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Authors

Tsiknia, Amaryllis A

Bergstrom, Jaclyn

Reas, Emilie T

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Original Article

Midlife omega-3 fatty acid intake predicts later life white matter microstructure in an age- and *APOE*-dependent manner

Amaryllis A. Tsiknia [1], Jaclyn Bergstrom [2], Emilie T. Reas [1,*]

[1]Department of Neurosciences, University of California, San Diego, CA 92093-0841, United States,
[2]School of Public Health and Human Longevity Science, University of California, San Diego, CA 92093, United States
*Corresponding author: Department of Neurosciences, Mail Code 0841, UCSD, 9500 Gilman Dr., La Jolla, CA 92093-0841, USA. Email: ereas@ucsd.edu

Omega-3 intake has been positively associated with healthy brain aging, yet it remains unclear whether high omega-3 intake beginning early in life may optimize its protective effects against brain aging. We examined whether omega-3 intake is associated with brain microstructure over 2 decades later among dementia-free older adults. The 128 participants (62% women; age at magnetic resonance imaging: 76.6 ± 7.9) from the Rancho Bernardo Study of Healthy Aging completed at least 1 dietary assessment between 1984 and 1996 and underwent restriction spectrum imaging (RSI) 22.8 ± 3.1 years later. We evaluated associations between prior omega-3 intake and RSI metrics of gray and white matter (WM) microstructure. Higher prior omega-3 intake was associated with greater restricted diffusion in the superior cortico-striatal fasciculus. A correlation between higher prior omega-3 intake and greater cingulum restricted diffusion was stronger among participants >80 years old. Higher omega-3 intake correlated with greater restricted diffusion in the inferior longitudinal and inferior fronto-occipital fasciculus more strongly for apolipoprotein E (*APOE*) ε4 carriers than noncarriers. Associations were not modified by adjustment for dietary pattern, health, or lifestyle. High omega-3 intake in midlife may help to maintain WM integrity into older age, particularly in the latest decades of life and among *APOE* ε4 carriers.

Key words: brain aging; brain microstructure; dietary patterns; diffusion MRI; omega-3 fatty acids; restriction spectrum imaging.

Introduction

Brain aging is a lifelong process that may begin decades before cognitive decline becomes evident. Therefore, health and lifestyle factors in midlife may be pivotal in modifying trajectories of brain aging. Evidence suggests that dietary patterns modify the risk for cognitive impairment (Scarmeas et al. 2018), although studies examining cognition in older age relative to midlife dietary patterns have been inconclusive (Dearborn–Tomazos et al. 2019; Hu et al. 2020). Despite mounting support for a protective effect of omega-3 fatty acids against age-related cognitive decline (Martí Del Moral and Fortique 2019), the brain mechanisms of this effect remain elusive.

A meta-analysis of effects of omega-3 fatty acids on brain and cognitive measures demonstrated substantial variability across findings, which may be due to differences in study populations, or the dosage and duration of omega-3 intake (McNamara et al. 2018). A supplementation trial on patients with moderate Alzheimer's disease found no beneficial effect of omega-3 fatty acids on gray matter (GM) atrophy and cognitive decline (Quinn et al. 2010), whereas trials in younger groups or dementia-free older adults observed greater GM volume and white matter (WM) integrity (Witte et al. 2014) in response to omega-3 supplementation. This discrepancy suggests that higher intake of omega-3 fatty acids during younger ages may impart a particular protective effect at preserving brain health into later life. Despite the inconclusiveness of prior findings, there is evidence that omega-3 fatty acid intake is most protective against volume loss within brain regions that are particularly vulnerable to age-related atrophy (Macaron et al. 2021). Thus, further investigation is needed to determine whether higher prior omega-3 intake is associated with preserved microstructural integrity in brain regions that are especially susceptible to aging-related injury that may accelerate in the final decades of life.

While some studies reported positive correlations between omega-3 fatty acids and GM volume but not WM integrity (Titova et al. 2013), others demonstrated that WM integrity mediates the protective effect of omega-3 fatty acids on cognitive decline (Bowman et al. 2013; Gu et al. 2016). These inconsistencies may be partly due to the limited sensitivity of conventional morphometric- and diffusion tensor-based imaging measures used in prior studies, which could be overcome by using multishell diffusion magnetic resonance imaging (MRI) techniques, such as restriction spectrum imaging (RSI). The multicompartment RSI model better characterizes microstructural properties than the diffusion tensor model due to its ability to separate intracellular,

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intraneurite, extracellular, and cerebrospinal fluid (CSF) signal within brain tissue (White et al. 2012). We previously demonstrated that RSI metrics are highly sensitive to microstructural brain changes associated with normal aging (Reas et al. 2020), vascular dysfunction (Reas et al. 2021), and prodromal Alzheimer's disease (Reas et al. 2017).

Several studies have suggested that apolipoprotein E (*APOE*) $\varepsilon 4$, the strongest genetic risk factor for Alzheimer's disease, modifies associations of omega-3 intake with brain health and cognitive aging. Whereas some studies have observed protective effects of omega-3 supplementation or intake against age-related cognitive decline and GM atrophy only among *APOE* $\varepsilon 4$ noncarriers (Daiello et al. 2015; Tomaszewski et al. 2020), a review suggested that omega-3 fatty acids may confer benefits to brain health among *APOE* $\varepsilon 4$ carriers if supplementation is implemented early in the aging process (Yassine et al. 2017). Therefore, further investigation of the effect of *APOE* $\varepsilon 4$ on the association between omega-3 intake at younger ages and brain aging in later life is warranted (Baker et al. 2016).

Several studies have reported sex differences in patterns of brain aging (Murphy et al. 1996; Reas et al. 2020; Reas et al. 2021) and in the effects of modifiable risk factors, such as dietary habits and exercise, on cognitive aging (Katsiardanis et al. 2013; Barha et al. 2017). Additionally, higher estradiol levels in women have been associated with more efficient omega-3 fatty acid metabolism (Giltay et al. 2004; Robinson et al. 2010). However, the effects of sex on the association between omega-3 intake and brain aging remain unclear.

In this study, we used RSI to investigate whether omega-3 intake is associated with GM and WM microstructure over 2 decades later among community-dwelling older adults without dementia. To determine whether associations between omega-3 intake and subsequent microstructure are modified by demographic risk factors known to influence patterns of brain aging and omega-3 fatty acid metabolism, we also examined potential modifying effects of age, sex, and *APOE* genotype. We hypothesized that higher prior omega-3 intake would correlate with greater brain microstructural integrity in later life, particularly in regions that demonstrate age-related vulnerability, and that the strength of associations would differ according to age, sex, and genetics.

Materials and methods

Participants

Eligible participants were 154 community-dwelling southern California residents enrolled in the Rancho Bernardo Study (RBS) of Healthy Aging who completed an MRI research visit in 2014–2016 and at least 1 prior dietary assessment between 1984 and 1996. Further details regarding the RBS and data access can be found at https://knit.ucsd.edu/ranchobernardostudy/. Participants were free of dementia at time of MRI and exclusion criteria included history of head injury, stroke, neurological disease, treatment for an alcohol use disorder, or safety contraindication for MRI. After excluding 19 participants due to missing dietary data, 6 individuals due to poor data quality, and 1 due to severe WM disease, the final sample included 128 participants (62% women; age at MRI: mean ± standard deviation [SD] = 76.6 ± 7.9, range: 56–99 years). Based on the participants' mean age at the dietary assessments they had completed, the time difference between dietary assessment and the MRI scan was mean ± SD: 22.8 ± 3.1 (range: 18.1–30.4) years. Study procedures were approved by the University of California, San Diego Human Research Protections Program Board, and participants provided informed written consent prior to participation.

Dietary, health, and lifestyle assessment

Dietary data were collected during 3 research visits in 1984–1987 ($n = 44$), 1988–1992 ($n = 70$), and 1992–1996 ($n = 103$). Participants completed the Willet Food Frequency Questionnaire (Willett et al. 1985) to indicate how frequently during the previous year they consumed a specified portion of foods commonly consumed in the United States, with choices ranging from "never or less than once per month" to "≥6 times per day" on a 9-point scale. Daily nutrient intakes, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA), and arachidonic acid (AA), were estimated in grams using the Harvard nutrient database program by multiplying the frequency of responses by the nutrient compositions of the corresponding food portion (HarvardSSFQ.5/93; Harvard TC Chan School of Public Health, Boston, MA, United States). Omega-3 fatty acid intake was computed as the sum of EPA and DHA (in grams), and omega-6 fatty acid was computed as the sum of LA and AA intake (in grams).

A modified Mediterranean diet score was computed based on the scale developed by Trichopoulou et al. (2003) and was adapted to the US population (Fung et al. 2005) by integrating the following components: alcohol, vegetables, legumes, fruits, nuts, whole grains, fish, red meat, and monounsaturated-to-saturated fat ratio. Individuals reporting an average alcohol intake of 5–15 g/day received 1 point, while those with alcohol intake outside of this range received 0 points. Participants with red meat intake below the sex-specific median received 1 point and 0 points otherwise. For the remaining components, those consuming above the sex-specific median received 1 point and 0 points otherwise. A Mediterranean diet score was computed as the sum of these component scores, ranging from 0 to 9, with higher scores representing stricter adherence to the Mediterranean diet.

Education level was assessed at enrollment and converted to years of education. Height and weight were measured to compute body mass index (BMI, kg/m^2). History of smoking (never vs. former), physical activity

(≥3 times per week, yes/no), alcohol consumption (nondrinker/drinker), and antihypertensive medication use (yes/no) were obtained from standard questionnaires. Blood pressure was measured in seated, resting participants by a trained nurse, and participants were considered hypertensive if they had an average systolic blood pressure ≥ 140, diastolic blood pressure ≥ 90, were taking antihypertensive medication, or reported a physician diagnosis of hypertension.

Cognitive assessment

A neuropsychological test battery was administered by a trained examiner on the same day as the MRI. The battery included: the 3MS, a cognitive screening tool assessing multiple cognitive domains; Trails B to assess executive function and processing speed; the Buschke Selective Reminding Test to assess verbal episodic memory; the Wechsler Visual Reproduction test to evaluate visual memory; and the Wechsler Logical Memory subtest, story A, to assess verbal story recall.

APOE genotyping

Genotyping was conducted by Diagnomics, Inc. using the Illumina Global Screening Array. *APOE* genotype was available on 121 participants, including 28, who carried at least 1 copy of the *APOE* $\varepsilon 4$ allele (27 $\varepsilon 3/\varepsilon 4$, 1 $\varepsilon 4/\varepsilon 4$) and 93 who carried no *APOE* $\varepsilon 4$ alleles. Participants were classified as $\varepsilon 4$ carriers or noncarriers for analysis.

Imaging data acquisition and processing

Imaging data were acquired on a 3T Discovery 750 scanner (GE Healthcare, Milwaukee, WI, United States) at the University of California, San Diego Center for Functional MRI, with an 8-channel phased array head coil. MRI sequences included a 3-plane localizer; a sagittal 3D fast spoiled gradient echo T_1-weighted structural scan (time echo [TE] = 3.2 ms, time repetition [TR] = 8.1 ms, inversion time = 600 ms, flip angle = 8°, FOV = 256 × 256 mm, matrix = 256 × 192, slice thickness = 1.2 mm); and an axial 2D single-shot pulsed-field gradient spin-echo echo-planar diffusion-weighted sequence (45 gradient directions, b-values = 0, 500, 1,500, 4,000 s/mm^2, 1 $b = 0$ volume and 15 gradient directions for each nonzero b-value; TE = 80.6 ms, TR = 7 s, FOV = 240 × 240 mm, matrix = 96 × 96, slice thickness = 2.5 mm, resampled to 1.875 × 1.875 × 2.5 mm).

MRI data were processed using an automated image processing pipeline using FreeSurfer (http://surfer.nmr.mgh.harvard.edu) and in-house software, as previously described (Hagler et al. 2019). Briefly, diffusion MRI data were corrected for distortions and visually inspected for artifacts (Jovicich et al. 2006; Holland et al. 2010). Tissue boundaries of GM, WM, and CSF were identified on T_1-weighted structural images using FreeSurfer and diffusion MRI data were automatically registered to T_1 images (Wells et al. 1996).

Computation of diffusion metrics

Computed RSI metrics included total restricted, hindered isotropic, and free water diffusion (White et al. 2012; Reas et al. 2020). Total restricted diffusion is the composite of isotropic and anisotropic restricted diffusion, accounting for crossing fibers, and thus reflects all intracellular diffusion within cell bodies, axons, and dendrites. This was computed as the aggregate of the zeroth (isotropic), second (anisotropic), and fourth (crossing fibers) spherical harmonic of the restricted compartment. Hindered isotropic diffusion was computed as the zeroth spherical harmonic of the hindered compartment, which presumably reflects diffusion within the extracellular space or within large cell bodies. Finally, the free water metric estimates diffusion from the CSF fraction.

Restricted and free water diffusion were computed in 15 WM tracts derived from a probabilistic atlas-based fiber tract segmentation (Hagler et al. 2009). Since WM is poorly characterized by the hindered fraction, we did not examine hindered diffusion in fiber tracts (White et al. 2012). Voxels containing primarily GM or CSF were excluded from WM tracts (Fischl et al. 2002). Global WM RSI measures were calculated as the mean across all tracts. Total restricted, hindered isotropic, and free water diffusion were measured along a vertex-wise map of the cortical GM surface. To minimize partial volume effects for cortical surface-based analyses, RSI metrics were sampled with linear interpolation ranging from 0.8 to 2.0 mm from the GM/WM boundary along the normal to the cortical ribbon and were combined using a weighted average based on the proportion of GM in each voxel. RSI cortical surface maps were registered to common space and smoothed with a full-width at half-maximum 10-mm kernel.

For comparison against conventional diffusion tensor imaging (DTI) studies, we also computed DTI measures of fractional anisotropy (FA) and mean diffusivity (MD) from the multishell RSI acquisition using a nonlinear fitting procedure.

Statistical analysis

Omega-3 and omega-6 consumption were computed for each participant as the mean of intake (in grams) across available dietary assessments from 1984 to 1996 (maximum of 3 dietary assessments). Omega-3/-6 ratios were computed as the ratio of the mean intake values. Associations between omega-3 intake and demographic, health, and lifestyle variables were computed using Pearson's correlations (continuous variables) or chi-squared tests (categorical variables). For analyses described below, omega-3 and omega-6 values were adjusted for total calorie intake by computing the linear regression residual.

To assess associations between omega-3 intake and cognition, partial correlations were conducted between prior omega-3 intake and cognitive test scores, adjusted for age, time between dietary assessment and cognitive assessment, sex, and education.

To assess associations between prior omega-3 intake and WM microstructure in later life, partial correlations were conducted between omega-3 intake and RSI measures in fiber tracts of interest. Vertex-wise linear regressions examined associations between omega-3 intake and cortical GM microstructure. Exploratory analyses additionally examined effects of omega-6 intake and omega-3/6 ratios on microstructure as well as effects of omega-3 intake on FA and MD.

Base models adjusted for age at MRI, time between dietary assessment and MRI, and sex. Secondary models further adjusted for demographic, health, or lifestyle factors that could influence brain microstructure or covary with omega-3 intake, including *APOE* status (ε4 carrier/noncarrier), education (years), smoking (never/ever), alcohol intake (nondrinker/drinker), physical activity (≥3 times per week, yes/no), hypertension (yes/no), and BMI. To assess whether effects of omega-3 intake were attributable to an overall healthy diet, fully adjusted models were additionally corrected for Mediterranean diet index. When significant correlations were present, linear regressions were computed, including the RSI measure as the dependent variable and omega-3 intake and all covariates from the fully adjusted models as independent variables, to assess the relative association strength for omega-3 intake compared to other health and lifestyle factors.

To assess whether associations differed by sex, *APOE* genotype, or age, partial correlations for WM tracts and cortical surface regressions were repeated stratified by sex, *APOE* status (ε4 carrier/noncarrier) and age (<80 or >80 years old at time of MRI). Covariates were as described above. For any RSI metric demonstrating a significant association in sex-, *APOE*-, or age-stratified analyses, correlation coefficients were normalized (Fisher *r*-to-z transformation) and the confidence interval (CI) of the difference between correlations was computed to compare correlation strengths between groups.

Cortical surface analyses were performed in FreeSurfer version 6.0 and all other analyses were conducted in SPSS version 28.0 (IBM Corp, Armonk, NY, United States). Significance was set to $P < 0.05$. Cortical surface GLMs were corrected with false discovery rate.

Results

Participants

Participant demographic and health characteristics at time of MRI, and their associations with prior omega-3 intake, are presented in Table 1. Participants ranged in age from 56 to 99 years and included 79 women and 49 men. Higher prior omega-3 intake correlated with lower BMI ($P = 0.04$) as well as higher calorie intake and adherence to the Mediterranean diet ($P < 0.001$). *APOE* ε4 carriers had higher mean omega-3 intake than noncarriers (288 ± 179 mg vs. 218 ± 136 mg; $P = 0.03$). Omega-3 levels were not associated with age at MRI or diet assessment, sex, education, hypertension, exercise, smoking, alcohol consumption, or performance on any cognitive test (Supplemental Table 1) (*P*s > 0.05).

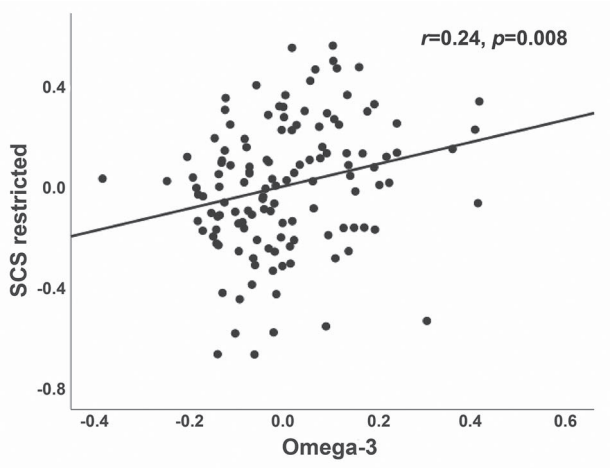

Fig. 1. Correlations between prior omega-3 intake (adjusted for calorie intake) and total restricted diffusion in the superior cortico-striatal fasciculus (SCS). Values are residuals, adjusted for age, time between dietary assessment and MRI, and sex.

Associations between midlife omega-3 intake and brain microstructure

Across all WM, prior omega-3 intake did not correlate with restricted ($r = 0.15$, $P = 0.09$) or free water diffusion ($r = 0.01$, $P = 0.92$) (adjusted for age, time between dietary assessment and MRI, and sex). Within specific fiber tracts, higher omega-3 intake correlated with higher restricted diffusion in the superior cortico-striatal fasciculus (SCS, $r = 0.24$, $P = 0.008$) (Fig. 1). This association remained significant ($r = 0.22$, $P = 0.02$) after further adjustment for *APOE*, education, alcohol consumption, smoking, physical activity, hypertension, and BMI, as well as after additional adjustment for Mediterranean diet index ($r = 0.20$, $P = 0.04$), and was also significant for omega-3/-6 ratios ($r = 0.19$, $P < 0.04$). Correlations between omega-3 intake and restricted diffusion are shown for all fiber tracts in Supplemental Table 2. Linear regressions, including all diet, health, demographic, and lifestyle predictors, revealed that age ($\beta = -0.001$; 95% CI: −0.002 to −0.001; $P < 0.001$), prior omega-3 intake ($\beta = 0.037$; 95% CI: 0.000 to −0.074; $P = 0.05$), and alcohol consumption ($\beta = -0.015$; 95% CI: −0.029 to −0.257; $P = 0.002$) were the strongest predictors of SCS-restricted diffusion (Supplemental Table 3). Omega-3 intake did not correlate with WM free water diffusion, or with DTI measures of FA and MD. Omega-6 did not correlate with microstructure (*P*s > 0.10).

Surface-based analyses revealed no substantial associations between omega-3 intake and cortical GM microstructure.

Differences in associations between omega-3 intake and brain microstructure by age, APOE, and sex

In age-stratified analyses, those older than 80 years of age ($n = 49$) demonstrated a stronger correlation ($z = 3.00$,

Table 1. Participant characteristics and associations between omega-3 intake and health or demographic variables.

Variable	Sample *N*	Mean ± SD or *N* (%)	Association with omega-3 intake
Age at MRI (years)	128	77.5 ± 7.7	$r = -0.01$, $P = 0.89$
Mean age at diet assessments (years)	128	54.7 ± 6.9	$r = -0.07$, $P = 0.45$
Time between mean diet assessments and MRI (years)	128	22.8 ± 3.1	$r = 0.12$, $P = 0.17$
Sex (women)	128	$N = 79$ (62%)	$F(1,126) = 0.06$, $P = 0.81$
Education (years)	128	14.9 ± 2.0	$r = 0.08$, $P = 0.39$
Hypertension	128	$n = 81$ (63%)	$F(1, 126) = 0.00$, $P = 0.99$
APOE ε4 carrier	121	$N = 28$ (23%)	**$F(1, 119) = 4.87$, $P = 0.03$**
Physical activity (3+ times/week)	127	$n = 96$ (76%)	$F(1, 125) = 1.64$, $P = 0.20$
BMI (kg/m^2)	128	25.9 ± 3.9	**$r = -0.19$, $P = 0.04$**
Smoking (ever)	127	$n = 52$ (41%)	$F(1, 125) = 0.83$, $P = 0.36$
Alcohol (current drinker)	127	$n = 106$ (83%)	$F(1, 125) = 3.44$, $P = 0.07$
Omega-3 (mg)	128	229 ± 149	N/A
Mediterranean diet index	128	3.8 ± 1.7	**$r = 0.37$, $P < 0.001$**
Calorie intake (kcal)	128	1796 ± 608	**$r = 0.32$, $P < 0.001$**

Statistic represents Pearson's correlation coefficients between continuous variables and omega-3 intake, or *F*-values for difference in omega-3 levels by group for categorical variables. Bold = significant ($P < 0.05$).

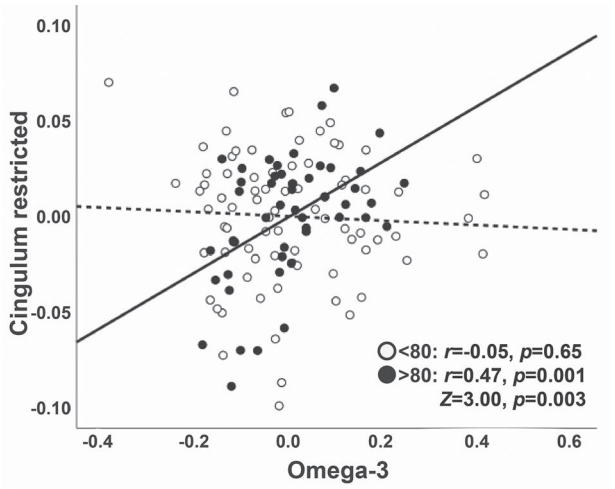

Fig. 2. Correlation between prior omega-3 intake (adjusted for calorie intake) and cingulum total restricted diffusion for those younger than ($N = 79$) or older than ($n = 49$) 80 years of age. Values are residuals, adjusted for age, time between dietary assessment and MRI, and sex.

$P = 0.003$, Fig. 2) between prior omega-3 intake and restricted diffusion in the cingulum ($r = 0.47$, $P = 0.001$) than for those below age 80 ($n = 79$; $r = -0.05$, $P = 0.65$). This was also present for omega-3/6 ratios ($z = 2.98$, $P = 0.003$) and was not modified by adjustment for demographic, health, and lifestyle covariates ($z = 2.99$, $P = 0.003$), or by further adjustment for Mediterranean diet ($z = 3.00$, $P = 0.003$). Among those older than 80 years, linear regressions, including all covariates, revealed that prior omega-3 intake was the only significant predictor of cingulum restricted diffusion ($\beta = 0.160$; 95% CI: 0.063–0.257; $P = 0.002$) (Supplemental Table 4). There were no age differences in associations between prior omega-3 intake and cortical GM microstructure.

Correlations between prior omega-3 intake and restricted diffusion in the inferior longitudinal fasciculus (ILF) were stronger ($z = 2.26$, $P = 0.02$) for *APOE* ε4 carriers ($n = 28$; $r = 0.50$, $P = 0.01$) than noncarriers ($n = 93$; $r = 0.04$, $P = 0.74$) (Fig. 3). This difference persisted after adjustment for demographic, health, and lifestyle covariates ($z = 2.57$, $P = 0.01$) and further adjustment for Mediterranean diet ($z = 2.12$, $P = 0.03$). There was a trend for a stronger correlation between omega-3 intake and restricted diffusion in the inferior frontal occipital fasciculus (IFO) ($z = 1.85$, $P = 0.06$) for *APOE* ε4 carriers ($r = 0.46$, $P = 0.02$) than noncarriers ($r = 0.08$, $P = 0.44$) that became significant in secondary ($z = 2.69$, $P = 0.007$) and fully adjusted ($z = 2.47$, $P = 0.01$) models. Among *APOE* ε4 carriers, fully adjusted linear regressions revealed that prior omega-3 intake was the second strongest predictor of ILF restricted diffusion ($\beta = 0.117$; 95% CI: 0.003–0.232; $P = 0.04$) after sex ($\beta = -0.045$; 95% CI: −0.083 to −0.008; $P = 0.02$) (Supplemental Table 5). Results for IFO restricted diffusion were similar, with omega-3 intake as the next strongest predictor ($\beta = 0.113$; 95% CI: 0.020–0.205; $P = 0.02$) after sex ($\beta = -0.050$; 95% CI: −0.080 to −0.020; $P = 0.003$) (Supplemental Table 6).

Associations between omega-3 intake and WM or GM microstructure did not differ by sex.

Discussion

In our sample of community-dwelling older adults, higher omega-3 intake approximately 25 years prior to MRI correlated with greater WM microstructural integrity in late-life. Omega-3 intake was positively associated with total restricted diffusion, an aggregate measure of all intracellular diffusion, in the SCS fasciculus. Additionally, we observed modifying effects of age and *APOE* ε4 on associations between prior omega-3 intake and microstructure. Higher omega-3 intake more strongly correlated with higher restricted diffusion in the cingulum among participants older than 80 years and more strongly correlated with higher restricted diffusion in the ILF and IFO for *APOE* ε4 carriers. The beneficial effect of omega-3 intake on WM integrity was not attributable to a healthy dietary pattern or lifestyle and was not clearly modified by sex.

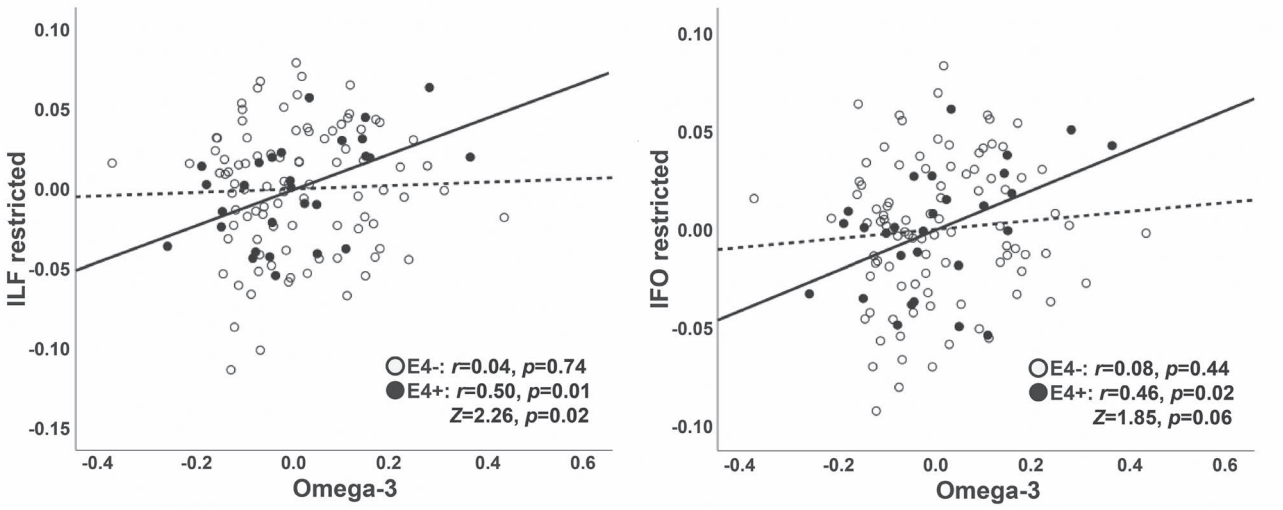

Fig. 3. Correlation between prior omega-3 intake (adjusted for calorie intake) and ILF (left) and inferior fronto-occipital fasciculus (IFO, right) total restricted diffusion for *APOE* ε4 carriers ($N = 28$) or noncarriers ($n = 93$). Values are residuals, adjusted for age, time between dietary assessment and MRI, and sex.

Our observations expand upon previous studies showing that higher omega-3 intake is associated with greater WM integrity in cognitively normal older adults (Witte et al. 2014; Gu et al. 2016) to report that these positive associations are present even for omega-3 intake 2 decades prior. Furthermore, we found that the protective effect of prior omega-3 intake was strongest in the SCS fasciculus, which may be particularly susceptible to age-related degradation (Sullivan et al. 2010). Thus, higher omega-3 intake may help preserve integrity of fibers that are particularly vulnerable to age-related damage. Further, prior omega-3 intake was more strongly associated with cingulum integrity among older (>80 years) than younger individuals. Considering evidence that association fibers, such as the cingulum, are exceptionally vulnerable to age-related damage, which accelerates at advanced ages (Bender et al. 2016), omega-3 intake may be particularly beneficial for slowing WM degeneration that accelerates in the final decades of life. Further, omega-3 intake did not clearly predict cognitive function, suggesting that RSI is sensitive to subtle cellular remodeling related to fatty acid intake that emerges prior to overt cognitive changes.

Associations between omega-3 intake and future microstructure were also modified by *APOE* ε4 status such that higher omega-3 intake more strongly correlated with higher restricted diffusion in the ILF and IFO among *APOE* ε4 carriers than noncarriers. Prior studies have shown that *APOE* ε4 is associated with inefficient lipolysis and delivery of omega-3 fatty acids in the brain, (Grimm et al. 2017) and fish oil supplementation has been associated with lower GM atrophy among *APOE* ε4 noncarriers only (Daiello et al. 2015). However, a comprehensive review of relevant literature suggests that omega-3 supplementation may effectively support healthy brain aging in *APOE* ε4 carriers if implemented earlier in the aging process (Yassine et al. 2017). Consistent with this hypothesis, 1 study found that moderate intake of polyunsaturated fatty acids at midlife was associated with decreased risk of dementia over an average follow-up of 21 years, and this effect was seen only among *APOE* ε4 carriers (Laitinen et al. 2006). Together with our findings, these reports suggest that midlife may constitute a critical time-period during which an omega-3-enriched diet could modify brain aging trajectories later in life, particularly for *APOE* ε4 carriers. Furthermore, a recent study reported that an omega-3-enriched diet effectively increased omega-3 levels among both *APOE* ε3- and *APOE* ε4-targeted replacement mice (Scheinman et al. 2021), suggesting that it may be possible to overcome the metabolic disadvantage for *APOE* ε4 carriers with sufficiently high omega-3 doses, warranting further research in human samples. Notably, *APOE* ε4 carriers in our study had higher omega-3 intake than noncarriers, which may have been sufficient to provide beneficial effects on WM microstructure. The modifying effect of *APOE* ε4 was observed specifically in the ILF and IFO. Prior research has found greater microstructural abnormalities in these fibers among older *APOE* ε4 carriers, compared to noncarriers (Cavedo et al. 2017), suggesting that sufficient omega-3 intake may help to counter injury to regions that are particularly vulnerable to *APOE* ε4-related damage.

Associations between prior omega-3 intake and WM integrity remained significant even after accounting for adherence to the Mediterranean diet as well as lifestyle and cardiovascular risk factors. Although some studies suggest that adherence to the Mediterranean diet is associated with greater WM integrity in cognitively normal older adults (Pelletier et al. 2015), others report no association (Karstens et al. 2019). Our findings, therefore, provide further support for a protective effect of omega-3 intake against age-related WM changes in late life that

exceeds the benefits conferred by a generally healthy dietary pattern.

We did not find an association between omega-3 intake and cortical GM microstructure, which is consistent with reports that more frequent fatty fish consumption was associated with greater WM but not GM integrity in cognitively normal older adults (Virtanen et al. 2008). However, other studies demonstrated the protective effects of omega-3 or fish oil supplementation against GM atrophy in older adults (Witte et al. 2014; Daiello et al. 2015; Köbe et al. 2016). One possible explanation for these discrepant findings is that higher doses of omega-3 may be required for measurable protection against age-related GM damage. Additionally, given that adherence to the Mediterranean diet has been associated with less GM atrophy (Ballarini et al. 2021), future investigations should consider the extent to which associations are attributable to overall healthy dietary patterns.

Various candidate mechanisms may explain the positive effects of omega-3 intake on WM integrity. Omega-3 fatty acids are structural components of phospholipid cell membranes and are involved in axonal myelination, synaptic transmission (Di Miceli et al. 2020), and mechanisms regulating microglial function and neuroinflammation (Layé et al. 2018). Additionally, omega-3 fatty acids may be involved in regulating cerebrovascular function. Omega-3 supplementation enhanced angiogenesis and cerebrovascular remodeling following cerebral ischemia in mice (Wang et al. 2014) and has been associated with greater cerebral blood flow in patients with mild cognitive impairment (Schwarz et al. 2018). Further investigation is warranted to clarify the mechanisms by which omega-3 consumption may be neuroprotective.

Despite evidence for sex differences in fatty acid metabolism (Decsi and Kennedy 2011), sex differences in associations between omega-3 fatty acids and brain aging have been minimally examined. Although we previously reported more pronounced age-related microstructural differences among women than men of the RBS (Reas et al. 2020), here, we did not observe sex differences in associations between omega-3 intake and brain microstructure. Thus, despite distinct patterns of age-related brain changes for men and women, omega-3 fatty acids may support healthy brain aging regardless of sex.

Our study has some limitations. Because our sample predominantly comprised highly educated, non-Hispanic White participants, our findings may not generalize to other populations. However, the relative homogeneity of our cohort limits potential confounding effects such as socioeconomic status. Omega-3 intake was estimated based on self-report of regular oily fish consumption. Although self-report may be less accurate than omega-3 measures obtained from blood samples, reported fish consumption correlates strongly with blood EPA and DHA levels (Sands et al. 2005). Additionally, if the most pronounced benefits of omega-3 fatty acids on brain aging emerge at very high levels of consumption, our ability to detect positive associations may have been limited by the range of omega-3 intake of our sample. Because imaging was conducted at a single timepoint, we were unable to examine whether omega-3 intake protects against subsequent age-related decline in microstructural integrity. Although omega-3 measures were highly stable across dietary assessments ($r = 0.86$, $P < 0.001$ between first and last assessment), omega-3 intake was not assessed at time of MRI. Therefore, we could not compare differences in associations of prior versus current omega-3 intake with brain microstructure. Future investigation will be important to monitor omega-3 levels together with longitudinal assessment of brain microstructure from midlife into older age.

In conclusion, our findings extend prior evidence of cross-sectional associations between omega-3 fatty acids and brain health to suggest that higher midlife omega-3 intake is associated with preserved WM microstructure over 2 decades later, independently of a healthy dietary pattern or other potentially confounding health and lifestyle factors. Sufficient omega-3 intake may be particularly protective against damage to WM association fibers, which accelerates in the final decades of life. Our findings support the consumption of high omega-3 fatty acid levels beginning in midlife as a preventative strategy to maintain healthy brain aging. Controlled randomized trials, including sex and genetic subgroup analyses, are recommended to determine optimal supplementation doses and to identify populations who may experience the greatest therapeutic benefit.

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Supplementary material

Supplementary material is available at *Cerebral Cortex* online.

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