
Neuritic plaque density ^c	0.0 [Reference]	-0.41 (-0.79 to -0.04)	-0.38 (-0.76 to 0.00)	.07	0.0 [Reference]	-0.00 (-0.38 to 0.38)	0.17 (-0.22 to 0.56)	.42
Neurofibrillary tangle severity ^d	0.0 [Reference]	-0.02 (-0.43 to 0.38)	-0.18 (-0.59 to 0.23)	.37	0.0 [Reference]	-0.04 (-0.44 to 0.35)	0.45 (0.05 to 0.86)	.04
Alzheimer disease diagnostic score ^e	0.0 [Reference]	-0.12 (-0.36 to 0.12)	-0.22 (-0.47 to 0.02)	.08	0.0 [Reference]	0.02 (-0.22 to 0.26)	0.13 (-0.12 to 0.37)	.33
Logistic Regression Models of Infarcts and Lewy Bodies, Odds Ratio (95% CI) ^f								
Macroinfarcts	1 [Reference]	1.01 (0.49 to 2.07)	0.48 (0.23 to 1.03)	.05	1 [Reference]	0.64 (0.31 to 3.05)	0.66 (0.31 to 1.33)	.25
Microinfarcts	1 [Reference]	0.37 (0.17 to 0.80)	0.37 (0.17 to 0.80)	.01	1 [Reference]	1.17 (0.56 to 2.48)	0.73 (0.33 to 1.63)	.49
Lewy bodies	1 [Reference]	0.71 (0.29 to 1.73)	0.86 (0.36 to 2.03)	.77	1 [Reference]	1.20 (0.47 to 3.03)	1.79 (0.73 to 4.39)	.21

Abbreviation: MAP, Memory and Aging Project.

^a Models were adjusted for age at death, sex, years of education, seafood consumption, total energy, and for Alzheimer disease neuropathology outcomes apolipoprotein E (*APOE* ε4) and seafood * *APOE* ε4).

^b Linear trend variable in which values within each tertile were scored at the median intake level for that tertile.

^c Consortium to Establish a Registry of Alzheimer Disease score range: 1 (no neuritic plaques) to 4 (frequent neuritic plaques).

^d Braak staging score range: 0 (no tangles in any region) to 6 (frequent tangles across multiple regions).

^e National Institute on Aging and the Ronald and Nancy Reagan Research Institute of the Alzheimer's Association score range: 1 (low Alzheimer disease pathology) to 4 (high Alzheimer disease pathology).

^f Lewy body staging range: 0 (no disease), 1 (nigra-predominant disease), 2 (limbic-type disease), 3 (neocortical disease).

Alzheimer disease diagnosis. The β-coefficients increased in magnitude and remained statistically significant between seafood consumption and global Alzheimer disease pathology (β, -0.88 score units [95% CI, -1.74 to -0.02]) and neuritic plaques (β, -0.83 score units [95% CI, -1.56 to -0.10]), were unchanged for Alzheimer disease diagnostic score (β, -0.53 score units [95% CI, -0.99 to -0.07]), and no longer statistically significant for neurofibrillary tangle severity (β, -0.73 score units [95% CI, -1.54 to 0.08]).

Brain Neuropathology and Brain Metal Concentrations

In further analyses, we examined whether brain concentrations of the neurotoxin mercury or the antioxidant trace element selenium were correlated with brain neuropathology in separate models adjusted for age at death, sex, education, and seafood consumption (Table 5; eTable 2 in the Supplement). There was no significant correlation between brain mercury levels and Alzheimer disease neuropathology. Brain mercury levels were significantly correlated with lower odds of macroinfarcts and microinfarcts. Brain selenium levels were not correlated with the neuropathologic markers except for neurofibrillary tangle severity in which higher selenium levels were correlated with higher tangle

severity. Tests for statistical interaction by *APOE* ε4 status on the brain metals and neuropathology did not reach statistical significance ($P > .26$).

We also investigated the possibility of statistical interaction between brain concentrations of mercury and selenium (an antagonist to mercury toxicity) on the neuropathologic markers.³¹ Tests for interaction between these 2 biometals on brain neuropathologies in the adjusted models did not reach statistical significance ($P > .14$).

Discussion

In this cross-sectional study of older Midwestern residents, weekly consumption of seafood and dietary intake of long-chain n-3 fatty acids were inversely correlated with Alzheimer disease neuropathology but only among *APOE* ε4 carriers. Dietary intakes of seafood and long-chain n-3 fatty acids were not correlated with brain infarcts or with Lewy bodies. Higher intake of α-linolenic acid, the shorter-chain n-3 fatty acid found in plants, was correlated with decreased risk of cerebral infarcts; however, there was no evidence of effect modification by *APOE* ε4 status. Although seafood consumption was correlated with

higher brain levels of mercury, the higher mercury levels were not significantly correlated with increased brain neuropathology.

In a large epidemiological study of middle-aged Dutch adults, dietary α -linolenic acid was associated with 35% to 50% reduction in incident strokes.³² A large number of epidemiological studies reported protective relations between seafood consumption and n-3 fatty acids with cognitive decline^{22,27,33-37} and incident dementia.^{1-5,7} Of those that investigated effect modification by *APOE* $\epsilon 4$ status, the findings are inconsistent. Some reported protective associations with *APOE* $\epsilon 4$ carriers,^{5,25} others with *APOE* $\epsilon 4$ noncarriers,^{2,27} and still others with no effect modification by *APOE* $\epsilon 4$.^{35,38,39} Of 3 randomized controlled trials of fish oil supplementation, 2 observed positive effects on cognitive change in healthy older adults who were *APOE* $\epsilon 4$ carriers,^{28,29} and the third observed positive effects in Alzheimer disease patients who were *APOE* $\epsilon 4$ noncarriers.²⁴ We did not observe a correlation between fish oil supplement use and brain neuropathology although use was very limited and not consistent over time. DHA, the primary lipid in the brain, decreases with age as a result of lipid peroxidation.⁴⁰ DHA metabolism may be affected by a host of factors including age, *APOE* genotype, body mass index, sex, and alcohol consumption, which may explain the inconsistencies across studies.⁴¹

Protection against Alzheimer disease neuropathology from dietary n-3 fatty acids in *APOE* $\epsilon 4$ carriers was also reported in an animal study that measured the effects of a fish oil diet vs control diet in *APOE* $\epsilon 4$ - and *APOE* $\epsilon 3$ -targeted mice.⁴² The study demonstrated the *APOE* $\epsilon 4$ phenotype of increased $A\beta_{42}$ (the abnormal protein characteristic of Alzheimer disease pathology), impaired synaptic function, and decreased learning and memory, and that these were prevented by a fish oil diet.⁴²

To our knowledge, this is the first study to report on the relationship between brain concentrations of mercury and brain neuropathology or diet. The finding of no deleterious correlations of mercury on the brain is supported by a number of case-control studies that found no difference between Alzheimer disease patients and controls in mercury concentrations in the brain,^{19,43,44} serum,⁴⁵ or whole blood.⁴⁶ A cross-sectional population study observed no association between blood concentrations of mercury and cognitive test scores.⁴⁷ In the MAP study, brain mercury levels trended in the protective direction with the neuropathologic markers. One cohort study reported a statistically significant inverse association between blood mercury levels and incident Alzheimer disease.³⁸

There is a vast literature on the benefits of n-3 fatty acids on neurocognitive development.⁴⁸ One study found that the cognitive benefits in early childhood associated with maternal fish consumption in utero were offset by higher erythrocyte mercury levels in the mothers.^{10,48} We did not observe a similar interaction between mercury and seafood consumption on brain health in the aged brain.

The study findings were from a cohort of individuals initially free of dementia who were observed until death. The design minimizes the chance of selection bias that

occurs in case-control studies. It also provides a wide spectrum of disease pathology from which to observe associations with environmental exposures. The comparatively large number of brains analyzed decreases the likelihood that the findings are due to chance and increased our ability to observe modifications in the correlations by *APOE* $\epsilon 4$ status. Nevertheless, insufficient statistical power may have prevented detection of small correlations of n-3 fatty acids on neuropathology in *APOE* $\epsilon 4$ noncarriers. Other strengths of the study include the validated and/or standardized measurements of diet, metal concentrations, and neuropathological measures. The measurements were based on averaged assessments over multiple years (diet) and brain regions (metal concentrations and neuropathologies), which help to minimize bias due to measurement error.

A major limitation of the study is the observational study design, which precludes causal interpretation of the data. In addition, the measurement of brain pathologies and mercury levels at the time of death prevents the assessment of temporality in relation to dietary intake levels. This limitation is mitigated by our finding in a previous study that weekly seafood consumption was associated with slower cognitive decline over a mean 4.9 years among 915 MAP participants.⁴⁹ Another limitation of the study is the subjective measure of dietary intake, although the dietary tool was validated in older adults.^{20,50} Further testament to the validity of the diet assessment is the positive correlation between brain mercury concentrations and seafood consumption. Another study limitation is that only half of the deceased MAP participants had both autopsy and dietary intake data for analyses. Concerns of sample bias are allayed by the fact that the analyzed sample was comparable with all deceased participants in age, sex, education, and *APOE* $\epsilon 4$ status. Seafood intake in the MAP study population was moderate, and therefore the findings cannot be generalized to populations with higher seafood consumption or with high mercury exposure. It is likely that types of fish consumed in this study reflect the top 10 consumed species in the United States, which have low-to-moderate levels of mercury.⁵¹ Nevertheless, the levels of mercury in the MAP study population were comparable with levels previously reported for cortical brain regions.¹⁹

The study findings were from a very old, largely non-Hispanic white cohort and may not be generalizable to younger adults or other racial or ethnic groups.¹² Brain concentrations of DHA decrease with older age because of lipid peroxidation,⁵² which is higher among *APOE* $\epsilon 4$ carriers.⁵³ Thus, fish consumption may be more beneficial with older age.

Conclusions

In cross-sectional analyses, moderate seafood consumption was correlated with lesser burden of brain Alzheimer disease neuropathology in *APOE* $\epsilon 4$ carriers. Although seafood consumption was correlated with higher brain levels of mercury, these levels were not correlated with brain neuropathology.

ARTICLE INFORMATION

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Administrative, technical, or material support: Brockman, Schneider, Bennett.

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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. A scientific travel grant from the Judith Zwart Foundation provided travel expenses for Dr van de Rest to Rush University Medical Center in Chicago. No other disclosures were reported.

Funding/Support: This study was supported by National Institutes of Health grants (R01AG031553, R21ES021290 and R01AG17917).

Role of the Funder/Sponsor: The funding sponsor had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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