Original Investigation

Association of Seafood Consumption, Brain Mercury Level, and *APOE ε4* Status With Brain Neuropathology in Older Adults

Martha Clare Morris, ScD; John Brockman, PhD; Julie A. Schneider, MD, MPH; Yamin Wang, PhD; David A. Bennett, MD; Christy C. Tangney, PhD; Ondine van de Rest, PhD

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 (ρ = 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 (β = −0.69 score units [95% CI, −1.34 to −0.04]), less severe and widespread neurofibrillary tangles (β = −0.77 score units [95% CI, −1.52 to −0.02]), and lower neuropathologically defined Alzheimer disease (β = −0.53 score units [95% CI, −0.96 to −0.10]) but only among apolipoprotein E (*APOE ε4*) 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

Editorial page 465

Author Video Interview and **JAMA Report Video** at jama.com

Supplemental content at jama.com

CME Quiz at jamanetworkcme.com and **CME Questions** page 513

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Martha Clare Morris, ScD, Director, Section on Nutrition and Nutritional Epidemiology, Department of Internal Medicine, Rush University Medical Center, 1645 W Jackson, Ste 675, Chicago, IL 60612 (martha_c_morris@rush.edu).

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-mm^2 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 criteria[16] 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/cm^2·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 ε4*). 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 correrelation coeffecient (ρ = 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 ε4* 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 ε4* 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 ε4* 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) µg/g in the midfrontal region, 0.25 (0.78) µg/g in the inferior temporal region, and 0.87 (1.80) µg/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 ε4* 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 ε4* 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 ε4* 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 ε4* carriers.

Further analyses examined correlations between the dietary variables and brain neuropathology stratified by *APOE ε4* status (**Table 4**). Individuals who were *APOE ε4* 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				
	Inferior Temporal	Midfrontal	Cerebellum	All Regions	*P* Value
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 ε4* 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 ε4-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 ε4-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 ε4-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 -ε4-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 ε4-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 ε4-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 ε4*, 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 ε4* 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 ε4* 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 ε4* status, the findings are inconsistent. Some reported protective associations with *APOE ε4* carriers,[5,25] others with *APOE ε4* noncarriers,[2,27] and still others with no effect modification by *APOE ε4*.[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 ε4* carriers,[28,29] and the third observed positive effects in Alzheimer disease patients who were *APOE ε4* 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 ε4* carriers was also reported in an animal study that measured the effects of a fish oil diet vs control diet in *APOE ε4*- and *APOE ε3*-targeted mice.[42] The study demonstrated the *APOE ε4* 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 ε4* status. Nevertheless, insufficient statistical power may have prevented detection of small correlations of n-3 fatty acids on neuropathology in *APOE ε4* 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 ε4* 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 ε4* 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 ε4* carriers. Although seafood consumption was correlated with higher brain levels of mercury, these levels were not correlated with brain neuropathology.

ARTICLE INFORMATION

Author Affiliations: Section on Nutrition and Nutritional Epidemiology, Department of Internal Medicine, Rush University Medical Center, Chicago, Illinois (Morris, Wang); Missouri University Research Reactor, Columbia (Brockman); Rush Alzheimer Disease Center, Rush University Medical Center, Chicago, Illinois (Schneider, Bennett); Department of Neurology, Rush University Medical Center, Chicago, Illinois (Schneider, Bennett); Department of Pathology, Rush University Medical Center, Chicago, Illinois (Schneider); Department of Clinical Nutrition, Rush University Medical Center, Chicago, Illinois (Tangney); Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands (van de Rest).

Author Contributions: Dr Morris had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Morris, Bennett.
Acquisition, analysis, or interpretation of data: Morris, Brockman, Schneider, Wang, Bennett, Tangney, van de Rest.
Drafting of the manuscript: Morris, van de Rest.
Critical revision of the manuscript for important intellectual content: Morris, Brockman, Schneider, Wang, Bennett, Tangney, van de Rest.
Statistical analysis: Morris, Wang, Tangney, van de Rest.
Obtained funding: Morris, Bennett.
Administrative, technical, or material support: Brockman, Schneider, Bennett.
Study supervision: Morris, Bennett.

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 Zwartz 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.

REFERENCES

1. Barberger-Gateau P, Letenneur L, Deschamps V, Pérès K, Dartigues JF, Renaud S. Fish, meat, and risk of dementia: cohort study. *BMJ*. 2002;325(7370):932-933.

2. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. *Neurology*. 2007;69(20):1921-1930.

3. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol*. 1997;42(5):776-782.

4. Huang TL, Zandi PP, Tucker KL, et al. Benefits of fatty fish on dementia risk are stronger for those without *APOE epsilon4*. *Neurology*. 2005;65(9):1409-1414.

5. Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. *Arch Neurol*. 2003;60(7):940-946.

6. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. *Arch Neurol*. 2006;63(11):1545-1550.

7. Larrieu S, Letenneur L, Helmer C, Dartigues JF, Barberger-Gateau P. Nutritional factors and risk of incident dementia in the PAQUID longitudinal cohort. *J Nutr Health Aging*. 2004;8(3):150-154.

8. Harris WS, Mozaffarian D, Lefevre M, et al. Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. *J Nutr*. 2009;139(4):804S-819S.

9. de Souza AS, Fernandes FS, do Carmo Md. Effects of maternal malnutrition and postnatal nutritional rehabilitation on brain fatty acids, learning, and memory. *Nutr Rev*. 2011;69(3):132-144.

10. Oken E, Radesky JS, Wright RO, et al. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *Am J Epidemiol*. 2008;167(10):1171-1181.

11. Bjørklund G. Selenium as an antidote in the treatment of mercury intoxication. *Biometals*. 2015;28(4):605-614.

12. Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS. Overview and findings from the rush memory and aging project. *Curr Alzheimer Res*. 2012;9(6):646-663.

13. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology*. 2006;66(12):1837-1844.

14. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD), part II: standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology*. 1991;41(4):479-486.

15. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;82(4):239-259.

16. The NIA-Reagan Working Group. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease: the National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging*. 1997;18(4)(suppl):S1-S2.

17. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA. Microinfarct pathology, dementia, and cognitive systems. *Stroke*. 2011;42(3):722-727.

18. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol*. 2009;66(2):200-208.

19. Samudralwar DL, Diprete CC, Ni BF, Ehmann WD, Markesbery WR. Elemental imbalances in the olfactory pathway in Alzheimer's disease. *J Neurol Sci*. 1995;130(2):139-145.

20. Morris MC, Tangney CC, Bienias JL, Evans DA, Wilson RS. Validity and reproducibility of a food frequency questionnaire by cognition in an older biracial sample. *Am J Epidemiol*. 2003;158(12):1213-1217.

21. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analysis. *Am J Epidemiol*. 1986;124(1):17-27.

22. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. *Arch Neurol*. 2005;62(12):1849-1853.

23. Barberger-Gateau P, Samieri C, Féart C, Plourde M. Dietary omega 3 polyunsaturated fatty acids and Alzheimer's disease: interaction with apolipoprotein E genotype. *Curr Alzheimer Res*. 2011;8(5):479-491.

24. Quinn JF, Raman R, Thomas RG, et al. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. *JAMA*. 2010;304(17):1903-1911.

25. Samieri C, Féart C, Proust-Lima C, et al. ω-3 Fatty acids and cognitive decline: modulation by ApoEε4 allele and depression. *Neurobiol Aging*. 2011;32(12):2317.e13-2317.e22.

26. Daiello LA, Gongvatana A, Dunsiger S, Cohen RA, Ott BR; Alzheimer's Disease Neuroimaging Initiative. Association of fish oil supplement use with preservation of brain volume and cognitive function. *Alzheimers Dement*. 2015;11(2):226-235.

27. Whalley LJ, Deary IJ, Starr JM, et al. n-3 Fatty acid erythrocyte membrane content, APOE varepsilon4, and cognitive variation: an observational follow-up study in late adulthood. *Am J Clin Nutr*. 2008;87(2):449-454.

28. van de Rest O, Geleijnse JM, Kok FJ, et al. Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. *Neurology*. 2008;71(6):430-438.

29. Stonehouse W, Conlon CA, Podd J, et al. DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial. *Am J Clin Nutr*. 2013;97(5):1134-1143.

30. Huang TL. Omega-3 fatty acids, cognitive decline, and Alzheimer's disease: a critical review and evaluation of the literature. *J Alzheimers Dis*. 2010;21(3):673-690.

31. Berry MJ, Ralston NV. Mercury toxicity and the mitigating role of selenium. *Ecohealth*. 2008;5(4):456-459.

32. de Goede J, Verschuren WM, Boer JM, Kromhout D, Geleijnse JM. Alpha-linolenic acid intake and 10-year incidence of coronary heart disease and stroke in 20 000 middle-aged men and women in the Netherlands. *PLoS One*. 2011;6(3):e17967.

33. Samieri C, Féart C, Letenneur L, et al. Low plasma eicosapentaenoic acid and depressive symptomatology are independent predictors of dementia risk. *Am J Clin Nutr*. 2008;88(3):714-721.

34. Eskelinen MH, Ngandu T, Helkala EL, et al. Fat intake at midlife and cognitive impairment later in life: a population-based CAIDE study. *Int J Geriatr Psychiatry*. 2008;23(7):741-747.

35. Beydoun MA, Kaufman JS, Satia JA, Rosamond W, Folsom AR. Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr*. 2007;85(4):1103-1111.

36. van Gelder BM, Tijhuis M, Kalmijn S, Kromhout D. Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. *Am J Clin Nutr*. 2007;85 (4):1142-1147.

37. Heude B, Ducimetière P, Berr C; EVA Study. Cognitive decline and fatty acid composition of erythrocyte membranes—the EVA study. *Am J Clin Nutr*. 2003;77(4):803-808.

38. Kröger E, Verreault R, Carmichael PH, et al. Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. *Am J Clin Nutr*. 2009;90(1):184-192.

39. Rönnemaa E, Zethelius B, Vessby B, Lannfelt L, Byberg L, Kilander L. Serum fatty-acid composition and the risk of Alzheimer's disease: a longitudinal population-based study. *Eur J Clin Nutr*. 2012;66(8): 885-890.

40. Barceló-Coblijn G, Högyes E, Kitajka K, et al. Modification by docosahexaenoic acid of age-induced alterations in gene expression and molecular composition of rat brain phospholipids. *Proc Natl Acad Sci U S A*. 2003;100(20):11321-11326.

41. Samieri C, Lorrain S, Buaud B, et al. Relationship between diet and plasma long-chain n-3 PUFAs in older people: impact of apolipoprotein E genotype. *J Lipid Res*. 2013;54(9):2559-2567.

42. Kariv-Inbal Z, Yacobson S, Berkecz R, et al. The isoform-specific pathological effects of apoε4 in vivo are prevented by a fish oil (DHA) diet and are modified by cholesterol. *J Alzheimers Dis*. 2012;28 (3):667-683.

43. Saxe SR, Wekstein MW, Kryscio RJ, et al. Alzheimer's disease, dental amalgam and mercury. *J Am Dent Assoc*. 1999;130(2):191-199.

44. Rusina R, Matěj R, Kašparová L, Kukal J, Urban P. Higher aluminum concentration in Alzheimer's disease after Box-Cox data transformation. *Neurotox Res*. 2011;20(4):329-333.

45. Park JH, Lee DW, Park KS, Joung H. Serum trace metal levels in Alzheimer's disease and normal control groups. *Am J Alzheimers Dis Other Demen*. 2014;29(1):76-83.

46. Fung YK, Meade AG, Rack EP, et al. Determination of blood mercury concentrations in Alzheimer's patients. *J Toxicol Clin Toxicol*. 1995; 33(3):243-247.

47. Weil M, Bressler J, Parsons P, Bolla K, Glass T, Schwartz B. Blood mercury levels and neurobehavioral function. *JAMA*. 2005;293(15): 1875-1882.

48. McNamara RK, Carlson SE. Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot Essent Fatty Acids*. 2006;75(4-5):329-349.

49. Van de Rest O, Tangney CC, Schneider J, et al. Fatty fish and long-chain n-3 fatty acids are associated with neurofibrillary tangle pathology and cognitive decline. *Alzheimers Dement*. 2014;10 (4 suppl):280-P281 doi:10.1016/j.jalz.2014.07.151.

50. Tangney CC, Bienias JL, Evans DA, Morris MC. Reasonable estimates of serum vitamin E, vitamin C, and beta-cryptoxanthin are obtained with a food frequency questionnaire in older black and white adults. *J Nutr*. 2004;134(4):927-934.

51. National Marine Fisheries Service, US Dept of Commerce. 2015. [serial online]. http://www.nmfs.noaa.gov/. Accessed January 12, 2016.

52. Cole GM, Ma QL, Frautschy SA. Dietary fatty acids and the aging brain. *Nutr Rev*. 2010; 68(suppl 2):S102-S111.

53. Dietrich M, Hu Y, Block G, et al. Associations between apolipoprotein E genotype and circulating F2-isoprostane levels in humans. *Lipids*. 2005;40 (4):329-334.