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1  
2 **Associations of erythrocyte omega-3 fatty acids with cognition, brain imaging**  
3 **and biomarkers in the Alzheimer's Disease Neuroimaging Initiative: cross-**  
4 **sectional and longitudinal retrospective analyses**  
5

6 Laure Rouch, PhD, PharmD\*<sup>1</sup>, Kelly Virecoulon Giudici, PhD\*<sup>1</sup>, Christelle Cantet, MSc<sup>1</sup>, Sophie  
7 Guyonnet, PhD<sup>1,2</sup>, Julien Delrieu, MD<sup>1,2,3</sup>, Philippe Legrand, PhD<sup>4</sup>, Daniel Catheline, PhD<sup>4</sup>,  
8 Sandrine Andrieu, MD, PhD<sup>2,5</sup>, Michael W. Weiner, MD, PhD<sup>6,7,8,9,10</sup>, Philippe de Souto Barreto,  
9 PhD<sup>1,2</sup>, Bruno Vellas, MD, PhD<sup>1,2</sup>, for the Alzheimer's Disease Neuroimaging Initiative\*\*  
10

11 \*Shared authorship

12 1: Gerontopole of Toulouse, Institute of Ageing, Toulouse University Hospital (CHU Toulouse),  
13 Toulouse, France.

14 2: CERPOP UMR1295, University of Toulouse III, Inserm, UPS, Toulouse, France.

15 3: Toulouse NeuroImaging Center, Université de Toulouse, Inserm, UPS, Toulouse, France.

16 4: Laboratory of Biochemistry and Human Nutrition, Institut Agro, INSERM 1241, Rennes,  
17 France.

18 5: Department of Epidemiology and Public Health, Toulouse University Hospital (CHU  
19 Toulouse), Toulouse, France.

20 6: Department of Veterans Affairs Medical Center, San Francisco, United States.

21 7: Department of Medicine, University of California, San Francisco, United States.

22 8: Department of Psychiatry, University of California, San Francisco, United States.

23 9: Department of Neurology, University of California, San Francisco, United States.

24 10: Department of Radiology and Biomedical Imaging, University of California, San Francisco,  
25 USA

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28 ADNI contributed to the design and implementation of ADNI and/or provided data but did not  
29 participate in analysis or writing of this report. A complete listing of ADNI investigators can be  
30 found at: [http://adni.loni.usc.edu/wp-](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)  
31 [content/uploads/how\\_to\\_apply/ADNI\\_Acknowledgement\\_List.pdf](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)

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39

### 40 **Corresponding author**

41 Dr. Laure ROUCH

42 Associate Professor

43 Gérontopôle of Toulouse, Institute of Aging, Toulouse University Hospital, Université Toulouse

44 III Paul Sabatier

45 37 Allées Jules Guesde

46 31000 Toulouse, France

47 E-mail: [rouch.l@chu-toulouse.fr](mailto:rouch.l@chu-toulouse.fr)

48 Phone: +33 6 71 34 51 23

49

### 50 **Running title**

51 Erythrocyte  $\omega$ -3 fatty acids, cognitive outcomes

52

53 **Abbreviations**

54  $\omega$ -3, omega-3; AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale  
55 cognitive subscale; ADCS-PACC, Alzheimer Disease Cooperative Study Preclinical Alzheimer  
56 Cognitive Composite; ADNI, Alzheimer's Disease Neuroimaging Initiative; ALA,  $\alpha$ -linolenic  
57 acid; ApoE, apolipoprotein E; BMI, body mass index; CDR, Clinical Dementia Rating; CN,  
58 cognitively normal; DHA, docosahexaenoic acid; DSST, Digit Symbol Substitution Test; EDTA,  
59 ethylenediamine tetraacetic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters;  
60 FCSRT, Free and Cued Selective Reminding Test; IDE, insulin-degrading enzyme; JNK, c-Jun N-  
61 terminal kinase; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination;  
62 MoCA, Montreal Cognitive Assessment; PET, positron emission tomography; PUFA,  
63 polyunsaturated fatty acids; RBC, red blood cells; RCT, randomized controlled trial; SAS,  
64 Statistical Analysis Software; SUV<sub>r</sub>, standard uptake value ratio; TIV, total intracranial volume;  
65 TMT-A, Trail Making Test A; TMT-B, Trail Making Test B.

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76 **Abstract**

77

78 **Background:** The association between  $\omega$ -3 polyunsaturated fatty acids and cognition, brain  
79 imaging and biomarkers is still not fully established.

80 **Objectives:** To analyze the cross-sectional and retrospective longitudinal associations between  
81 erythrocyte  $\omega$ -3 index and cognition, brain imaging and biomarkers among older adults.

82 **Methods:** 832 Alzheimer's Disease Neuroimaging Initiative 3 participants, mean (SD) age 74.0  
83 (7.9) years, 50.8% female, 55.9% cognitively normal, 32.7% with mild cognitive impairment,  
84 11.4% with Alzheimer's disease (AD) were included. Low  $\omega$ -3 index (% eicosapentaenoic acid  
85 EPA + % docosahexaenoic acid DHA) was defined as the lowest quartile ( $\leq 3.70\%$ ). Cognitive  
86 tests (composite score; AD Assessment Scale cognitive (ADAS-Cog); Wechsler Memory Scale  
87 (WMS); Trail Making Test; Category fluency; Mini Mental State Examination; Montreal  
88 Cognitive Assessment) and brain variables (hippocampal volume, white matter hyperintensities  
89 (WMH), PET amyloid- $\beta$  and Tau) were considered as outcomes in regression models.

90 **Results:** Low  $\omega$ -3 index was not associated with cognition, hippocampal and WMH volume, brain  
91 A $\beta$  and Tau after adjustment for demographics, ApoE  $\epsilon 4$ , cardiovascular disease, BMI and total  
92 intracranial volume in the cross-sectional analysis. In the retrospective analysis, low  $\omega$ -3 index  
93 was associated with greater A $\beta$  accumulation (adjusted  $\beta = 0.02$ , 95% CI [0.01, 0.03];  $p = 0.003$ ).  
94 The composite cognitive score did not differ between groups; however, low  $\omega$ -3 index was  
95 significantly associated with greater WMS-Delayed recall cognitive decline (adjusted  $\beta = -1.18$ ,  
96 95% CI [-2.16, -0.19];  $p = 0.019$ ) but unexpectedly lower total ADAS-Cog cognitive decline. Low  
97  $\omega$ -3 index was cross-sectionally associated with lower WMS performance (adjusted  $\beta = -1.81$ ,  
98 SE=0.73,  $p = 0.014$ ) and higher Tau accumulation among ApoE  $\epsilon 4$  carriers.

99 **Conclusions:** Longitudinally, low  $\omega$ -3 index was associated with greater A $\beta$  accumulation and  
100 WMS cognitive decline but unexpectedly with lower total ADAS-Cog cognitive decline. Although

101 no associations were cross-sectionally found in the whole population, low  $\omega$ -3 index was  
102 associated with lower WMS cognition and higher Tau accumulation among ApoE  $\epsilon$ 4 carriers.

103

104 Alzheimer's Disease Neuroimaging Initiative (ADNI, ClinicalTrials.gov identifier,  
105 NCT00106899).

106

107 **Keywords:** omega-3, cognition, brain imaging, biomarkers, Alzheimer's disease, mild cognitive  
108 impairment, docosahexaenoic acid, eicosapentaenoic acid.

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110

## 111 **Introduction**

112 Omega-3 ( $\omega$ -3) polyunsaturated fatty acids (PUFA), mainly docosahexaenoic acid (DHA)  
113 and eicosapentaenoic acid (EPA), have received substantial attention due to their beneficial effect  
114 on brain functioning (1). Several studies reported associations between higher  $\omega$ -3 PUFA intake or  
115 blood concentrations and lower cognitive decline, lower risk of dementia or preserved brain  
116 structure (2); however, much less is known about the association of erythrocyte  $\omega$ -3 fatty acids  
117 with brain imaging and biomarkers of cognitive function.

118 Erythrocyte  $\omega$ -3 index, defined as the sum of EPA and DHA proportion from the total  
119 membrane lipid content, represents a more reliable measurement of nutritional status since fatty  
120 acids are stable in the erythrocytes' membranes for up to 3 months (3). In the Multidomain  
121 Alzheimer Preventive Trial (MAPT), dementia-free older adults with low  $\omega$ -3 index were at  
122 greater risk of cognitive decline (4). Consistent results were found among dementia-free  
123 Framingham Study participants, with lower erythrocyte DHA being associated with smaller brain  
124 volumes and a vascular pattern of cognitive impairment (5). In animal studies, DHA and EPA

125 have also been shown to reduce amyloid- $\beta$  (A $\beta$ ) peptide production, particularly by altering  
126 amyloid precursor protein (APP) processing (6). In the MAPT Study, multidomain interventions  
127 both with and without  $\omega$ -3 PUFA supplementation were associated with lower cerebral A $\beta$  (7). To  
128 our knowledge, only one study investigated the association between serum DHA levels and A $\beta$   
129 positron-emission tomography (PET) imaging, and reported an inverse correlation with brain A $\beta$   
130 load (8). Although even less is known, DHA could also additionally contribute to the inhibition of  
131 tau phosphorylation (9). Finally, the beneficial effects of  $\omega$ -3 PUFA on cognition have been most  
132 often seen in cognitively healthy individuals or individuals with mild cognitive impairment (MCI)  
133 (10). A recent Cochrane meta-analysis found no convincing evidence for the efficacy of  $\omega$ -3  
134 PUFA supplements in the treatment of mild to moderate Alzheimer's disease (AD) (11).  
135 Interestingly, previous studies also showed that the presence of Apolipoprotein E (ApoE)  $\epsilon$ 4 allele  
136 might modify the relationship between  $\omega$ -3 PUFA and brain functioning, but results are still  
137 conflicting (12–14). Overall, in the literature, most studies were only cross-sectional, investigated  
138  $\omega$ -3 intake or plasma/serum measurements and their association with cognitive function, with very  
139 few analyses on brain imaging, biomarkers especially PET-Tau, and on the potential modifying  
140 effect of ApoE  $\epsilon$ 4 genotype.

141 We therefore aimed to assess the cross-sectional and retrospective longitudinal associations  
142 between erythrocyte  $\omega$ -3 index, cognition and neuroimaging biomarkers (hippocampal and white  
143 matter hyperintensities – WMH volume, amyloid and Tau PET) among community-dwelling older  
144 adults. Sub-group analyses were also conducted according to cognitive status (cognitively normal  
145 CN, MCI and AD) and ApoE  $\epsilon$ 4 genotype. We hypothesized that participants with low erythrocyte  
146  $\omega$ -3 index would present lower cognitive function, lower hippocampal volume, higher WMH  
147 volume, brain amyloid and Tau accumulation.

148

## 149 **Methods**

150 *Study design and population*

151 Data used in the preparation of this article were obtained from the Alzheimer's Disease  
152 Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI, a multi-center  
153 observational study conducted in 60 sites in the United States and Canada (15), was launched in  
154 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The  
155 primary goal of ADNI was to test whether serial magnetic resonance imaging (MRI), PET, other  
156 biological markers, and clinical and neuropsychological assessment could be combined to measure  
157 the progression of MCI and early AD. After three waves (ADNI-1, ADNI-Grand Opportunity –  
158 ADNI-GO, and ADNI-2), the ongoing ADNI-3 wave began on August 1, 2016. Older adults aged  
159 55 to 97 years with CN status (with or without subjective memory concerns), MCI (both early and  
160 late MCI) or AD, newly enrolled in ADNI-3 or previously recruited in other ADNI waves, were  
161 included in our study. Main exclusion criteria were having a major neurologic or psychiatric  
162 illness; a history of substance abuse; a screening MRI showing evidence of infection, infarction, or  
163 other focal lesions (including multiple lacunes, or lacunes in a critical memory structure). Detailed  
164 inclusion and exclusion criteria for joining ADNI according to each of these three cognitive status'  
165 categories are described elsewhere (16). Study flowchart is presented in **Figure 1**. Among the 849  
166 participants with fatty acids measurements during the ADNI-3 wave, 832 were included in our  
167 cross-sectional analysis (cognition, brain imaging and biomarkers measured simultaneously in the  
168 ADNI-3 wave). After exclusion of 482 participants without ADNI-1, ADNI-GO or ADNI-2 data,  
169 350 participants were included in our retrospective longitudinal analysis (cognition, brain imaging  
170 and biomarkers assessed over time using data from all waves). ADNI-3 was registered in  
171 www.ClinicalTrials.gov under the protocol NCT02854033. All subjects were informed about the  
172 aims of the study and signed a consent form.

173

174 *Isolation of erythrocytes and fatty acids measurement*



175 Fatty acids were measured during the ADNI-3 wave. Red blood cells (RBC) were isolated  
176 from the whole blood (10 mL) collected into ethylenediamine tetra-acetic acid (EDTA) tubes,  
177 according to the standardized procedures at ADNI sites ( $\omega$ -3 PUFA measurement is not affected  
178 by the presence of anti-coagulant). The blood was centrifuged at 3,000 gravitational force (g) for  
179 15 minutes at 4°C. This results in the formation of a RBC pellet, an intermediate layer containing  
180 the leukocytes and platelets (buffy coat) and an upper phase comprising plasma. Following  
181 removal of the plasma and buffy coat, RBC was stored immediately at -80°C in EDTA tubes.  
182 Fatty acids analysis was performed at biochemical laboratory of Institut Agro Rennes, France  
183 according to previous publication (17). Briefly, lipids were extracted twice from RBC (475 to 525  
184 mg) samples with a mixture of hexane/isopropanol (3:2 v/v), after acidification with 1 mL  
185 hydrochloric acid (HCl) 3M (18), containing 5 ppm butylated hydroxytoluene (BHT). Margarinic  
186 acid (C17:0) was added as internal standard. Total lipid extracts were saponified with 1mL of  
187 0.5M sodium hydroxide (NaOH) in methanol for 30 minutes at 70°C and methylated with 1 mL of  
188 boron trifluoride (BF<sub>3</sub>) methanol solution (12% w/v in methanol) for 15 min at 70°C. Fatty acid  
189 methyl esters (FAME) were extracted twice with pentane. Solvent was removed under nitrogen  
190 and FAME were redissolved in 200 $\mu$ L of hexane. Analyses were performed using an Agilent  
191 Technologies 7890N gas chromatograph (Bios Analytic, Toulouse, France) fitted with a split  
192 injector (10:1) at 250°C (injection volume 1 $\mu$ L) and a bonded silica capillary column (BPX 70,  
193 60m long 250 $\mu$ m inner diameter and 0.25 $\mu$ m film thickness; SGE, Villeneuve-St Georges,  
194 France). Helium was used as a carrier gas (constant flow: 1.8 mL/min, 36 cm/sec). The column  
195 temperature program started at 170°C, increased by 4°C/min to 250°C and held at 250°C for 2  
196 minutes. Transfer line was at 270°C. Mass spectra were obtained with an Agilent 5975C  
197 spectrometer used in electron impact mode (EI) with 70 eV energy; source temperature was set at  
198 150°C and quadrupole at 230°C. Acquisition was performed in the full scan mode ranging from 50  
199 to 500 amu (3 scan/s). Identification of FAME was based on retention times obtained for FAME

200 prepared from fatty acid standards and confirmed by comparison of their MS spectra with those of  
201 the NIST bank (V.2.2, 2014). Quantification was achieved by determining the area under the  
202 peaks with Mass Hunter software (version B.07.00 SP2, 2015, Agilent).

203 Results of EPA and DHA were expressed as % of total identified fatty acids.  $\omega$ -3 index  
204 was calculated as the sum of %EPA and %DHA, and thus also expressed in % from total fatty  
205 acids. Low  $\omega$ -3 index was characterized by the lowest quartile within the populations investigated  
206 ( $\leq 3.70\%$  for the cross-sectional analysis and  $\leq 3.62\%$  for the retrospective longitudinal analysis).  
207 Distribution of  $\omega$ -3 index is shown in **Supplementary Figure 1**.

208

### 209 *Cognition, brain imaging and biomarkers*

210 Cognitive, brain imaging and biomarkers variables were collected over time in the  
211 different ADNI waves. The primary outcome was cognitive function which was assessed using a  
212 modified form of the Alzheimer's Disease Cooperative Study Preclinical Alzheimer Cognitive  
213 Composite (ADCS-PACC) (19). The modified composite score included the delayed word recall  
214 from the Alzheimer's Disease Assessment Scale – Cognitive Subscale (ADAS-Cog) (20), the Trail  
215 Making Test B (TMT-B) (21), the Mini Mental State Examination (MMSE) total score (22) and  
216 the Wechsler Memory Scale (WMS) delayed recall (23). The modified composite score was  
217 determined using standardized Z-scores. They were calculated by dividing each of the component  
218 scores by the sample standard deviation of that component and by subtracting each of the  
219 component scores by the mean of that component. The Z-scores of delayed recall from the ADAS-  
220 Cog and from the TMT-B were then multiplied by -1 to account for the fact that, differently than  
221 the other tests, higher scores mean worse cognitive performance. All four Z-scores were then  
222 summed to form the composite score. For all subjects, the MMSE total score, WMS delayed  
223 recall, WMS immediate recall, Category Fluency test (24), Montreal Cognitive Assessment  
224 (MoCA) (25) (higher scores mean better cognitive function for these 5 tests) and delayed word

225 recall ADAS-Cog, total ADAS-Cog, TMT-A, TMT-B, Clinical Dementia Rating Sum of Boxes  
226 (CDR-SB) (26) (higher scores mean worse cognitive function for these 5 tests) were also  
227 examined. Total MMSE, ADAS-Cog, MOCA and CDR-SB were used to assess global cognition;  
228 ADAS-Cog delayed recall, WMS immediate and delayed recall were used to assess memory and  
229 category fluency, TMT-A and TMT-B tests were used to assess executive functions.

230 Secondary outcomes were brain imaging and biomarkers variables and included  
231 hippocampal subcortical volume (in left hemisphere, right hemisphere, and total), WMH volume  
232 (27), assessed by 3 Tesla (3T) MRI, and brain A $\beta$  and hyper-phosphorylated paired helical  
233 filaments (PHF) tau, measured by PET. MRI was performed using unified parameter scanning  
234 protocols available on: <https://adni.loni.usc.edu/methods/documents/mri-protocols/>. Amyloid  
235 positive status (A $\beta$ +) was defined as <sup>18</sup>F-florbetapir PET cortical standardized uptake value ratio  
236 (SUV<sub>r</sub>) normalized by the whole cerebellum reference region (SUV<sub>r</sub>) >1.11 for the cross-sectional  
237 analysis and SUV<sub>r</sub> based on the composite reference region (SUV<sub>r</sub> > 0.78) for the longitudinal  
238 analysis (28). Tau PET was assessed during the ADNI-3 wave in meta-temporal region and in  
239 Braak stages 1 to 6 (29) after intravenous bolus injection of [<sup>18</sup>F]-radiolabeled AV1451.

#### 240 *Potential confounders*

241 Potential confounders included: age (years), education (years), body mass index (BMI,  
242 calculated as body weight in kg divided by height<sup>2</sup> in m<sup>2</sup>), ApoE  $\epsilon$ 4 genotype (carriers, non-  
243 carriers), history of cardiovascular disease (yes, no), and total intracranial volume (TIV) (cm<sup>3</sup>),  
244 this last only for WMH and hippocampal volume.

245

#### 246 *Statistical analysis*

247 Characteristics of the population were presented as mean and standard deviation (SD) for  
248 continuous variables and as frequencies and percentages for categorical variables. They were  
249 compared according to  $\omega$ -3 index (1<sup>st</sup> quartile vs. others) using Student t-test for continuous

250 variables (with log transformation if necessary) with normal distribution (or non-parametric  
251 Kruskal-Wallis test for other quantitative variables) and Pearson's chi-squared test for qualitative  
252 variables. In cross-sectional analyses, linear regression models were performed to assess the  
253 association between  $\omega$ -3 index (1<sup>st</sup> quartile vs. others), cognitive, brain imaging and biomarkers  
254 variables and whether the associations differed according to cognitive status (CN, MCI, and AD)  
255 or ApoE4 genotype (carriers vs. non-carriers). For this purpose, an interaction in each model was  
256 introduced between  $\omega$ -3 index and cognitive status or ApoE  $\epsilon$ 4 genotype. If interactions were  
257 significant or marginally significant ( $p < 0.10$ ), within-group estimates were further explored using  
258 model estimates with interaction. Linear mixed models with random intercepts and slopes were  
259 used in the longitudinal analyses. Differences in retrospective change of cognitive, brain imaging  
260 and biomarkers variables between participants in the lowest omega-3 index quartile and those in  
261 the other 3 quartiles were assessed at 24, 36, 60 and 120 months except for MoCA, WMH volume  
262 and amyloid PET (whose maximum retrospective follow-up was 6 years). Analyses were adjusted  
263 for potential confounders (age, education, BMI, ApoE  $\epsilon$ 4 genotype, history of cardiovascular  
264 disease and TIV (the last one only for WMH and hippocampal volume). Given the exploratory  
265 nature of our study, we did not correct for multiplicity. We finally conducted cross-sectional and  
266 longitudinal sensitivity analyses by excluding participants with AD and also investigated among  
267 cognitively normal and individuals with MCI whether the associations differed according to  
268 amyloid status (amyloid positive status, A $\beta$ <sup>+</sup> vs. amyloid negative status, A $\beta$ <sup>-</sup>). Analyses were  
269 performed using the Statistical Analysis Software (SAS) version 9.4 (Cary, NC, USA), with a  
270 significance level established as 5%.

271

## 272 **Results**

273

274 We included 832 individuals (mean (SD) age 74.0 (7.9) years; 50.8% female; 37.7% ApoE  $\epsilon$ 4  
275 carriers): 465 (55.9%) had a CN status, 272 (32.7%) had MCI and 95 (11.4%) had AD. Mean  $\omega$ -3  
276 index was 4.85% (SD=1.52). The distribution was as follows: median, 4.68%; interquartile range,  
277 3.70%-5.83%; minimum, 1.77%; maximum, 11.37%. Among fish oil supplements users, only 45  
278 (13.2%) were in the lowest  $\omega$ -3 index quartile compared to 162 (33.1%) among non-users  
279 ( $p<0.001$ ). Participants in the lowest  $\omega$ -3 index quartile ( $\leq 3.70\%$ ) were less educated (mean (SD)  
280 years of education: 15.8 (2.4) vs. 16.8 (2.4);  $p<0.0001$ ) than those in the 3 other quartiles. No  
281 other differences were found in terms of population characteristics. The proportion of participants  
282 with low  $\omega$ -3 index did not significantly differ according to cognitive status or ApoE  $\epsilon$ 4 genotype  
283 (**Table 1**).

284  
285 In our cross-sectional analysis, low  $\omega$ -3 index was not significantly associated with  
286 cognitive function (composite cognitive score or any cognitive test), hippocampal volume, WMH,  
287 brain A $\beta$  and tau accumulation in unadjusted models or after adjustment for age, education, ApoE  
288  $\epsilon$ 4 genotype, history of cardiovascular disease, BMI and TIV (the last one only for WMH and  
289 hippocampal volume) (**Table 1**). Interactions were found between low  $\omega$ -3 index and ApoE  $\epsilon$ 4  
290 genotype (**Table 2**). Among the subgroup of ApoE  $\epsilon$ 4 carriers, low  $\omega$ -3 index was significantly  
291 associated in unadjusted models with lower performance on WMS-Delayed and Immediate recall  
292 and with higher tau accumulation in several regions. After adjustment, a similar result with lower  
293 performance on WMS-Delayed recall ( $\beta=-1.81$ , SE=0.73,  $p=0.014$ ) was found and a tendency was  
294 observed with higher tau accumulation (**Table 3**). Interactions were also found between low  $\omega$ -3  
295 index and cognitive status on MMSE, MoCA, hippocampal volume, and Tau accumulation  
296 (**Supplementary Table 1**), with significant better cognitive, brain imaging and biomarkers  
297 conditions among the subgroup of AD if  $\omega$ -3 index was low (**Supplementary Table 2**). In our  
298 cross-sectional sensitivity analyses after exclusion of participants with AD, low  $\omega$ -3 index was not

299 significantly associated with cognitive function, WMH, brain A $\beta$  load (SUVr) and tau  
 300 accumulation after adjustment but was significantly associated with lower right hippocampal  
 301 volume ( $\beta$ =-85.64, SE=43.03,  $p$ =0.047). Interactions were found between low  $\omega$ -3 index, ApoE  $\epsilon$ 4  
 302 genotype and amyloid status but not cognitive status. Among ApoE  $\epsilon$ 4 carriers only, low  $\omega$ -3  
 303 index was significantly associated with lower right hippocampal volume after adjustment ( $\beta$ =-  
 304 212.50, SE=73.39,  $p$ =0.004) and with higher Tau accumulation in several regions. Among A $\beta$ +  
 305 participants only, we observed a trend toward statistical significance after adjustment between low  
 306  $\omega$ -3 index and higher tau accumulation (Tau  $^{18}$ F-AV1451 Braak stage 2:  $\beta$ =0.08, SE=0.04,  
 307  $p$ =0.078).

308         In our retrospective longitudinal analysis, consistent across all time periods ( $p$ =0.003),  
 309 individuals in the lowest  $\omega$ -3 index quartile had significantly greater A $\beta$  accumulation over time  
 310 compared to those in the 3 other quartiles (adjusted  $\beta$ = 0.02, 95% CI [0.01, 0.03];  $p$ =0.003  
 311 between M-60 and M0 months) (**Table 4** and **Figure 2**). No significant differences were observed  
 312 between the two  $\omega$ -3 index groups for WMH volume or the composite cognitive score. WMS-  
 313 Delayed recall cognitive trajectories tended to differ (global  $p$ =0.066) between individuals in the  
 314 lowest  $\omega$ -3 index quartile versus those in the 3 other quartiles (**Table 4** and **Supplementary**  
 315 **Figure 2**). In both groups, cognitive performances declined between M-60 and M0 months (lowest  
 316  $\omega$ -3 index quartile: unadjusted  $\beta$ = -1.25, 95% CI [-2.12, -0.38]; 3 highest  $\omega$ -3 index quartiles:  
 317 unadjusted  $\beta$ = -0.08, 95% CI [-0.59, 0.42]). Compared to those in the 3 highest  $\omega$ -3 index  
 318 quartiles, individuals in the lowest  $\omega$ -3 index quartile had a significant greater cognitive decline  
 319 (adjusted  $\beta$ = -1.18, 95% CI [-2.16, -0.19];  $p$ =0.019). Total ADAS-Cog cognitive trajectories were  
 320 also different between the two  $\omega$ -3 index groups, with low  $\omega$ -3 index unexpectedly associated with  
 321 lower cognitive decline across certain time periods (**Table 4** and **Supplementary Figure 2**). In  
 322 our retrospective longitudinal analysis, cognitive, brain imaging and biomarkers trajectories  
 323 between participants in the 2  $\omega$ -3 index groups did not statistically differ according to ApoE  $\epsilon$ 4

324 genotype (**Supplementary Table 3**). Total MMSE, TMT-A, TMT-B and MoCA cognitive  
325 trajectories between participants in the 2  $\omega$ -3 index groups significantly differed according to  
326 cognitive status, particularly in participants with AD compared to those with a CN status  
327 (**Supplementary Table 4** and **Supplementary Figure 3**). Unlike individuals with MCI or a CN  
328 status, whose cognitive trajectories did not significantly differ between  $\omega$ -3 index groups,  
329 participants with AD in the lowest  $\omega$ -3 index quartile compared to those in the 3 highest quartiles  
330 had a significant lower cognitive decline in our retrospective analysis (**Supplementary Table 4**  
331 and **Supplementary Figure 3**). In our retrospective longitudinal sensitivity analyses after  
332 exclusion of participants with AD, consistent across all time periods ( $p=0.038$ ), individuals in the  
333 lowest  $\omega$ -3 index quartile had significantly greater  $A\beta$  accumulation (adjusted  $\beta= 0.01$ , 95% CI  
334 [0.00, 0.03];  $p=0.038$  between M-60 and M0 months). MOCA trajectories also differed between  
335 the 2 omega-3 index groups ( $p=0.053$  across all time periods). Individuals in the lowest  $\omega$ -3 index  
336 quartile had a borderline significant greater cognitive decline between M-60 and M0 months  
337 (adjusted  $\beta= -0.53$ , 95% CI [-1.07, 0.01];  $p=0.053$ ). Although not significant across all time  
338 periods, individuals in the lowest  $\omega$ -3 index quartile had a significant greater cognitive decline  
339 between M-60 and M0 months on the WMS-immediate recall (adjusted  $\beta= -1.00$ , 95% CI [-1.88, -  
340 0.13];  $p=0.025$ ) and on the WMS-delayed recall (adjusted  $\beta= -0.94$ , 95% CI [-1.87, -0.00];  
341  $p=0.050$ ). Although not significant across all time periods, individuals in the lowest  $\omega$ -3 index  
342 quartile had an unexpected lower cognitive decline on total ADAS-Cog between M-60 and M0  
343 months (adjusted  $\beta= -0.11$ , 95% CI [-0.22, -0.01];  $p=0.030$ ). No interactions were found between  
344 low  $\omega$ -3 index, cognitive or amyloid status. However, unlike non-carriers, ApoE  $\epsilon$ 4 carriers in the  
345 lowest  $\omega$ -3 index quartile experienced a significant higher cognitive decline on MMSE between  
346 M-60 and M0 months (adjusted  $\beta= -0.56$ , 95% CI [-1.12, -0.01];  $p=0.045$ ).

347

348 **Discussion**

349 Our study explored the cross-sectional and retrospective longitudinal associations between  
350 erythrocyte  $\omega$ -3 index and cognitive tests, MRI data, PET A $\beta$  and tau accumulation among  
351 community-dwelling older adults. In the cross-sectional analysis, we did not find any significant  
352 association between low  $\omega$ -3 index and cognitive or brain outcomes in the whole population. In  
353 the retrospective longitudinal analysis, low  $\omega$ -3 index was significantly associated with greater A $\beta$   
354 accumulation over time. Although no differences were observed on the composite cognitive  
355 scores, individuals with low  $\omega$ -3 index had greater cognitive decline on the WMS-Delayed recall  
356 but unexpectedly lower cognitive decline on the total ADAS-Cog.

357 Several studies explored the association between erythrocyte omega-3 index and cognitive  
358 outcomes (**Table 5**); however, to our knowledge, our study is the first to investigate the  
359 association between erythrocyte  $\omega$ -3 index and both PET A $\beta$  and tau accumulation. Only one  
360 cross-sectional study with 61 older adults reported an inverse correlation between serum DHA  
361 levels and brain amyloid load on PET imaging (8); however, the longitudinal association had  
362 never been investigated. Although there is evidence suggesting that DHA could confer neuro-  
363 protection in part through the direct inhibition of Tau phosphorylation (9,30), we did not find any  
364 significant association between erythrocyte  $\omega$ -3 index and Tau PET. Further longitudinal studies  
365 on omega-3 fatty acids and Tau PET are needed to assess this important relationship.

366 Studies investigating the relationship between  $\omega$ -3 PUFA, brain morphology and volumes  
367 have also provided mixed findings (10). Higher erythrocyte  $\omega$ -3 index was correlated with larger  
368 total normal brain volume and hippocampal volume in females after menopause from the  
369 Women's Health Initiative Memory Study (31). However, no relationships were observed between  
370 erythrocyte omega-3 index and total hippocampal volume among dementia-free Framingham  
371 Study participants (5). Although accumulating evidence has reported a beneficial role of  $\omega$ -3  
372 PUFA on cognitive aging, previous studies have examined different exposures and findings have  
373 been mixed overall (32). Consistent with our findings on WMS-Delayed recall, higher levels of



374 plasma  $\omega$ -3 index were associated with lower cognitive decline and risk of dementia among older  
375 adults from the Three-City (3C) Study (2). Conversely, in a recent meta-analysis including  
376 181,580 participants from 21 prospective studies (33), DHA intake (but not blood DHA) was  
377 associated with lower risk of cognitive impairment or dementia, and total blood  $\omega$ -3 PUFA were  
378 not related to dementia outcomes. Finally, our unexpected results on the total ADAS-Cog need  
379 further investigation. The ADAS-Cog is not an optimal outcome measure for pre-dementia studies  
380 and is limited in measuring progression of cognitive impairment over the course of the disease  
381 progression (34). Its low sensitivity is primarily due to most of its items suffering from either floor  
382 or ceiling effects.

383         Although we did not find any association between low  $\omega$ -3 index and cognitive or brain  
384 outcomes in the whole population, our cross-sectional (but not retrospective longitudinal)  
385 findings, reported a significant interaction between low  $\omega$ -3 index and ApoE  $\epsilon$ 4 genotype on  
386 certain cognitive tests and tau accumulation. Accumulating evidence also suggests that ApoE  
387 genotype could be one of the most important moderators of  $\omega$ -3 PUFA effectiveness in the aging  
388 brain. Some studies reported associations of better cognitive effectiveness in ApoE  $\epsilon$ 4 non carriers  
389 (35–38), whereas others showed benefits among ApoE  $\epsilon$ 4 carriers only (13). In the Three-City  
390 (3C) Study, greater plasma DHA and EPA were associated with lower cognitive decline in ApoE  
391  $\epsilon$ 4 carriers (39). Similar findings were observed in secondary analyses from the Rush Memory and  
392 Aging Project, with ApoE  $\epsilon$ 4 carriers exhibiting slower rates of cognitive decline upon weekly  
393 seafood consumption (40). This is consistent with the study by Morris et al. (41) in which  
394 consumption of  $\alpha$ -linolenic acid (ALA) was only protective towards incident AD in ApoE  $\epsilon$ 4  
395 carriers. In cognitively unimpaired ApoE  $\epsilon$ 4 homozygotes from the Alzheimer and Families study  
396 (ALFA), dietary DHA intake also related to structural patterns that may result in greater resilience  
397 to AD pathology (42). Finally, very recent findings from the Framingham Offspring Cohort  
398 showed that risk for incident AD in the highest red blood cell DHA quintile was 49% lower

399 compared with the lowest quintile and that ApoE  $\epsilon$ 4 carriers may benefit more from higher DHA  
400 levels than non-carriers (43). There is a biological rationale for a vulnerability of ApoE  $\epsilon$ 4 carriers  
401 to lower  $\omega$ -3 PUFA status, especially in early AD, since several mechanisms linked ApoE  $\epsilon$ 4 with  
402 reduced brain DHA metabolism (13). It has been suggested that ApoE  $\epsilon$ 4 carriers  $\beta$ -oxidize DHA  
403 at greater rates than non-carriers (44), and are more likely to exhibit dysfunctions in blood-brain  
404 barrier (45), which can impair the DHA delivery to the brain (44,46).

405 In spite of the aforementioned interesting findings, we observed in our cross-sectional  
406 study an unexpected association between low  $\omega$ -3 index and better cognition, higher total  
407 hippocampal volume and lower Tau accumulation in patients with AD only. Similarly,  
408 participants with AD in the lowest  $\omega$ -3 index quartile exhibited lower cognitive decline than those  
409 in the 3 highest quartiles in our retrospective longitudinal analysis. Given the distributions of the  
410 cognitive tests among participants with AD, we ruled out the possibility of a floor effect, which  
411 might have partly explained some of these unexpected findings. The existing literature shows that  
412 the association between  $\omega$ -3 PUFA and brain outcomes may differ according to the cognitive  
413 status. A recent systematic review of clinical trials showed that interventions were promising for  
414 people with mild or subjective cognitive impairment but not in those already diagnosed with  
415 dementia (47). Further in-depth investigations of the differential effects of  $\omega$ -3 PUFAs according  
416 to cognitive status are required.

417 Sensitivity analyses after exclusion of participants with AD did not notably change our  
418 findings. They strengthened the negative effects of low omega-3 index on cognitive health,  
419 including on A $\beta$  accumulation and confirmed the greater vulnerability of ApoE4  $\epsilon$ 4 carriers to  
420 lower  $\omega$ -3 PUFA status. We also observed a trend towards higher tau accumulation among A $\beta$ +  
421 participants with low omega-3 index, which was not confirmed in the retrospective longitudinal  
422 analysis. Finally, low omega-3 index was overall mostly associated with lower global cognitive  
423 performances and complex memory tests. Other studies (48) showed that EPA and DHA could

424 benefit executive functions but not overall cognition. Further studies are needed to clarify the  
425 effects of  $\omega$ -3 PUFA on different cognitive domains.

426         The present study adds important elements to the field by evaluating several cognitive  
427 outcomes (including several clinical tests comprising different cognitive domains, MRI data and  
428 brain A $\beta$  and tau accumulation) and their association with  $\omega$ -3 PUFA status among a large sample  
429 of older adults with different cognitive and ApoE  $\epsilon$ 4 status. We were also able to analyze not only  
430 the cross-sectional but also the retrospective longitudinal associations between erythrocyte  $\omega$ -3  
431 index, cognition and neuroimaging biomarkers. Another strength to be noted is the assessment of  
432  $\omega$ -3 status through erythrocyte fatty acid concentrations, considered a more reliable measurement  
433 of  $\omega$ -3 PUFA intake compared to plasma or serum measurements (since fatty acids are stable in  
434 erythrocyte membranes over its lifespan, i.e. up to 3 months) (3,49), and known to reflect fatty  
435 acids concentrations of other tissues (50). However, some limitations must be considered. Our  
436 findings are based on an observational study, and residual confounding may still exist despite  
437 extensive efforts to account for possible confounders. ADNI participants were predominantly  
438 white, highly educated, with relatively few comorbidities, which may limit the generalizability of  
439 our findings. PET scan and MRI were not performed for all subjects; in addition, hippocampal  
440 volume and Tau PET were not assessed in our retrospective longitudinal analysis because of  
441 differences in methods used over time, which could have affected the comparability of the results.  
442 Our sub-group analyses according to cognitive status included few individuals with AD in the  
443 longitudinal analysis, therefore, the results should be taken with caution. Erythrocyte fatty acids  
444 assessment was only performed once at baseline during ADNI-3 wave, on defreeze samples after  
445 blood collection, which might have underestimated  $\omega$ -3 PUFA values due to partial oxidative  
446 degradation (51). However, the storage under  $-80^{\circ}\text{C}$  might have minimized such possible  
447 occurrence. In addition, fish oil consumption was associated with higher omega-3 index,  
448 supporting the reliability of our erythrocyte fatty acids assessment. However, although our

449 analytical method, which is the most widely used in the research setting, makes findings internally  
450 valid, they can only be compared to studies that used the same analytic approach. Finally, the  
451 analytical variability has been determined formerly in a precedent trial and was about 2%, which  
452 is very low and negligible compared to the patients' value variability (52).

453

#### 454 **Conclusions**

455 Our cross-sectional analysis did not find any significant association between erythrocyte  $\omega$ -3  
456 index and cognitive tests, brain imaging and biomarkers in the general sample of older adults with  
457 different cognitive status. In our retrospective longitudinal analysis, low  $\omega$ -3 index was associated  
458 with greater A $\beta$  accumulation over time and cognitive decline on the WMS test, but unexpectedly  
459 with lower cognitive decline on the total ADAS-Cog. Low  $\omega$ -3 index was associated with lower  
460 cognition and higher Tau accumulation in certain brain regions among ApoE  $\epsilon$ 4 carriers in the  
461 cross-sectional analysis. Our findings are consistent with the vulnerability of ApoE  $\epsilon$ 4 carriers to  
462 lower  $\omega$ -3 PUFA status reported in the literature and reinforce the group of evidence supporting  
463 the potential use of  $\omega$ -3 PUFA for cognitive decline and AD prevention among ApoE  $\epsilon$ 4 carriers.  
464 Identifying individuals who may benefit more from this dietary and/or supplementation approach  
465 is of high interest, given that increasing  $\omega$ -3 intake is a generally safe, simple and well-tolerated  
466 intervention. This is critical from a public health perspective, not only in terms of preserving  
467 cognition but also given the potential benefits on other health conditions such as inflammation and  
468 cardiovascular events (53). Our analysis was exploratory, and prospective longitudinal studies  
469 further evaluating the topic are encouraged. They should be conducted with large sample sizes,  
470 sufficient follow-ups, detailed dietary information and greater heterogeneity of participants,  
471 especially those with MCI and AD.

472

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500

### 501 **Authors' contributions**

502 LR and KVG interpreted data and wrote the manuscript. CC performed the statistical analyses,  
503 interpreted the data, and revised the draft critically for important intellectual content. PL and DC  
504 performed fatty acid analysis, interpreted analytical data and revised the draft critically on this  
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508 authors have read and approved the final version of the manuscript.

509

### 510 **Conflict of interest statement**

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518 with personal compensation. The other authors declare no conflict of interest.

519

### 520 **Data availability**

521 The data set is owned by a third-party organization; the Alzheimer's Disease Neuroimaging  
522 Initiative (ADNI). Data are publicly available at <http://adni.loni.usc.edu/data-samples/access-data/>

523 Institutional Data Access / Ethics Committee (contact via <http://adni.loni.usc.edu/data->  
524 [samples/access-data/](http://adni.loni.usc.edu/data-samples/access-data/)) upon sending a request composed by proposed analysis plan and the named  
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526

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725

726 **Figure 1.** Study flowchart

727 ADNI: Alzheimer’s Disease Neuroimaging Initiative

728

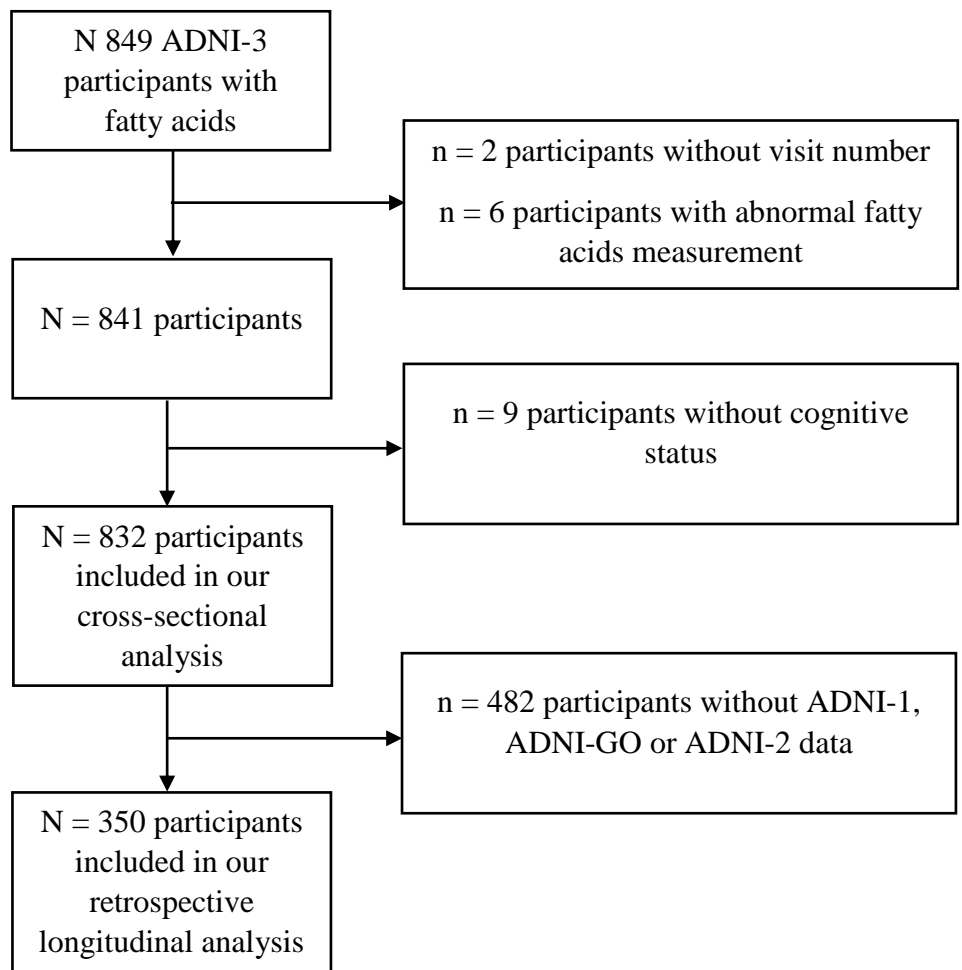
729 **Figure 2.** A $\beta$  trajectories over time according to the lowest vs. highest  $\omega$ -3 index quartiles  
730 (retrospective longitudinal analysis - whole population)

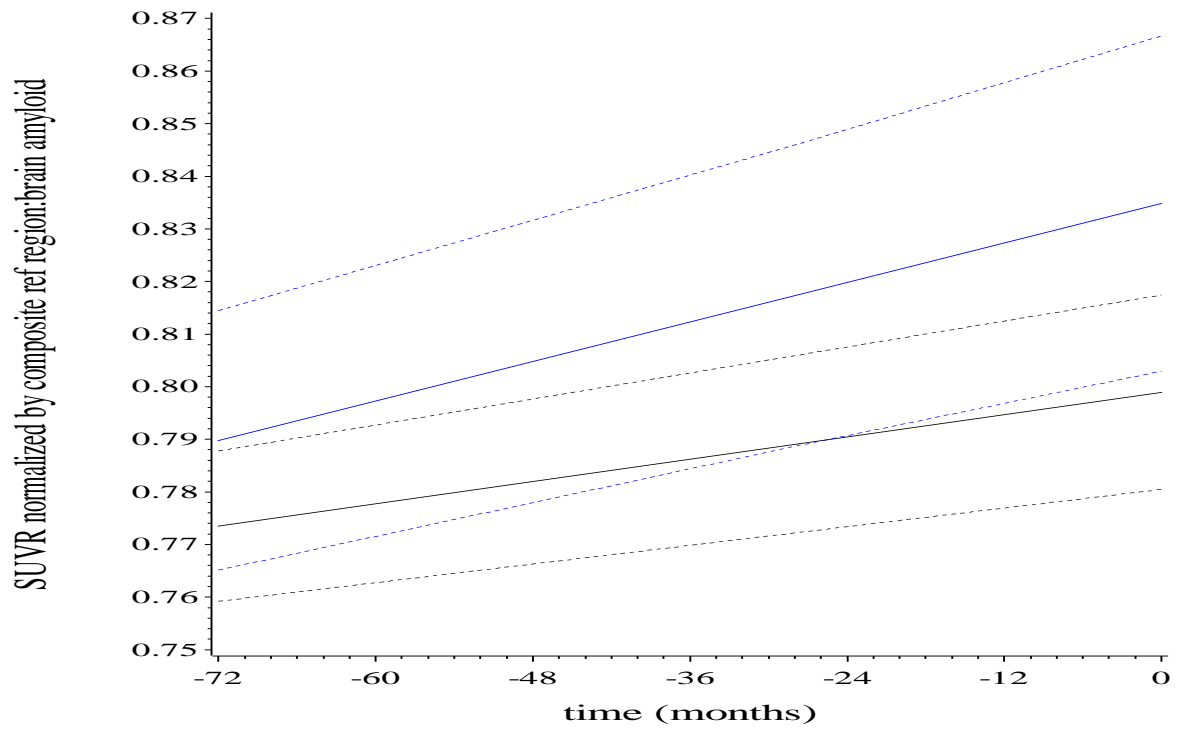
731 SUVr, standard uptake value ratio (brain A $\beta$  load)

732 Results from mixed linear regression models (mean estimate and 95% CI)

733







Omega-3 index assessed at time 0:

— 3 highest quartiles	- - - - - lower 95% CI	- - - - - upper 95% CI
— lowest quartile	- - - - - lower 95% CI	- - - - - upper 95% CI

**Table 1.** Population characteristics, cognitive, brain imaging and biomarkers variables according to  $\omega$ -3 index status (cross-sectional analysis)

	<b>Total</b>	<b>Lowest <math>\omega</math>-3 index quartile (<math>\leq 3.70\%</math>)</b>	<b>3 highest <math>\omega</math>-3 index quartiles (<math>&gt; 3.70\%</math>)</b>	<b>unadjusted p-value</b>	<b>adjusted p-value</b>
	<b>n = 832</b>	<b>n = 209</b>	<b>n = 623</b>		
	<b>mean (SD)<sup>1</sup></b>	<b>mean (SD)<sup>1</sup></b>	<b>mean (SD)<sup>1</sup></b>		
Female sex	423 (50.8%)	109 (52.2%)	314 (50.4%)	0.661 <sup>4</sup>	-
Age (years)	74.0 (7.9)	73.4 (8.6)	74.3 (7.7)	0.200 <sup>5</sup>	-
Education (years)	16.5 (2.5)	15.8 (2.4)	16.8 (2.4)	<b>&lt;0.0001<sup>6</sup></b>	-
Body mass index (kg/m <sup>2</sup> ) (n = 822)	27.3 (5.2)	27.8 (5.6)	27.1 (5.1)	0.077 <sup>5</sup>	-
Cognitive status					-
Cognitively normal	465 (55.9%)	117 (56.0%)	348 (55.9%)	0.682 <sup>4</sup>	
Mild cognitive impairment	272 (32.7%)	65 (31.1%)	207 (33.2%)		
Alzheimer's disease	95 (11.4%)	27 (12.9%)	68 (10.9%)		
ApoE $\epsilon$ 4 genotype carriers (n = 794)	299 (37.7%)	69 (34.2%)	230 (38.9%)	0.235 <sup>4</sup>	
History of cardiovascular disease (n=820)	573 (69.9%)	141 (68.5%)	432 (70.4%)	0.605 <sup>4</sup>	-

**Cognitive tests**

Composite cognitive score <sup>2</sup> (n=801)	0.00 (3.31)	-0.13 (3.29)	0.04 (3.31)	0.534 <sup>5</sup>	0.882
MMSE score (n = 828)	27.8 (3.0)	27.9 (2.9)	27.8 (3.1)	0.907 <sup>6</sup>	0.350
ADAS-Cog delayed recall (n=831)	3.9 (2.6)	3.8 (2.5)	3.9 (2.6)	0.987 <sup>6</sup>	0.552
Total ADAS-Cog (n=823) <sup>3</sup>	17.2 (8.6)	17.5 (8.7)	17.1 (8.6)	0.465 <sup>5</sup>	0.968
Wechsler Memory Scale-delayed recall (n = 826)	10.5 (5.6)	10.0 (5.5)	10.7 (5.6)	0.109 <sup>5</sup>	0.293
Wechsler Memory Scale-immediate recall (n = 828)	12.1 (5.2)	11.8 (5.0)	12.2 (5.2)	0.349 <sup>5</sup>	0.949
Category Fluency test (words) (n = 829)	19.4 (6.1)	18.8 (5.4)	19.6 (6.3)	0.089 <sup>5</sup>	0.574
TMT-A (seconds) (n = 825) <sup>3</sup>	37.4 (21.2)	38.1 (21.1)	37.2 (21.3)	0.351 <sup>5</sup>	0.513
TMT-B (seconds) (n = 808) <sup>3</sup>	97.4 (62.9)	98.5 (63.5)	97.0 (62.8)	0.714 <sup>5</sup>	0.768
MoCA total score (n = 810)	24.2 (4.5)	24.0 (4.3)	24.2 (4.5)	0.508 <sup>5</sup>	0.819
CDR sum of boxes (n = 829)	1.15 (2.05)	1.18 (2.09)	1.15 (2.04)	0.759 <sup>6</sup>	0.995
CDR score > 0 (n=829)	362 (43.7)	93 (25.7)	269 (74.3)	0.780 <sup>4</sup>	0.892

**Brain imaging and biomarkers variables**

Total hippocampal volume (mm <sup>3</sup> ) (n=651)	7168.8 (1090.8)	7203.7 (1034.1)	7156.9 (1110.1)	0.634 <sup>5</sup>	0.699
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Left hippocampal subcortical volume (mm <sup>3</sup> )	3525.2 (546.5)	3547.3 (515.7)	3517.7 (556.8)	0.548 <sup>5</sup>	0.504
Right hippocampal subcortical volume (mm <sup>3</sup> )	3643.5 (571.2)	3656.4 (546.8)	3639.2 (579.7)	0.739 <sup>5</sup>	0.933
White matter hyperintensities volume (cm <sup>3</sup> ) (n=773) <sup>3</sup>	5.6 (10.6)	5.1 (11.4)	5.7 (10.4)	0.294 <sup>5</sup>	0.422
Cortical SUVR (n=472) <sup>3</sup>	1.18 (0.24)	1.18 (0.24)	1.18 (0.24)	0.896 <sup>5</sup>	0.561
Amyloid status (n=472)					
A $\beta$ positive (SUVR > 1.11)	218 (46.2%)	57 (44.2%)	161 (46.9%)		
A $\beta$ negative (SUVR $\leq$ 1.11)	254 (53.8%)	72 (55.8%)	182 (53.1%)	0.593 <sup>4</sup>	0.837
Tau <sup>18</sup> F-AV1451 meta-temporal region (n=693) <sup>3</sup>	1.93 (0.51)	1.94 (0.51)	1.93 (0.52)	0.761 <sup>5</sup>	0.947
Tau <sup>18</sup> F-AV1451 Braak stage 1 (n=693) <sup>3</sup>	2.19 (0.71)	2.18 (0.69)	2.19 (0.72)	0.955 <sup>5</sup>	0.812
Tau <sup>18</sup> F-AV1451 Braak stage 2 (n=693)	1.46 (0.27)	1.46 (0.27)	1.46 (0.27)	0.819 <sup>5</sup>	0.650
Tau <sup>18</sup> F-AV1451 Braak stages 3 and 4 (n=693) <sup>3</sup>	1.76 (0.39)	1.77 (0.39)	1.76 (0.39)	0.727 <sup>5</sup>	0.919
Tau <sup>18</sup> F-AV1451 Braak stages 5 and 6 (n=693)	1.79 (0.37)	1.82 (0.43)	1.78 (0.34)	0.285 <sup>5</sup>	0.806

A $\beta$ , amyloid- $\beta$ ; ADAS, Alzheimer's disease assessment scale; ApoE, apolipoprotein E; CDR, Clinical Dementia Rating; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SD, standard deviation; SUVR, standard uptake value ratio normalized by whole cerebellum; <sup>1</sup>except otherwise specified; <sup>2</sup>The modified composite score included the delayed word recall from the Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-Cog), the Trail Making Test B (TMT-B), the MMSE total score and the Wechsler Memory Scale delayed recall;  $\omega$ -3 index

defined as erythrocyte % docosahexaenoic acid (DHA) + % eicosapentaenoic acid (EPA); p-values based on Student's T-test <sup>5</sup>, Kruskal-Wallis test <sup>6</sup> or Chi-square test <sup>4</sup>, after log transformation when needed; Significant p-values are presented in bold, and marginally significant p-values are presented in italics; <sup>3</sup>variables with log transformation; Analyses adjusted for age, education, ApoE ε4 genotype, history of cardiovascular disease, body mass index and total intracranial volume (the last one only for white matter hyperintensities and hippocampal volume) with a linear regression model for the quantitative outcomes and with a logistic regression model for the qualitative outcomes.

**Table 2.** Interactions between  $\omega$ -3 index (lowest vs. 3 highest quartiles) and ApoE  $\epsilon$ 4 genotype (carriers vs. non-carriers) on cognitive, brain imaging and biomarkers variables among community-dwelling older adults (cross-sectional analysis)

	n	ApoE $\epsilon$ 4 non-carrier	$\omega$ -3 index x		
			ApoE $\epsilon$ 4 carrier		
			$\beta$	SE	p-value
<b>Model 1 (unadjusted)</b>					
Composite cognitive score	764	Ref.	-0.92	0.56	0.104
MMSE score	790	Ref.	-0.37	0.52	0.479
Total ADAS-Cog <sup>1</sup>	785	Ref.	0.02	0.08	0.758
ADAS-Cog delayed recall	793	Ref.	0.39	0.44	0.373
Wechsler Memory Scale-Delayed recall	788	Ref.	<b>-2.07</b>	<b>0.94</b>	<b>0.028</b>
Wechsler Memory Scale-Immediate recall	790	Ref.	-1.49	0.87	0.088
Category Fluency test (words)	791	Ref.	-0.21	1.04	0.836
TMT-A (seconds) <sup>1</sup>	787	Ref.	0.02	0.07	0.769
TMT-B (seconds) <sup>1</sup>	771	Ref.	0.08	0.09	0.366

MoCA total score	775	Ref.	-0.23	0.76	0.764
CDR sum of boxes	791	Ref.	0.32	0.34	0.346
Total hippocampal volume (mm <sup>3</sup> )	624	Ref.	-62.43	210.92	0.767
Left hippocampal subcortical volume (mm <sup>3</sup> )	624	Ref.	-8.00	105.81	0.940
Right hippocampal subcortical volume (mm <sup>3</sup> )	624	Ref.	-54.43	110.34	0.622
White matter hyperintensities volume (cm <sup>3</sup> ) <sup>1</sup>	736	Ref.	0.15	0.28	0.591
Cortical SUVR <sup>1</sup>	459	Ref.	0.02	0.04	0.608
Tau 18F-AV1451 meta-temporal region <sup>1</sup>	660	Ref.	<b>0.10</b>	<b>0.04</b>	<b>0.010</b>
Tau 18F-AV1451 Braak stage 1 <sup>1</sup>	660	Ref.	<b>0.12</b>	<b>0.05</b>	<b>0.019</b>
Tau 18F-AV1451 Braak stage 2	660	Ref.	<b>0.10</b>	<b>0.05</b>	<b>0.038</b>
Tau 18F-AV1451 Braak stages 3 and 4 <sup>1</sup>	660	Ref.	<b>0.09</b>	<b>0.03</b>	<b>0.009</b>
Tau 18F-AV1451 Braak stages 5 and 6	660	Ref.	<b>0.17</b>	<b>0.07</b>	<b>0.011</b>
<b>Model 2 (adjusted)</b>					
Composite cognitive score	745	Ref.	-0.92	0.53	0.081
MMSE score	769	Ref.	-0.35	0.51	0.494
Total ADAS-Cog <sup>1</sup>	765	Ref.	0.05	0.07	0.543



ADAS-Cog delayed recall	773	Ref.	0.47	0.42	0.267
Wechsler Memory Scale-Delayed recall	769	Ref.	<b>-2.09</b>	<b>0.91</b>	<b>0.022</b>
Wechsler Memory Scale-Immediate recall	771	Ref.	<i>-1.47</i>	<i>0.84</i>	<i>0.081</i>
Category Fluency test (words)	771	Ref.	-0.47	0.97	0.630
TMT-A (seconds) <sup>1</sup>	767	Ref.	0.04	0.07	0.560
TMT-B (seconds) <sup>1</sup>	751	Ref.	0.09	0.08	0.263
MoCA total score	754	Ref.	-0.33	0.72	0.652
CDR sum of boxes	770	Ref.	0.38	0.34	0.263
Total hippocampal volume (mm <sup>3</sup> )	608	Ref.	-85.33	182.39	0.640
Left hippocampal subcortical volume (mm <sup>3</sup> )	608	Ref.	-13.10	93.45	0.889
Right hippocampal subcortical volume (mm <sup>3</sup> )	608	Ref.	-72.24	95.04	0.448
White matter hyperintensities (cm <sup>3</sup> ) <sup>1</sup>	718	Ref.	0.18	0.25	0.478
Cortical SUVR <sup>1</sup>	454	Ref.	0.01	0.04	0.783
Tau <sup>18</sup> F-AV1451 meta-temporal region <sup>1</sup>	648	Ref.	<b>0.09</b>	<b>0.04</b>	<b>0.023</b>
Tau <sup>18</sup> F-AV1451 Braak stage 1 <sup>1</sup>	648	Ref.	<b>0.11</b>	<b>0.05</b>	<b>0.023</b>
Tau <sup>18</sup> F-AV1451 Braak stage 2	648	Ref.	<i>0.09</i>	<i>0.05</i>	<i>0.060</i>
Tau <sup>18</sup> F-AV1451 Braak stages 3 and 4 <sup>1</sup>	648	Ref.	<b>0.08</b>	<b>0.03</b>	<b>0.022</b>

Tau <sup>18</sup> F-AV1451 Braak stages 5 and 6	648	Ref.	<b>0.14</b>	<b>0.07</b>	<b>0.040</b>
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ADAS, Alzheimer's disease assessment scale; CDR, Clinical Dementia Rating; MoCA, Montreal Cognitive Assessment; MMSE, Mini Mental State Examination; TMT, Trail Making Test; SE, standard error; SUV<sub>r</sub>, standard uptake value ratio normalized by whole cerebellum. The modified composite score included the delayed word recall from the Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-Cog), the Trail Making Test B (TMT-B), the MMSE total score and the Wechsler Memory Scale delayed recall; ω-3 index defined as erythrocyte % docosahexaenoic acid (DHA) + % eicosapentaenoic acid (EPA). Model 2 was adjusted for age, education, history of cardiovascular disease, body mass index and total intracranial volume (this last only for white matter hyperintensities and hippocampal volume); <sup>1</sup>Variable with log transformation; Significant p-values are presented in bold, and marginally significant p-values are presented in italics. Results from linear regression models.

**Table 3.** Associations between  $\omega$ -3 index (lowest vs. 3 highest quartiles) and cognitive and brain biomarkers variables among community-dwelling older adults ApoE  $\epsilon$ 4 carriers and non-carriers (within-group estimates according to the interaction between  $\omega$ -3 index and ApoE  $\epsilon$ 4 status presented in Table 2) (cross-sectional analysis)

	ApoE $\epsilon$ 4 non-carriers			ApoE $\epsilon$ 4 carriers		
	$\beta$	SE	p-value	$\beta$	SE	p-value
<b>Model 1 (unadjusted)</b>						
Wechsler Memory Scale-Delayed recall	-0.22	0.56	0.699	<b>-2.29</b>	<b>0.76</b>	<b>0.003</b>
Wechsler Memory Scale-Immediate recall	0.01	0.52	0.985	<b>-1.48</b>	<b>0.70</b>	<b>0.035</b>
Tau 18F-AV1451 meta-temporal region <sup>1</sup>	-0.03	0.02	0.294	<b>0.08</b>	<b>0.03</b>	<b>0.014</b>
Tau 18F-AV1451 Braak stage 1 <sup>1</sup>	-0.04	0.03	0.236	<b>0.08</b>	<b>0.04</b>	<b>0.040</b>
Tau 18F-AV1451 Braak stage 2	-0.04	0.03	0.196	0.06	0.04	0.105
Tau 18F-AV1451 Braak stages 3 and 4 <sup>1</sup>	-0.02	0.02	0.306	<b>0.07</b>	<b>0.03</b>	<b>0.013</b>
Tau 18F-AV1451 Braak stages 5 and 6	-0.02	0.04	0.686	<b>0.15</b>	<b>0.05</b>	<b>0.004</b>

**Model 2 (adjusted)**

Wechsler Memory Scale-Delayed recall	0.28	0.55	0.613	<b>-1.81</b>	<b>0.73</b>	<b>0.014</b>
Wechsler Memory Scale-Immediate recall	0.55	0.51	0.279	-0.92	0.68	0.178
Tau 18F-AV1451 meta-temporal region <sup>1</sup>	-0.03	0.02	0.192	<i>0.06</i>	<i>0.03</i>	<i>0.066</i>
Tau 18F-AV1451 Braak stage 1 <sup>1</sup>	-0.04	0.03	0.242	<i>0.08</i>	<i>0.04</i>	<i>0.052</i>
Tau 18F-AV1451 Braak stage 2	-0.02	0.03	0.448	<i>0.06</i>	<i>0.04</i>	<i>0.078</i>
Tau 18F-AV1451 Braak stages 3 and 4 <sup>1</sup>	-0.03	0.02	0.198	<i>0.05</i>	<i>0.03</i>	<i>0.061</i>
Tau 18F-AV1451 Braak stages 5 and 6	-0.04	0.04	0.303	<i>0.09</i>	<i>0.05</i>	<i>0.076</i>

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SE, standard error;  $\omega$ -3 index defined as erythrocyte % docosahexaenoic acid (DHA) + % eicosapentaenoic acid (EPA). Model 2 was adjusted for age, education, history of cardiovascular disease, body mass index and total intracranial volume (this last only for white matter hyperintensities and hippocampal volume); <sup>1</sup>Variable with log transformation; Significant p-values are presented in bold, and marginally significant p-values are presented in italics. Results from linear regression models.

**Table 4.** Differences in retrospective change of cognitive, brain imaging and biomarkers variables between the lowest  $\omega$ -3 index quartile and the 3 highest quartiles (longitudinal analysis)

Outcome	Time	Lowest $\omega$ -3 index quartile		3 highest $\omega$ -3 index quartiles			Lowest vs highest $\omega$ -3 index quartiles			
		unadjusted		unadjusted			adjusted <sup>1</sup>			
		N	$\beta$	95% CI	N	$\beta$	95% CI	$\beta$	95% CI	p-value
Composite cognitive score	M0	83			250					
	M0 - M-24	44	-0.88	[-1.28, -0.47]	120	-0.68	[-0.91, -0.44]	-0.19	[-0.67, 0.29]	0.436
	M0 - M-36	31	-1.25	[-1.82, -0.68]	94	-0.95	[-1.28, -0.62]	-0.27	[-0.93, 0.39]	0.426
	M0 - M-60	34	-1.87	[-2.68, -1.05]	125	-1.37	[-1.85, -0.90]	-0.40	[-1.33, 0.54]	0.408
	M0 - M-120	14	-2.66	[-3.84, -1.48]	28	-1.69	[-2.37, -1.00]	-0.53	[-1.90, 0.83]	0.440
	Whatever time									0.701
Total MMSE	M0	88			259					
	M0 - M-24	46	-0.47	[-0.76, -0.18]	127	-0.43	[-0.59, -0.26]	0.00	[-0.34, 0.35]	0.985
	M0 - M-36	34	-0.68	[-1.09, -0.27]	97	-0.60	[-0.84, -0.37]	-0.01	[-0.49, 0.48]	0.977
	M0 - M-60	52	-1.04	[-1.62, -0.47]	167	-0.90	[-1.23, -0.56]	-0.05	[-0.74, 0.63]	0.880
	M0 - M-120	18	-1.64	[-2.36, -0.92]	35	-1.24	[-1.65, -0.82]	-0.31	[-1.15, 0.53]	0.471
	Whatever time									0.379
<u>Total ADAS-Cog<sup>2</sup></u>	M0	88			257					

	M0 - M-24	44	0.32	[0.25, 0.38]	127	0.43	[0.39, 0.47]	-0.10	[-0.19, -0.02]	<b>0.017</b>
	M0 - M-36	34	0.40	[0.32, 0.47]	97	0.52	[0.48, 0.57]	-0.12	[-0.21, -0.02]	<b>0.018</b>
	M0 - M-60	52	0.45	[0.38, 0.53]	167	0.57	[0.52, 0.61]	-0.09	[-0.19, 0.01]	0.073
	M0 - M-120	18	0.58	[0.43, 0.72]	35	0.50	[0.42, 0.58]	0.04	[-0.16, 0.24]	0.689
	Whatever time									0.092
	M0	88			261					
ADAS-Cog delayed recall	M0 - M-24	46	0.14	[-0.06, 0.35]	127	0.29	[0.18, 0.41]	-0.11	[-0.35, 0.14]	0.398
	M0 - M-36	34	0.22	[-0.06, 0.50]	97	0.41	[0.25, 0.57]	-0.14	[-0.47, 0.19]	0.400
	M0 - M-60	52	0.39	[0.01, 0.77]	167	0.60	[0.38, 0.82]	-0.18	[-0.64, 0.27]	0.421
	M0 - M-120	18	0.89	[0.23, 1.55]	35	0.75	[0.36, 1.14]	-0.11	[-0.92, 0.70]	0.787
	Whatever time									0.700
	M0	87			258					
Wechsler Memory Scale-Delayed recall	M0 - M-24	46	-0.73	[-1.18, -0.27]	123	-0.19	[-0.45, 0.08]	-0.61	[-1.13, -0.08]	<b>0.024</b>
	M0 - M-36	34	-0.98	[-1.60, -0.35]	94	-0.21	[-0.57, 0.16]	-0.84	[-1.55, -0.13]	<b>0.021</b>
	M0 - M-60	36	-1.25	[-2.12, -0.38]	125	-0.08	[-0.59, 0.42]	-1.18	[-2.16, -0.19]	<b>0.019</b>
	M0 - M-120	14	-0.62	[-2.17, 0.93]	29	1.13	[0.21, 2.05]	-1.23	[-3.05, 0.58]	0.180
	Whatever time									0.066

	M0	87			260					
	M0 - M-24	46	-0.55	[-0.97, -0.14]	124	-0.11	[-0.35, 0.13]	-0.43	[-0.92, 0.05]	0.079
	M0 - M-36	34	-0.77	[-1.34, -0.20]	95	-0.12	[-0.45, 0.21]	-0.62	[-1.29, 0.04]	0.065
Wechsler Memory Scale-Immediate recall	M0 - M-60	36	-1.10	[-1.90, -0.30]	125	-0.02	[-0.49, 0.44]	-0.96	[-1.88, -0.04]	<b>0.042</b>
	M0 - M-120	14	-1.26	[-2.55, 0.03]	29	0.81	[0.05, 1.56]	-1.52	[-3.04, 0.01]	0.052
	Whatever time									0.097
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	M0	88			259					
	M0 - M-24	46	-0.52	[-0.78, -0.26]	125	-0.45	[-0.60, -0.30]	-0.07	[-0.38, 0.24]	0.644
	M0 - M-36	34	-0.78	[-1.17, -0.39]	96	-0.68	[-0.91, -0.45]	-0.11	[-0.58, 0.36]	0.644
Category Fluency test (words)	M0 - M-60	52	-1.30	[-1.95, -0.65]	167	-1.13	[-1.51, -0.74]	-0.18	[-0.96, 0.59]	0.644
	M0 - M-120	18	-2.61	[-3.90, -1.31]	35	-2.25	[-3.02, -1.49]	-0.37	[-1.92, 1.19]	0.644
	Whatever time									0.644
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	M0	88			258					
	M0 - M-24	46	0.05	[0.02, 0.09]	124	0.03	[0.01, 0.05]	0.02	[-0.02, 0.06]	0.354
	M0 - M-36	34	0.07	[0.02, 0.12]	95	0.05	[0.02, 0.08]	0.02	[-0.03, 0.08]	0.395
TMT-A (seconds) <sup>2</sup>	M0 - M-60	52	0.09	[0.03, 0.16]	167	0.07	[0.03, 0.11]	0.02	[-0.05, 0.10]	0.529
	M0 - M-120	18	0.07	[-0.03, 0.16]	35	0.10	[0.04, 0.15]	-0.03	[-0.14, 0.09]	0.637

	Whatever time									0.352
	M0	84			254					
	M0 - M-24	44	0.09	[0.05, 0.14]	121	0.06	[0.04, 0.09]	0.03	[-0.02, 0.08]	0.287
	M0 - M-36	32	0.13	[0.07, 0.19]	96	0.09	[0.06, 0.13]	0.03	[-0.03, 0.10]	0.329
TMT-B (seconds) <sup>2</sup>	M0 - M-60	50	0.19	[0.11, 0.27]	167	0.14	[0.10, 0.19]	0.03	[-0.06, 0.13]	0.467
	M0 - M-120	18	0.25	[0.14, 0.35]	33	0.22	[0.16, 0.28]	-0.04	[-0.17, 0.09]	0.558
	Whatever time									0.206
	M0	87			257					
	M0 - M-24	46	-0.74	[-1.17, -0.31]	123	-0.51	[-0.76, -0.26]	-0.35	[-0.82, 0.11]	0.137
MoCA total score	M0 - M-36	34	-1.02	[-1.58, -0.46]	96	-0.68	[-1.00, -0.36]	-0.47	[-1.07, 0.13]	0.122
	M0 - M-60	51	-1.41	[-2.10, -0.72]	165	-0.85	[-1.25, -0.45]	-0.60	[-1.36, 0.17]	0.127
	Whatever time									0.288
	M0	88			259					
	M0 - M-24	46	0.53	[0.31, 0.74]	125	0.38	[0.25, 0.51]	0.20	[-0.06, 0.45]	0.134
CDR sum of boxes	M0 - M-36	35	0.68	[0.39, 0.97]	95	0.49	[0.33, 0.66]	0.23	[-0.11, 0.58]	0.180
	M0 - M-60	52	0.83	[0.43, 1.23]	166	0.62	[0.39, 0.86]	0.22	[-0.24, 0.69]	0.349
	M0 - M-120	17	1.12	[0.60, 1.63]	35	0.85	[0.55, 1.15]	0.03	[-0.57, 0.63]	0.927



	Whatever time									0.101
	M0	77			226					
	M0 - M-24	43	-0.33	[-0.54, -0.11]	117	-0.34	[-0.46, -0.21]	0.00	[-0.23, 0.24]	0.970
White matter hyperintensities (cm <sup>3</sup> ) <sup>2</sup>	M0 - M-36	24	-0.25	[-0.46, -0.03]	52	-0.30	[-0.42, -0.18]	0.06	[-0.18, 0.29]	0.638
	M0 - M-60	36	-0.03	[-0.22, 0.15]	115	-0.13	[-0.24, -0.03]	0.11	[-0.09, 0.32]	0.279
	Whatever time									0.287
	M0	74			215					
	M0 - M-24	44	0.02	[0.01, 0.02]	120	0.01	[0.01, 0.01]	0.01	[0.00, 0.01]	<b>0.003</b>
Cortical SUVR (brain A $\beta$ load)	M0 - M-36	24	0.02	[0.02, 0.03]	71	0.01	[0.01, 0.02]	0.01	[0.00, 0.02]	<b>0.003</b>
	M0 - M-60	34	0.04	[0.03, 0.05]	77	0.02	[0.02, 0.03]	0.02	[0.01, 0.03]	<b>0.003</b>
	Whatever time									<b>0.003</b>

<sup>1</sup>Adjustments: age (years), Education participant (years), apoE4 (yes vs no), History of cardiovascular disease (yes vs no), BMI (Kg/m<sup>2</sup>) + Intra-Cranial Volume (ICV only for WMH) and their interaction with time; ADAS, Alzheimer's disease assessment scale; CDR, Clinical Dementia Rating; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SUVR, standard uptake value ratio. Results from mixed linear regression models. <sup>2</sup>variable with log transformation;

**Table 5.** Studies exploring erythrocyte omega-3 index (% of DHA+EPA from total lipids in erythrocyte membranes) and cognitive outcomes.

<b>Publication</b>	<b>Population</b>	<b>Study design</b>	<b>Cognitive outcomes / Main findings</b>
Amen et al., 2017 (54)	166 individuals from a psychiatric referral clinical	Cross-sectional	Neurocognitive status assessed by computerized testing (WebNeuro). / Omega-3 index correlated to the feeling subsection of the WebNeuro ( $r = 0.25$ , $p = 0.01$ ).
Ammann et al., 2017 (55)	6,706 women $\geq 65$ years, participants of the Women's Health Initiative Memory Study (WHIMS)	Prospective observational study	Risk of dementia. / After adjusting for demographic, clinical, and behavioral risk factors, 1 SD increase in omega-3 index was associated with a lower risk of dementia (HR = 0.92, 95% CI: 0.84, 1.00; $p < 0.05$ ).
Bigornia et al., 2018 (56)	1,032 subjects (45–75 years)	Prospective observational study	Verbal fluency, executive function, memory, global cognitive function. / Erythrocyte omega-3 index and omega-3 very-long-chain fatty acids (EPA+DHA+DPA) were associated with better executive function (P-trend $< 0.02$ ).
Coley et al., 2018 (4)	724 older adults ( $\geq 70$ years) participants of the MAPT Study	Secondary exploratory analysis of a RCT with	Memory, lexical fluency, attention, executive function. / Participants receiving placebo in the lowest quartile of baseline omega-3 index ( $\leq 4.83\%$ ) underwent more 3-year cognitive decline than the other

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		omega-3 supplementation	quartiles (mean composite cognitive score difference 0.14, 95%CI [0.00, 0.28], p=0.048).
Cook et al., 2017 (57)	299 healthy women (18-35 years)	Cross-sectional	Impulsivity, attention, information processing, memory and executive function. / Adjustment for omega-3 index weakened the interaction effect between BMI and cognitive domains.
Cook et al., 2019 (58)	299 healthy women (18-35 years)	Cross-sectional	Impulsivity, attention, information processing, memory and executive function. / Participants in the low omega-3 index tertile group scored significantly lower on attention than the other tertile groups in adjusted analysis.
Coro et al., 2022 (59)	76 Australian survivors of breast and colorectal cancer mean age 57.5 ( $\pm$ 10.2) years	Cross-sectional	Visual memory, learning, visuospatial memory, executive function, attention, short-term visual memory, sustained attention, executive function, working memory. / Omega-3 index was not associated with cognitive outcomes.
Dretsch et al., 2014 (60)	78 American service members deployed to Iraq (18-55 years)	RCT with omega-3 supplementation	Reaction time, processing speed, cognitive flexibility, executive functioning, verbal memory, working memory. / Changes in omega-3 index were not associated with changes in cognitive outcomes.

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Erhardt et al., 2021 (61)	142 Australian community-dwelling older adults (60-85 years)	Cross-sectional	Memory, language, executive function, visuospatial skills. / Erythrocyte omega-3 index was not associated with cognitive performance.
Johnston et al., 2013 (62)	78 American service members deployed to Iraq (20-54 years)	Cross-sectional	Processing speed, complex attention, reaction time, cognitive flexibility, and executive function. / The omega-3 index was positively associated with cognitive flexibility ( $p < 0.02$ ) and executive function ( $p=0.01$ ).
Lukaschek et al., 2016 (63)	720 older adults (68-92 years)	Cross-sectional	Cognitive status. / In model adjusted by sex and age, subjects with a low omega-3 index were at a significantly higher risk for cognitive impairment (OR: 1.77, 95% CI: 1.15-2.73, $p = 0.009$ ). The association remained stable after further adjusting for educational level, metabolic risk factors and affective disorders.
Tan et al., 2012 (5)	1,575 individuals aged $67 \pm 9$ years	Cross-sectional	Visual memory, executive function, abstract thinking. / Participants with lower omega-3 index (Q1 vs Q2-4) had lower scores on tests of visual memory ( $\beta \pm SE = -0.47 \pm 0.18$ ; $p = 0.008$ ), executive function ( $\beta \pm SE = -0.07 \pm 0.03$ ; $p = 0.004$ ), and abstract thinking ( $\beta \pm SE = -0.52 \pm 0.18$ ; $p = 0.004$ ).

Thomas et al., 2020 (2) <sup>1</sup>	1,279 older adults from the Three-City (3C) Study	Prospective observational study	Risk of dementia, global cognition, memory. / Higher levels of plasma omega-3 index were consistently associated with a lower risk of dementia (HR for 1 SD = 0.87, 0.76-0.98), and a lower decline in global cognition (p = 0.04 for change over time) and memory (p = 0.06).
Witte et al., 2014 (64)	65 subjects (50-75 years)	RCT with omega-3 supplementation	Executive function, memory performance, sensorimotor speed, attention. / Changes in omega-3-index correlated positively with improvements in executive functions.

BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; MAPT, Multidomain Alzheimer Preventive Trial; OR, odds ratio; RCT, randomized controlled trial; SD, standard deviation. <sup>1</sup>This study analyzed omega-3 index in plasma, not in erythrocytes.

