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Title: Blood polyunsaturated omega-3 fatty acids, brain atrophy, cognitive decline and dementia risk

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Declaration of interest:

A. Thomas, M. Baillet, C. Proust-Lima, C. Helmer, G. Catheline, and C. Samieri report no conflict of interest. A. Foubert-Samier received fees from LVL medical. C. Féart received fees for conferences from Danone Research and Nutricia.

ABSTRACT

INTRODUCTION: We searched for consistent associations of an omega-3 index in plasma (sum of eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) with several dementia-related outcomes in a large cohort of older adults.

METHODS: We included 1279 participants from the Three-City study, non-demented at the time of blood measurements at baseline, with face-to-face neuropsychological assessment and systematic detection of incident dementia over a 17-year follow-up. An ancillary study included 467 participants with up to three repeated brain imaging exams over 10 years.

RESULTS: In multivariable models, higher levels of plasma EPA+DHA were consistently associated with a lower risk of dementia (hazard ratio for 1 standard deviation = 0.87 [95% confidence interval, 0.76-0.98]), and a lower decline in global cognition (P = .04 for change over time), memory (P = .06) and medial temporal lobe volume (P = .02).

DISCUSSION: This prospective study provides compelling evidence for a relationship between long-chain omega-3 fatty acids levels and lower risks for dementia and related outcomes.

Key words: Eicosapentaenoic acid; Docosahexaenoic acid; Dementia; Cognitive decline; Atrophy; Magnetic Resonance Imaging; Prospective studies; Risk factors in epidemiology

1. Introduction

The decreasing incidence of dementia recently observed in many countries, collectively attributed to a general improvement of educational level and health risk factors in the last decades, has provided empiric demonstration that dementia may be efficiently preventable [1]. Nutrition has raised interest for dementia prevention, and the two long-chain omega-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), mainly provided together by fish intake in the human diet, are promising candidates. Indeed, DHA represents up to 60% of lipids incorporated in neural membranes and is implicated in many aspects of the neuronal machinery, including synaptic plasticity and hippocampal neurogenesis [2]. Both EPA and DHA are involved in anti-inflammatory pathways that may preserve brain vasculature and counteract neuro-inflammation in cognitive aging and dementia [2,3].

Epidemiological studies reported associations between higher fish or long-chain n-3 PUFA intakes, or higher blood concentrations of n-3 PUFA, and lower cognitive decline [4,5], lower risk of dementia [6–8] or preserved brain structure at brain imaging [9,10]. However, evidence has been inconsistent according to the type (e.g., EPA, DHA or total n-3 PUFA) and/or the exposures (intake versus blood status) investigated [11–13]. Among limitations of previous research, including our own work [5,8,9], were prospective designs of moderate duration (< 10 years) [11,13], and a relative heterogeneity in the studied outcomes.

Long-chain n-3 PUFA, EPA and DHA, share complementary neuroprotective properties and may collectively contribute to lower neurodegeneration and maintain cognitive functioning during aging. In this study, we took advantage of the long follow-up for dementia and related outcomes in a large cohort of older persons, the Three-City (3C) study, to look for robust associations of the combination of EPA and DHA, as represented by a plasma omega-3 index (EPA+DHA), with the long-term evolution of three complementary outcomes over up to 17

years: incidence of dementia, cognitive decline and atrophy of the medial temporal lobe (MTL, a biomarker of dementia [14]).

2. Methods

2.1. Study population

The 3C study is a population-based prospective cohort initiated in 1999-2000, including 9294 non-institutionalized community dwellers aged 65 years or older from three French cities (Bordeaux n = 2104, Dijon n = 4931, and Montpellier n = 2259) [15]. At baseline, data were collected by face-to-face interviews and included sociodemographic, lifestyle and medical information; a brief food frequency questionnaire; anthropometric and blood pressure measurements; neuropsychological testing; and blood sampling. Seven follow-up visits, with repeated cognitive evaluations through a battery of neurocognitive tests, were performed at home every 2 to 3 years until 2018.

In Bordeaux, 1811 participants were included in a comprehensive nutritional survey, and 1416 of them had plasma fatty acids measured at baseline (**Figure S1 in supporting information**). We excluded 137 individuals with dementia at baseline, leaving 1279 participants for the analysis of dementia risk. Among them, 94 participants had incomplete cognitive battery at baseline (required for computation of composite cognitive scores over follow-up), leaving 1185 participants for the analysis of cognitive decline (n = 1245 for memory). Moreover, among the 1279 dementia-free individuals at baseline, 459 were included in an ancillary brain imaging study. Three Magnetic Resonance Imaging (MRI) exams were performed in 2000-2001, 2004-2006 and 2010-2011. Participants were excluded if they presented major brain pathologies (n = 15; e.g. meningioma or major cerebrovascular pathology) or major acquisition artefacts on MRI scans and post-processing failure (n = 16,

e.g. excessive movements), leading to 467 participants for brain atrophy analyses (57% had >1 MRI).

The protocol of the 3C study was approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research at Kremlin-Bicêtre University Hospital (Paris, France), and all participants provided written informed consent.

2.2. Assessment of cognitive function and diagnosis of dementia

We used incidence of dementia, cognitive decline and MTL atrophy as three co-primary outcomes. For dementia, all participants at baseline, and those suspected of dementia based on their neuropsychological performances at each follow-up visit, were examined by a neurologist to establish a provisional diagnosis. An independent committee of neurologists reviewed all potential cases of dementia to obtain a consensus on the diagnosis according to criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition [16]. Incident cases were adjudicated until year 2013-2014.

For analyses of cognitive decline, we assessed both global cognition and memory through composite scores, as used previously [17]. A global cognitive score was calculated at each repeated visit from baseline (1999-2000) to 2017-2018, as the mean of z-scores of four cognitive tests assessing various cognitive domains: (i) the Mini-Mental State Examination (MMSE) [18], which assesses global cognition; (ii) the Isaacs' Set Test (IST) [19] for verbal semantic fluency; (iii) the Benton Visual Retention Test (BVRT) [20] assessing visual working memory and attention; and (iv) the Trail Making Test A (TMT-A) [21] for executive functioning (see **Supplementary Methods in supporting information** for details). For memory, we calculated a composite memory score defined as the mean of z-scores of the BVRT and a subset of the MMSE (the sum of items related to orientation to time and the three-word recall task [22]).

2.3. Assessment of brain atrophy

The first two MRI exams were performed on a 1.5-T Gyroscan Interra system (Philips Medical System, The Netherlands) while the third one was acquired on a 3-T Achieva (Philips Medical System, The Netherlands). The protocol for MRI acquisition is described in S**upplementary Methods**. FreeSurfer 5.1 software was used for cortical surface reconstruction and for estimation of grey matter volumes for each region of the Destrieux parcellation atlas [23]. MTL volume was defined as the sum of amygdalar, parahippocampal, and hippocampal volumes of both hemispheres.

2.4. Assessment of plasma long-chain ω3 fatty acids

Fasting blood samples were collected at baseline in heparinized vacutainers and centrifuged at 1000 x g for 10 minutes. Plasma fatty acid composition was assessed by separation of isopropyl esters using gas chromatography after lipid extraction from plasma with 5 mL of hexane/isopropanol (3:2, by vol), as previously detailed [8]. We focused our analyses on an omega-3 index in plasma, defined as the sum of the two main long-chain n-3 PUFAs, namely EPA and DHA, expressed as a percentage of total fatty acids.

2.5. Other variables

Sociodemographic and lifestyle variables were derived from baseline evaluation and included age, sex, educational level, smoking status (never, former, current), alcohol consumption (never, former, current), and regular physical activity (defined as practicing a sport or an intensive leisure activity [e.g., hiking] ≥1 hour per week and/or engaging in a more moderate activity [e.g., walking or household] ≥1 hour per day). Apolipoprotein E (*APOE*) ε4 allele carrier status was considered dichotomously (carrying at least one ε4 allele versus no ε4 allele). Vascular risk factors included history of cardiovascular or cerebrovascular disease, hypertension (blood pressure ≥140/90 mmHg, or treated), hypercholesterolemia (plasma total cholesterol ≥6.2 mmol/L, or treated), diabetes (fasting blood glucose ≥7.0 mmol/L, or

treated), and body mass index (BMI, body weight/height² in kg/m²). Depressive symptoms were recorded using the Center for Epidemiologic Studies-Depression (CES-D) scale [24]; high depressive symptoms were defined as a CES-D score ≥17 for men and ≥23 for women, or being too depressed to answer [25].

2.6. Statistical analyses

In statistical models, the plasma omega-3 index EPA+DHA was transformed into a z-score and used as a continuous variable (i.e. for 1 standard deviation [SD] increase of EPA+DHA), to account for the entire continuum of exposure. For descriptive analyses, we categorized EPA+DHA in four categories around mean±1SD.

The association between plasma EPA+DHA and the risk of dementia was estimated by a Cox proportional-hazards model with age as time scale and delayed entry, adjusted for covariates. For analyses of cognitive decline, the trajectories of each cognitive score were estimated using a linear mixed model. We used natural cubic splines to approximate the nonlinear shape of cognitive trajectory with time (see **Supplementary Methods**). Models included: an indicator for the first cognitive assessment; an intercept representing the cognitive score at baseline and the splines functions of time (with corresponding correlated individual random effects); EPA+DHA, covariates, and their interactions with splines' functions of time.

Similarly, we estimated MTL volume change over the three repeated MRI exams with a linear mixed model, but limiting our analysis to linear trajectories according to time since baseline (as only three repeated measures were available, precluding any analysis by more complex functions of time). To account for the change of protocol from a 1.5T to a 3T scanner at the last MRI examination, we added a last visit indicator (identifying a mean difference in volumes measured by the 3T scanner) and a scanner-specific variance for the measurement error (which captures a difference in the uncertainty of the volumes measured by the 3T and

1.5T scanners; the robustness of this strategy had been evaluated in preliminary analyses by comparing the approach to a latent process modeling strategy, specifically developed to handle change in measurement tools in cohort studies [26]; results available upon request). Thus, the regression included: an intercept representing the MTL volume at baseline and the linear function of time (with corresponding random effects); an indicator for the last MRI visit (fixed and independent random effects); EPA+DHA, covariates, and their interactions with time.

2.7. Supplementary analyses

We performed a series of secondary analyses. First, to complement the primary analysis on continuous exposure, we run models using n-3 PUFA levels categorized into quintiles. We also examined the specificity of EPA, DHA and the ratios EPA/Arachidonic Acid (AA) and DHA/AA.

Second, we evaluated the ability of our unique measurement at baseline to reflect longer-term exposures. Although exposures earlier than baseline were not accessible in our cohort, we had repeated information on fish intakes during follow-up; thus we evaluated the ability of the n-3 PUFA plasma measurement at baseline to reflect moderate to long-term fish intakes at each follow-up.

Third, we ran supplementary models: (i) investigating the relation of EPA+DHA to mortality risk, to evaluate the possibility of competing risk by death in interval-censored time-to-event analyses; (ii) taking into account attrition over follow-up, using joint models for cognitive scores or MTL volumes and time to either dropout or death, whichever occurred first [27]; (iii) further adjusting for other dietary factors (fruits, vegetables, legumes and meat consumptions; a Mediterranean diet score); and (iv) testing the interactions with *APOEε*4 status.

In Cox models, the log-linearity hypothesis was assessed using restricted cubic splines [28], and the proportional-hazards assumption was investigated with Schoenfeld residuals. Missing data for covariates were imputed by multiple imputations (using chained equations with fully conditional specification method; M = 5 imputations). Multiple comparisons were not address in this study, which carefully limited the number of tests performed by focusing on three co-primary exposures evaluating different and complementary questions (statistical significance threshold: $\alpha = .05$). Statistical analyses were performed using SAS v9.4 (SAS Institute Inc), and R v3.5.3 (R Foundation).

3. Results

Among the 1279 participants included, the mean age was 74.3 (SD, 4.9) years and the mean plasma EPA+DHA level at baseline was 3.39 (SD, 1.25) % of total fatty acids (**Table 1**). Participants with higher levels of baseline EPA+DHA were more often female, had higher educational level, had higher fish intake, tended to practice more regular physical activity, were less often diabetic, and had slightly better cognitive performance at baseline.

3.1. Long-chain ω3 fatty acids and risk of dementia

A total of 271 participants were diagnosed with dementia after a median follow-up of 9.8 years (range, 0.8 to 14.9 years). The incidence rate of dementia decreased by increasing levels of plasma EPA+DHA (**Table 2**). For example, among participants with low EPA+DHA levels (<2.2% of total fatty acids [i.e. mean-1SD]) the incidence rate of dementia was 2.66 per 100 person-years (95% confidence interval [CI], 1.90; 3.42), versus 1.99 per 100 person-years (1.34; 2.64) for those with high levels (≥4.6% [i.e. mean+1SD]); with an absolute difference of -0.67 per 100 person-years (-1.18; -0.16).

The relationship between EPA+DHA and risk of dementia was log-linear (**Figure S2A in supporting information**). In multivariable analyses, higher plasma EPA+DHA levels were associated with a reduced risk of dementia (**Figure 1**). Each increase of 1SD of EPA+DHA (i.e. 1.25%) was associated with a hazard ratio (HR) of 0.87 (95% CI, 0.76; 0.98) for dementia risk. The trends were similar when examining separately probable/possible Alzheimer's Disease (AD) and vascular/mixed dementia, although power was more limited in these subgroups (adjusted HR = 0.88 [0.76; 1.03] for AD, 0.92 [0.70; 1.21] for vascular/mixed dementia, and 0.86 [0.75; 1.00] for mixed dementia/AD).

3.2. Long-chain ω3 fatty acids and cognitive decline

Cognitive status was evaluated for a maximum of 18.6 years (median follow-up: 11.7 years). A higher plasma EPA+DHA level was significantly associated with a slower global cognitive decline (**Figure 2A**, $P = .04$ for EPA+DHA-by-splines interaction term). For global cognition, the estimated difference in cognitive score for each increase of 1SD of EPA+DHA was 0.048 (95% CI, 0.016; 0.081) standard units (SU) at baseline, 0.042 (-0.006; 0.090) SU at year 9 and 0.137 (0.036; 0.239) SU at year 18. Thus, as presented in **Figure 2**, a woman with a EPA+DHA level of 0.9% (i.e. mean-2SD) would reach a global cognitive level of -0.5 SU about 6.5 years after inclusion, whereas a woman with a similar profile but a EPA+DHA level of 5.9% (i.e. mean+2SD) would reach that cognitive level 11.3 years after inclusion.

A similar trend was found with memory decline (**Figure 2B**, $P = .06$). The estimated difference in memory score for each increase of 1SD of EPA+DHA was 0.016 (-0.028; 0.061) SU at baseline, 0.043 (-0.015; 0.101) SU at year 9 and 0.185 (0.064; 0.305) SU at year 18.

3.3. Long-chain ω3 fatty acids and atrophy of the medial temporal lobe

MTL volume trajectories were assessed over a maximum of 10.8 years (median follow-up 4.0 years). A higher EPA+DHA level was significantly associated with a lower rate of MTL

atrophy (**Figure 3**, $P = .02$). The difference in mean MTL volume change was 0.02 (0.004; 0.04) cm^3/year for each increase of 1SD of EPA+DHA. The estimated difference in MTL volume for each increase of 1SD of EPA+DHA was 0.17 (0.02; 0.32) cm^3 at baseline, 0.28 (0.12; 0.44) cm^3 at year 5 and 0.38 (0.17; 0.60) cm^3 at year 10.

3.4. Supplementary analyses

Categorizing EPA+DHA into quintiles yielded results similar to our primary analysis based on continuous exposures, albeit with more limited power (**Tables S1 to S3 and Figures S3 and S4 in supporting information**). Investigating EPA and DHA separately, EPA was only significantly associated with reduced MTL atrophy, while DHA was associated with lower risk/decline for all three outcomes (**Table S3, Figures S5 and S6 in supporting information**). The ratios EPA/AA and DHA/AA were not significantly associated with the outcomes.

When we investigated the ability of the unique plasma measurement to reflect habitual dietary exposures, plasma EPA+DHA levels at baseline were strongly associated to fish intakes at every follow-up visit up to 12 years after. In supplementary analyses, there was no association of EPA+DHA with mortality risk. Moreover, the use of joint models to take into account attrition or further adjustments for dietary factors did not meaningfully change the results. Associations were not modulated by *APOE*ε4 status ($P \geq .29$ for interaction tests on the co-primary outcomes), including ε4 homozygosity (results available upon request).

4. Discussion

In this large cohort of older adults, a higher plasma omega-3 index at baseline was consistently associated with a lower risk of dementia, less cognitive decline and slower atrophy of the MTL in the following 10 to 17 years. Compared with individuals in the upper quintile of the plasma omega-3 index, those in the lower quintile had a 1.6 times higher risk of

dementia (HR = 1.62 [95% CI, 1.11; 2.38] in multivariable model) – approximately the effect estimate of the *APOE*ε4 allele in our study. Based on our findings, we estimated that the number of expected cases of dementia at the age of 80 would be n = 165 if all our study participants were in the lower quintile of omega-3 index, versus n = 105 if they were all in the upper quintile (i.e. a 36% decreasing number of cases). When separating EPA and DHA, we found consistent associations for both species, albeit with some differences across endpoints (i.e. stronger associations of EPA with brain imaging, and of DHA with clinical outcomes). Taken together, these findings support the relevance of the plasma omega-3 index as a composite marker of long-chain n-3 PUFAs status, in relation to brain aging.

These results are in agreement with accumulating evidence for a beneficial role of long-chain n-3 PUFA on cognitive aging; although previous studies have examined various primary exposures and findings have been mixed overall [29]. A recent meta-analysis including 181,580 participants from 21 prospective cohorts with 2 to 20 years of follow-up, reported that DHA intake, but not blood DHA, was associated with reduced risk of cognitive impairment or dementia [11]. Moreover, blood levels of EPA and total n-3 PUFA (including the precursor α-linolenic acid) were not related to dementia outcomes. However, existing studies on blood biomarkers were limited (n = 7) and mostly with moderate follow-up (5/7 with follow-up less than 5 years); thus, power was moderate in the pooled analysis of biomarker studies. In addition, the meta-analysis did not investigate the combination of EPA+DHA. However, five out of the six studies exploring the omega-3 index (generally measured within erythrocytes) found associations with better cognition or slower cognitive decline [4,30–34]. Moreover, consistent with our findings, the Women's Health Initiative Memory Study (WHIMS, n = 6706) reported a 8% lower risk of dementia for each 1SD increase in the omega-3 index, over a median follow-up of 9.8 years [6]. Collectively, these findings suggest that the blood omega-3 index may be a relevant biological measure of long-chain n-3 PUFA in relation to cognitive aging.

Intending to translate our findings for the design of a supplementation trial, we estimated that a sample size of n = 1636 would be needed in both EPA+DHA and placebo arms to evidence a difference in dementia incidence over 5 years equivalent to the one we found between highest and lowest quintiles of omega-3 index (**Supplementary Methods**). However, both average levels and variability of n-3 PUFA exposure are moderate in observational studies (e.g., average EPA+DHA intake, 416 g/day; minimum, 0; maximum, 12850 in 3C), leading to generally smaller differences than that found in trials. Therefore, using the difference in dementia rate obtained in an observational study as a theoretical expected difference may overestimate the sample size needed for an omega-3 trial.

We found no evidence of interaction between the plasma omega-3 index and *APOE*ε4 status. There is a biological rationale for a vulnerability of *APOE*ε4 carriers to lower DHA status, especially in early AD [35]. However, interactions between fish/n-3 PUFA and *APOE*ε4 in relation to dementia outcomes have been inconsistent in epidemiological literature (including our own findings based on the first 4 years of follow-up in 3C), with studies reporting association limited to *APOE*ε4 carriers [5,7,36,37], others reporting association in *APOE*ε4 non-carriers [11,38–40], and many studies reporting no interaction [11].

As with cognitive decline and dementia, the majority of studies on brain structure reported associations between higher blood n-3 PUFA and higher grey matter volumes [9,10,34,41–43], although most studies were cross-sectional and some inconsistent findings were also reported [44–46]. Among the few large longitudinal studies, the Cardiovascular Health study (n = 2293) did not find any association between plasma long-chain n-3 PUFA and whole brain atrophy over 5 years [45]. In contrast, the WHIMS (n = 1111) reported an association between erythrocyte EPA+DHA and higher hippocampal volumes 8 years later [10]. Our longitudinal study with a long follow-up period extends these previous findings showing the potential involvement of all medial temporal structures in the relationship between long-chain n-3 PUFA and the risk of dementia and cognitive decline.

Our study has several major strengths, including a large population-based sample with three complementary dementia outcomes evaluated over up to 17 years, and a clinical diagnosis of dementia based on in-home cognitive testing and adjudication by an expert committee. Moreover, we evaluated long-chain n-3 PUFA exposure through blood biomarkers, limiting measurement errors in diet assessment [47]. Finally, our analyses were controlled for a large number of potential confounders, including lifestyle and diet quality.

The main limitation of our study is the use of a single measurement of plasma n-3 PUFA at baseline, which might cause misclassification in the assessment of dietary exposures. However, the validity of plasma n-3 measurements as a biomarker of moderate-term dietary intakes is established [48], and healthy older adults have relatively stable dietary habits [49,50]. In addition, although we could not verify, with our data, the ability of the single baseline measurement to reflect past exposures, we showed that plasma n-3 PUFA levels at baseline ranked individuals reasonably well according to their consumption of fish over the subsequent 12 years. Another limitation, specifically for the imaging ancillary study, is the inclusion of healthier individuals than the overall cohort population. However, selection toward healthier participants in prospective observational studies generally leads to underestimation of associations.

In conclusion, in this large cohort of older persons, we found consistent associations between higher levels of plasma long-chain n-3 PUFA and lower rate or decline of three important dementia outcomes (incidence of dementia, cognitive decline and atrophy of the MTL) over up to 17 years follow-up. The efficacy of EPA+DHA supplementation for the primary prevention of dementia and its endophenotypes remains to be established in a clinical study.

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Table 1. Baseline characteristics of participants, the 3C Bordeaux study, 1999-2018 (n = 1279)

Characteristics	Total population	Plasma EPA+DHA (% of total fatty acids) *			
		<2.2 <mean -1SD (N = 191)	[2.2; 3.4[[mean -1SD; mean[(N = 499)	[3.4; 4.6[[mean; mean +1SD[(N = 397)	≥4.6 ≥mean +1SD (N = 192)
Age (years), mean (SD)	74.3 (4.9)	74.2 (5.1)	74.3 (5.0)	74.3 (4.8)	74.7 (4.6)
Female, n (%)	786 (61.5)	118 (61.8)	300 (60.1)	239 (60.2)	129 (67.2)
Educational level (≤ secondary), n (%)	770 (60.5)	124 (65.6)	307 (61.9)	238 (60.1)	101 (52.9)
*APOE*ε4, n (%)	246 (19.4)	39 (20.7)	91 (18.3)	83 (21.0)	33 (17.3)
Regular exercise, n (%)	358 (32.2)	56 (33.9)	119 (27.7)	120 (34.9)	63 (36.6)
Smoking, n (%)					
Never	833 (65.2)	126 (66.0)	316 (63.3)	262 (66.2)	129 (67.2)
Ex-smoker	388 (30.4)	53 (27.7)	161 (32.3)	119 (30.1)	55 (28.6)
Current smoker	57 (4.5)	12 (6.3)	22 (4.4)	15 (3.8)	8 (4.2)
Alcohol consumption, n (%)					
Never	229 (17.9)	39 (20.5)	104 (20.8)	56 (14.1)	30 (15.6)
Former	38 (3.0)	7 (3.7)	19 (3.8)	7 (1.8)	5 (2.6)
Current	1010 (79.1)	144 (75.8)	376 (75.4)	333 (84.1)	157 (81.8)
Fish intake (servings/week), mean (SD)	1.9 (1.2)	1.5 (1.0)	1.6 (1.1)	2.1 (1.2)	2.6 (1.5)
Body mass Index (kg/m²), mean (SD)	26.4 (4.2)	26.3 (4.1)	26.9 (4.2)	26.2 (3.8)	25.8 (4.6)
Diabetes, n (%)	125 (9.9)	23 (12.2)	57 (11.6)	35 (9.0)	10 (5.3)
Hypertension, n (%)	1000 (78.2)	148 (77.5)	400 (80.2)	293 (73.8)	159 (82.8)
Hypercholesterolemia, n (%)	729 (57.1)	107 (56.0)	269 (54.1)	240 (60.6)	113 (58.9)
History of cardiovascular diseases, n (%)	400 (31.3)	72 (37.7)	144 (28.9)	117 (29.5)	67 (34.9)
High depressive symptoms, n (%)	100 (7.8)	14 (7.3)	43 (8.6)	28 (7.1)	15 (7.8)
MMSE score† (range, 0-30), mean (SD)	27.5 (1.9)	27.5 (1.8)	27.4 (2.1)	27.5 (1.9)	27.9 (1.6)
BVRT score† (range, 0-12), mean (SD)	11.4 (2.1)	11.3 (1.9)	11.3 (2.2)	11.5 (2.0)	11.6 (2.1)
IST score†, mean (SD)	29.8 (6.2)	29.3 (6.1)	29.5 (6.2)	29.8 (6.3)	30.9 (6.0)
TMT-A score†, mean (SD)	27.1 (9.5)	26.5 (9.2)	26.2 (9.0)	27.7 (9.9)	28.8 (10.1)
Total GM volume‡ (cm^3), mean (SD)	471.6 (40.6)	469.3 (38.4)	468.6 (38.6)	475.7 (43.8)	471.7 (39.6)

MTL volume‡ (cm^3), mean (SD)	15.6 (1.8)	15.6 (1.7)	15.3 (1.8)	15.7 (1.9)	15.7 (1.6)
Amygdalar volume‡ (cm^3), mean (SD)	2.6 (0.4)	2.6 (0.3)	2.5 (0.4)	2.6 (0.3)	2.6 (0.4)
Parahippocampal volume‡ (cm^3), mean (SD)	6.4 (1.0)	6.6 (1.1)	6.3 (1.0)	6.4 (1.0)	6.4 (0.9)
Hippocampal volume‡ (cm^3), mean (SD)	6.6 (0.8)	6.5 (0.7)	6.5 (0.8)	6.7 (0.8)	6.7 (0.7)

Abbreviations: 3C, Three-City; *APOE*ε4, ε4 allele of the apolipoprotein E gene; BVRT, Benton Visual Retention Test; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GM, gray matter; IST, Isaacs Set Test; MMSE, Mini-Mental State Examination; MTL, medial temporal lobe; SD, standard deviation; TMT-A, Trail Making Test part A.

*Cut-off defined according to the distribution of continuous plasma EPA+DHA values: < mean -1SD, [mean –1SD; mean[, [mean; mean +1SD[, and ≥ mean +1SD. Mean plasma EPA+DHA = 3.39 (SD, 1.25) % of total fatty acids.

† Among secondary study sample for cognitive decline (n=1185).

‡ Among secondary study sample for brain atrophy (n=467). Baseline values were missing for 10.3% of participants. Amygdalar, parahippocampal, and hippocampal volumes were defined as the sum of both hemispheres.

NOTE. Means and percentages are of non-missing values. Missing baseline values: 0.1% for smoking status, 0.2% for alcohol consumption, fish intakes and hypercholesterolemia, 0.5% for educational level, 0.7% for *APOE*ε4 status, 1.4% for diabetes, 1.6% for body mass index, and 13.1% for regular exercise.

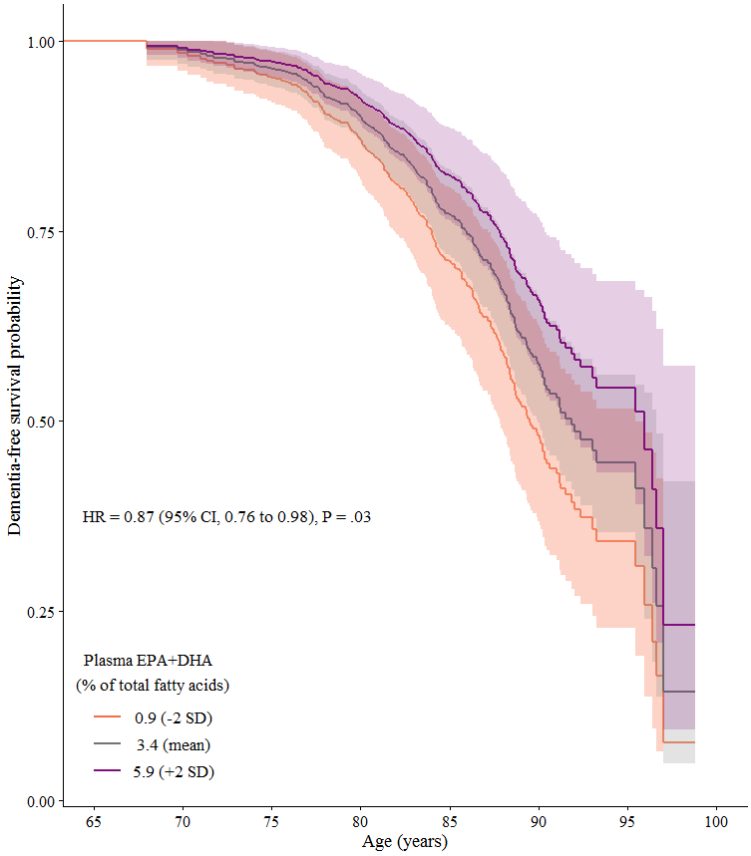
Table 2. Incidence rates of dementia by increasing levels of baseline plasma EPA+DHA, the 3C Bordeaux study, 1999-2014 (n = 1279)

Dementia	Total population	Plasma EPA+DHA (% of total fatty acids) *			
		<2.2 <mean -1SD	[2.2; 3.4[[mean -1SD; mean[	[3.4; 4.6[[mean; mean +1SD[	≥4.6 ≥mean +1SD
N of incident cases/total N (%)	271/1279 (21.2)	47/191 (24.6)	105/499 (21.0)	83/397 (20.9)	36/192 (18.8)
Incidence rate per 100 person-years (95% CI)	2.29 (2.02-2.57)	2.66 (1.90; 3.42)	2.31 (1.87; 2.75)	2.24 (1.76; 2.73)	1.99 (1.34; 2.64)
Absolute rate difference per 100 person-years (95% CI)		Ref	-0.35 (-0.80; 0.10)	-0.42 (-0.87; 0.04)	-0.67 (-1.18; -0.16)

Abbreviations: 3C, Three-City; 95% CI, 95% confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

*Cut-off defined according to the distribution of continuous plasma EPA+DHA values: < mean -1SD, [mean -1SD; mean[, [mean; mean +1SD[, and ≥ mean +1SD.

Figure 1. Dementia-free survival estimated by a multivariable Cox model*, according to increasing levels of baseline plasma EPA+DHA, the 3C Bordeaux study, 1999-2014 (n = 1279)

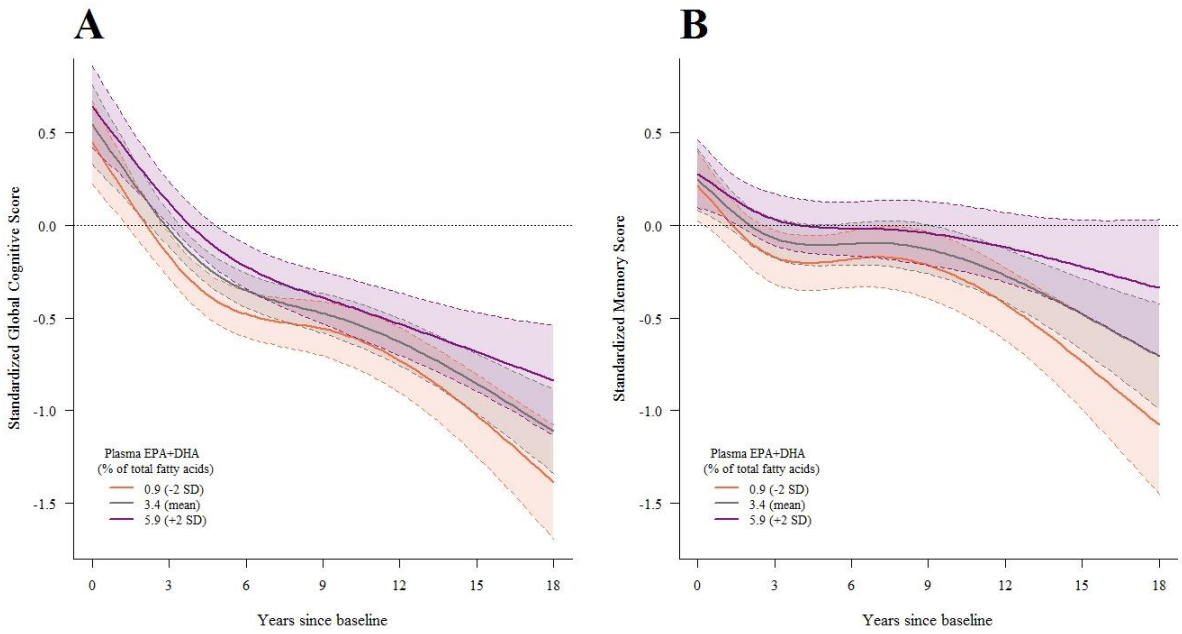


* Dementia-free survival with 95% CI (indicated by shading) was estimated by a Cox proportional-hazard model with delayed entry and age as time scale, adjusted for sex, status for ε4 allele of the apolipoprotein E (*APOE*ε4) gene, educational level, body mass index, smoking status, alcohol consumption, practice of regular physical activity, diabetes, history of cerebral and cardiovascular diseases, hypertension, hypercholesterolemia and high depressive symptoms.

NOTE. Curves were plotted for a chosen profile of covariates; we chose three representative levels of continuous plasma EPA+DHA values (mean ±2 SD) of an average study participant profile (a woman, with no higher than primary education level, *APOE*ε4 non-carrier, who drinks ≥1 alcoholic beverages per week, does not smoke or practice regular physical activity, with a body mass index of 26 kg/m², without history of cerebral or cardiovascular diseases, diabetes or high depressive symptoms, with hypertension and hypercholesterolemia). Note that the choice of profile is made to optimize graphical representation and has no influence on the differences in HR estimated by the model (calculated for each increase of 1 SD of EPA+DHA taken as a continuous variable).

Abbreviations: 3C, Three-City; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; SD, standard deviation.

Figure 2. Mean trajectories of global cognition (panel A, n = 1185) and memory (panel B, n = 1245) estimated by multivariable linear mixed models*, according to increasing levels of baseline plasma EPA+DHA, the 3C Bordeaux study, 1999-2018

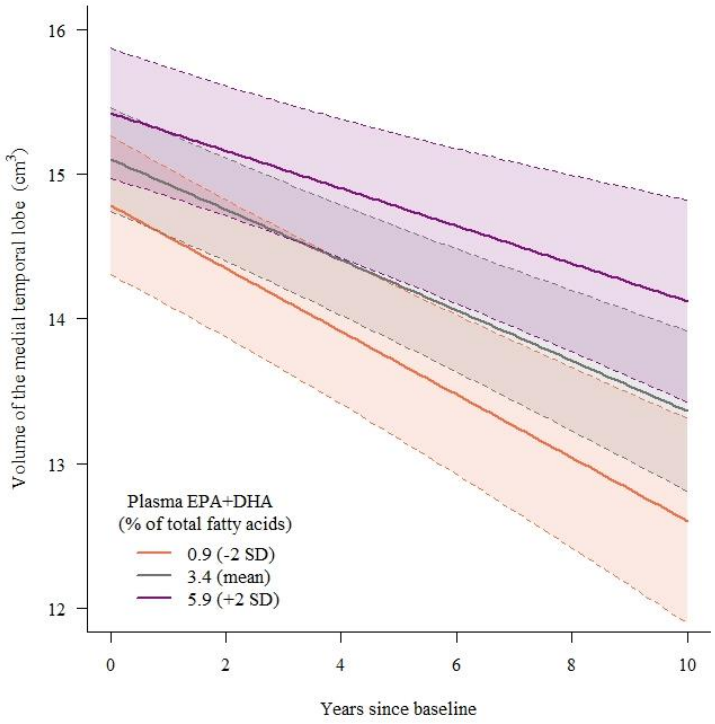


* Trajectories of change in global cognition (panel A) and memory (panel B) were estimated using linear mixed models across repeated cognitive visits for computation of the composite score of global cognition and of memory. Models considered a nonlinear trajectory with time approximated by natural cubic splines (two internal knots placed at tertiles of measurement times), with corresponding random effects; they also included: an intercept representing the cognitive score at baseline(corresponding random effect); an indicator for the first cognitive visit; EPA+DHA (continuous, standardized), covariates (age, sex, status for ε4 allele of the apolipoprotein E (*APOE*ε4) gene, educational level, body mass index, smoking status, alcohol consumption, practice of regular physical activity, diabetes, history of cerebral and cardiovascular diseases, hypertension, hypercholesterolemia, and high depressive symptoms) and their interactions with time. Composite scores for memory were normalized using latent process mixed modeling and standardized before being entered as dependent variables in the mixed model.

NOTE. The mean predicted trajectories (solid lines) with 95% Confidence Intervals (indicated with shading) were plotted for a chosen profile of covariates; we chose three representative levels of continuous plasma EPA+DHA values (mean ±2 SD) of an average study participant profile (a woman aged 72 years at study baseline, with no higher than primary education level, *APOE*ε4 non-carrier, who drinks ≥1 alcoholic beverages per week, does not smoke or practice regular physical activity, with a body mass index of 26 kg/m², without history of cerebral or cardiovascular diseases, diabetes or high depressive symptoms, with hypertension and hypercholesterolemia). Note that the choice of profile is made to optimize graphical representation and has no influence on the differences in trajectories estimated by the model (calculated for each increase of 1 SD of EPA+DHA taken as a continuous variable).

Abbreviations: 3C, Three-City; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SD, standard deviation.

Figure 3. Mean trajectories of medial temporal lobe volume estimated by a multivariable linear mixed model*, according to increasing levels of baseline plasma EPA+DHA, the 3C Bordeaux study, 1999-2011 (n = 467)



* Trajectories of change in medial temporal lobe (MTL) volume were estimated using a linear mixed model across three repeated MRI examinations. The model considered a linear function of time, with corresponding random effect; it also included: an intercept representing the MTL volume at baseline(and corresponding random effect); EPA+DHA (continuous, standardized), covariates (age, sex, status for ε4 allele of the apolipoprotein E (*APOE*ε4) gene, educational level, body mass index, smoking status, alcohol consumption, practice of regular physical activity, diabetes, history of cerebral and cardiovascular diseases, hypertension, hypercholesterolemia, and high depressive symptoms), and their interactions with time. To account for the change of protocol from a 1.5T to a 3T scanner at the third MRI examination, a third visit indicator and a scanner-specific variance for the measurement error were added to the model.

NOTE. The mean predicted trajectories (solid lines) with 95% Confidence Intervals (indicated with shading) were plotted for a chosen profile of covariates; we chose three representative levels of continuous plasma EPA+DHA values (mean ±2 SD) of an average study participant profile (a woman aged 72 years at study baseline, with no higher than primary education level, *APOE*ε4 non-carrier, who drinks ≥1 alcoholic beverages per week, does not smoke or practice regular physical activity, with a body mass index of 26 kg/m², without history of cerebral or cardiovascular diseases, diabetes or high depressive symptoms, with hypertension and hypercholesterolemia). Note that the choice of profile is made to optimize graphical representation and has no influence on the differences in trajectories estimated by the model (calculated for each increase of 1 SD of EPA+DHA taken as a continuous variable).

Abbreviations: 3C, Three-City; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MRI: Magnetic Resonance Imaging; SD, standard deviation