1. Supplementary Methods

1.1. Assessment of cognitive function

For analyses of cognitive decline, composite scores (with higher scores indicating better cognition) were calculated at each follow-up visit, as the mean of z-scores of various cognitive tests (using the mean and standard error at baseline), as previously done in the 3C study [1].

For the global cognitive score, four cognitive domains were assessed: (i) global cognition, (ii) verbal semantic fluency, (iii) working memory and attention, and (iv) executive functioning. Global cognition was assessed using the Mini-Mental State Examination (MMSE) [2] with a total score ranging from 0 to 30 (with higher scores indicating better performances). For verbal semantic fluency we used the Isaacs' Set Test [3], in which participants have 15 seconds to cite as many words as possible belonging to a specific semantic category (four successive categories were used: cities, fruits, animals, and colors; range, 0-40). Working memory and attention were assessed by the Benton Visual Retention Test (BVRT) [4], in which a stimulus card displaying a geometric figure is presented for 10 seconds, after which participants are asked to identify the initial figure among four possibilities (15 figures are successively presented; range, 0-15). For executive functioning, we used the Trail Making Test A [5], which consists in connecting numbers from 1 to 25 in an ascending manner; here, we used the number of correct displacements divided by the time (in minutes) required to perform the test (range, 0-76; higher scores indicating better performances).

For the memory score, two memory tests were combined, as done in a previous study [1]: the BVRT and a subset of the MMSE (defined as the sum of items related to orientation to time and the 3-word recall task; range, 0-8). The subset of the MMSE is used as a proxy of episodic memory, as validated in a study showing a correlation (ρ>0.40) between this sub-score and scores obtained on the Free and Cued Selective Reminding Test (FCSRT, a validated test of episodic memory [6]) [7].

1.2. Assessment of brain atrophy

MRI acquisition

The first two MRI acquisitions (in 2000-2001 and 2004-2006) were performed on a 1.5-T Gyroscan Interra system (Philips Medical System, Netherlands) equipped with a quadrature head coil. Anatomical high resolution MRI volumes were acquired in transverse plane using a T1 (3D magnetization prepared rapid gradient echo [MPRAGE]) weighted sequence, with the following parameters: repetition time [TR]/echo time [TE] 8.5/3.9 ms, flip angle 10°, matrix size 256 x256, field of view [FOV] 240 mm, yielding 124 slices and slice thickness of 1 mm, voxel size 0.94x0.94x1 mm^3. T2 Head motions were minimized by the use of tightly padded clamps attached to the head coil. All acquisitions were aligned on the anterior commissure-posterior commissure plane.

In 2010-2011, the third MRI exam was performed using an ACHIEVA 3T scanner (Philips Medical System, Netherlands) equipped with a SENSE 8-channel head coil. Anatomical high resolution MRI volumes were acquired in transverse plane using a three-dimensional MPRAGE

weighted-T1 sequence with the following parameters: TR/TE=8.2/3.5 ms, 7-degree flip angle, FOV 256×256 mm^2 to cover the whole brain, yielding 180 contiguous slices, voxel size 1×1×1 mm^3. All acquisitions were aligned on the anterior commissure/posterior commissure plane.

MRI processing

Each subject's anatomical images were processed using cortical segmentation of Freesurfer (v5.1, http://surfer.nmr.mgh.harvard.edu) with the Destrieux parcellation atlas [8]. Hippocampal and amygdalar parcellations were done using fMRIB's Integrated Registration and Segmentation Tool [9], part of FSL [10]. Results of the segmentation were checked for global accuracy of the anatomical delineation: images with potential segmentation errors were identified based on the description of grey matter and hippocampal volumes and discarded if necessary after visual inspection by three trained-operators.

1.3. Statistical analyses

For analyses of cognitive decline, associations between EPA+DHA and the trajectories of each cognitive score were estimated using a linear mixed model. The linear mixed model included an intercept representing the cognitive score at baseline and a slope representing the annual change in scores over time, with corresponding random intercept and slope to account for inter individual variability. We used natural cubic splines with two internal knots (at tertiles of measurement times) to approximate the nonlinear shape of cognitive trajectory with time since baseline (Akaike information criterion confirmed the better fit of the spline-based versus linear trajectory: 6390 and 6435, respectively, for global score, 14,494 and 14,554 for memory score).

1.4. Hypothetical sample size calculation

A sample size was calculated for the number of subjects needed in a hypothetical clinical trial of the effect of supplementation in EPA+DHA on dementia incidence, versus placebo. For calculation we considered an expected absolute difference in dementia risk between intervention and placebo groups equivalent to the difference observed between quintile 5 (Q5) and quintile 1 (Q1) of plasma omega-3 index in our study.

The number of subjects needed in each group was calculated using the formula for two incidence rates in follow-up studies described in *Lwanga and Lemeshow* [11] and using the R package epiR (function epi.sscohortt).

$$n = \frac{\left(z_{1-\frac{\alpha}{2}} \sqrt{2 \times \frac{\overline{IR}^3 \times t}{\overline{IR} \times t - 1 + e^{-\overline{IR} \times t}}} + z_{1-\beta} \sqrt{\frac{IR_1^3 \times t}{IR_1 \times t - 1 + e^{-IR_1 \times t}} + \frac{IR_0^3 \times t}{IR_0 \times t - 1 + e^{-IR_0 \times t}}} \right)^2}{\Delta^2}$$

With:

n, number of subjects needed in each group; t, duration of the study (in years); IR_1, incidence rate of dementia expected in intervention group (i.e., observed in Q5 $IR_1 = 1.83$ per 100 person-year [**Supplementary Table 2**]); IR_0, incidence rate of dementia expected in placebo group (i.e., observed in Q1 $IR_0 = 2.79$ per 100 person-year); $\overline{IR}$, mean incidence rate of dementia between the intervention and placebo group, $\overline{IR} = (IR_1 + IR_0)/2 = 2.31$ per 100 person-year; Δ, expected absolute rate difference of dementia (i.e., observed absolute different rate between Q1 and Q5 $\Delta = 0.96$ per 100 person-year); type I error $\alpha = 5\%$; and power $(1-\beta) = 80\%$.

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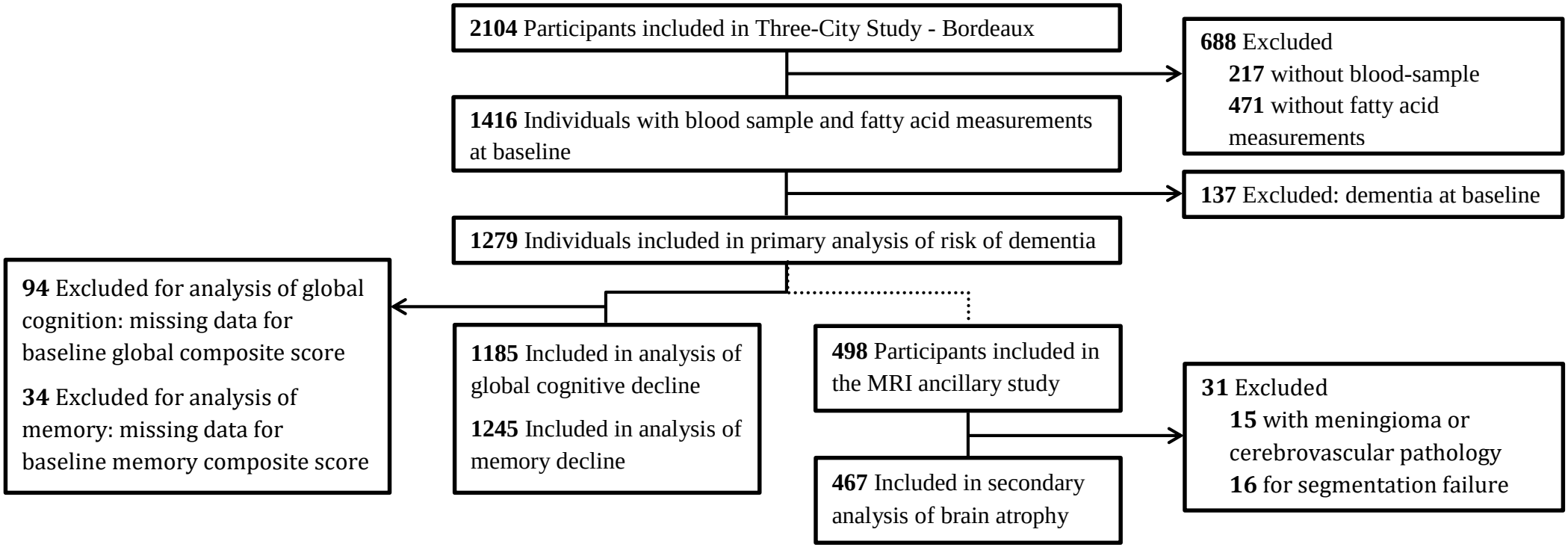
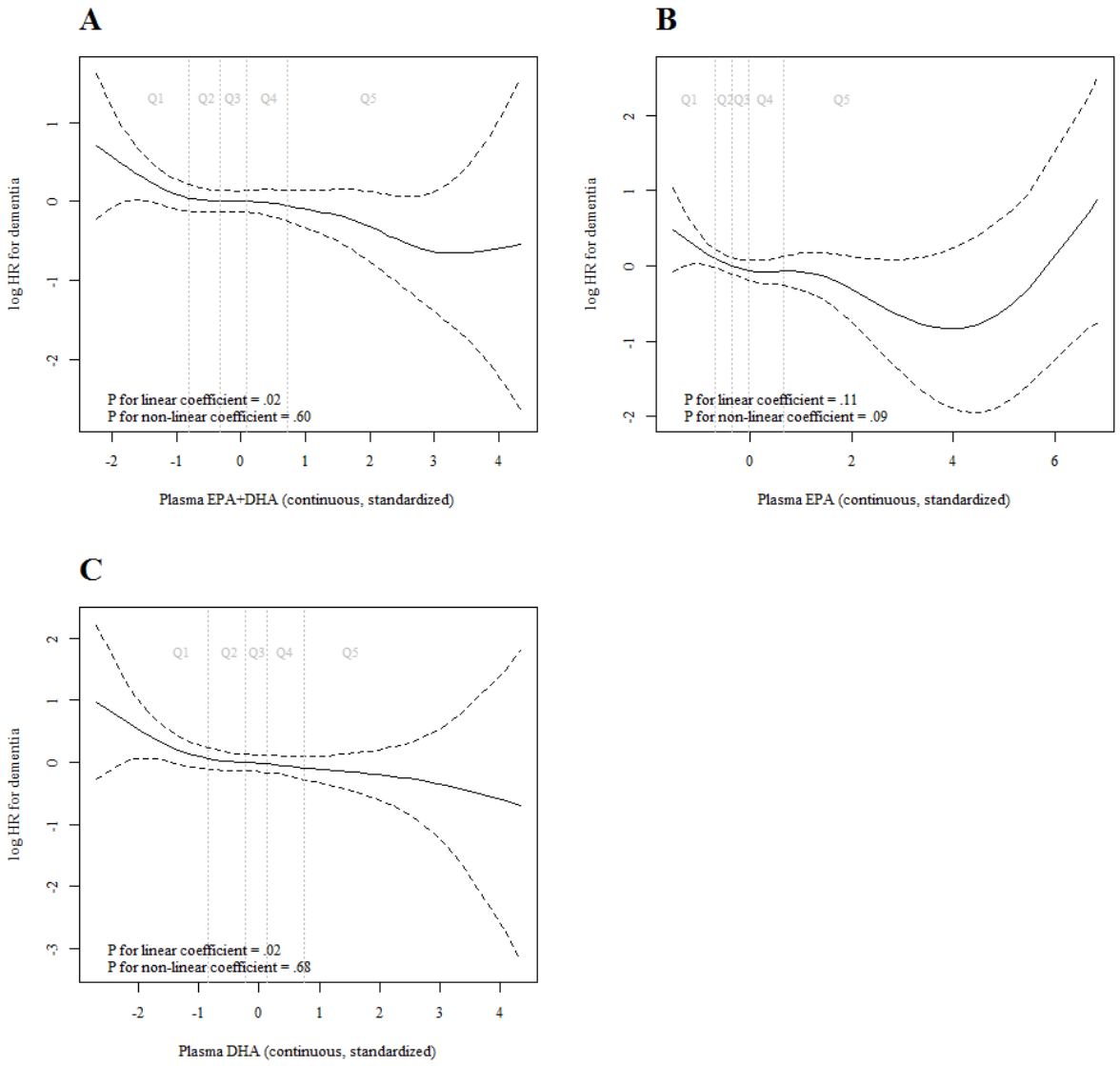
Supplementary Figure 1. Flow chart of participants included in the study, the Three-City Bordeaux study, 1999-2018



Supplementary Figure 2. Dose-response relationships between plasma EPA+DHA (panel A), EPA alone (panel B) and DHA alone (panel C) and hazard ratios for dementia estimated by restricted cubic splines in Cox proportional hazard models, the 3C Bordeaux study, 1999-2014 (n=1279)



NOTE. P for linear coefficient is the *P*-value for the test of a linear association against a null association. P for non-linear coefficient is the *P*-value for the test of a non-linear association against a linear association. Vertical dashed lines indicate quintiles of distribution for EPA+DHA, EPA or DHA.
Abbreviations: 3C, Three-City; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Supplementary Table 1. Baseline cognitive scores and brain volumes by quintiles of baseline plasma EPA+DHA, the 3C Bordeaux study

Characteristics	Quintiles of plasma EPA+DHA (% of total fatty acids)					P*
	<2.4	[2.4-3.0[	[3.0-3.5[	[3.5-4.0[	≥4.0	
MMSE score† (range, 0-30)						
Mean (SD)	27.4 (2.0)	27.4 (2.1)	27.6 (2.0)	27.5 (1.9)	27.7 (1.8)	0.25
Cohen's *d* (95% CI)§	Ref	-0.01 (-0.20; 0.17)	0.09 (-0.09; 0.28)	0.07 (-0.11; 0.25)	0.18 (0.00; 0.36)	
BVRT score† (range, 0-12)						
Mean (SD)	11.2 (2.0)	11.4 (2.2)	11.3 (2.2)	11.5 (2.0)	11.7 (2.0)	0.08
Cohen's *d* (95% CI)§	Ref	0.12 (-0.06; 0.31)	0.05 (-0.14; 0.23)	0.16 (-0.02; 0.34)	0.25 (0.07; 0.44)	
IST score†						
Mean (SD)	28.8 (6.1)	29.6 (5.7)	29.8 (6.6)	29.8 (6.3)	30.7 (6.1)	0.02
Cohen's *d* (95% CI)§	Ref	0.13 (-0.06; 0.32)	0.16 (-0.02; 0.34)	0.16 (-0.02; 0.34)	0.31 (0.13; 0.50)	
TMT-A score†						
Mean (SD)	26.3 (9.1)	26.3 (9.0)	26.1 (9.2)	28.1 (9.9)	28.3 (10.1)	0.01
Cohen's *d* (95% CI)§	Ref	0.00 (-0.19; 0.18)	-0.02 (-0.21; 0.16)	0.19 (0.01; 0.37)	0.21 (0.02; 0.39)	
Total GM volume‡ (cm^3)						
Mean (SD)	469.0 (39.0)	468.5 (43.2)	468.7 (34.3)	478.0 (46.4)	471.9 (37.7)	0.47
Cohen's *d* (95% CI)§	Ref	-0.01 (-0.33; 0.31)	-0.01 (-0.32; 0.31)	0.21 (-0.09; 0.50)	0.08 (-0.22; 0.38)	
MTL volume‡ (cm^3), mean (SD)						
Mean (SD)	15.6 (1.9)	15.3 (1.9)	15.5 (1.7)	15.6 (1.8)	15.8 (1.6)	0.41
Cohen's *d* (95% CI)§	Ref	-0.17 (-0.49; 0.15)	-0.07 (-0.38; 0.25)	0.02 (-0.27; 0.32)	0.13 (-0.17; 0.43)	
Amygdalar volume‡ (cm^3)						
Mean (SD)	2.6 (0.3)	2.5 (0.4)	2.5 (0.3)	2.6 (0.3)	2.6 (0.4)	0.44
Cohen's *d* (95% CI)§	Ref	-0.05 (-0.37; 0.27)	-0.05 (-0.37; 0.26)	0.18 (-0.11; 0.47)	0.14 (-0.16; 0.44)	
Parahippocampal volume‡ (cm^3)						
Mean (SD)	6.6 (1.1)	6.3 (1.0)	6.3 (1.0)	6.4 (1.1)	6.5 (1.0)	0.39
Cohen's *d* (95% CI)§	Ref	-0.29 (-0.61 ; 0.03)	-0.22 (-0.54 ; 0.10)	-0.14 (-0.43; 0.16)	-0.11 (-0.41; 0.19)	
Hippocampal volume‡ (cm^3)						
Mean (SD)	6.5 (0.7)	6.5 (0.8)	6.6 (0.8)	6.6 (0.7)	6.8 (0.7)	0.09

Cohen's *d* (95% CI)[§]	Ref	0.02 (-0.30; 0.34)	0.17 (-0.14; 0.49)	0.17 (-0.12; 0.46)	0.39 (0.09; 0.69)

Abbreviations: 3C, Three-City; BVRT, Benton Visual Retention Test; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GM, Grey matter; IST, Isaacs Set Test; MMSE, Mini-Mental State Examination; MTL, medial temporal lobe; SD, standard deviation; TMT-A, Trail Making Test part A.

[*] *P*-values estimated by analysis of variance.

[†] 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.

[§] Effect size estimated by Cohen's d ($[Mean_1\text{-}Mean_2] / SD_{pooled}$)

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

Dementia	Quintiles of plasma EPA+DHA (% of total fatty acids)				
	<2.4	[2.4-3.0[	[3.0-3.5[	[3.5-4.0[	≥4.0
N of incident cases/ total N (%)	61/242 (25.2)	42/235 (17.9)	59/260 (22.7)	61/274 (22.3)	48/268 (17.9)
Incidence rate per 100 person-years (95% CI)	2.79 (2.09; 3.49)	1.89 (1.32; 2.46)	2.63 (1.96; 3.31)	2.39 (1.79; 2.99)	1.83 (1.31; 2.35)
Rate difference per 100 person-years (95% CI)	Ref	-0.90 (-1.36; -0.44)	-0.16 (-0.65; 0.34)	-0.40 (-0.87; 0.07)	-0.96 (-1.41; -0.52)

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

Supplementary Table 3. Multivariable-adjusted associations between baseline plasma n-3 PUFA categorized into quintiles and the risk of dementia, the 3C Bordeaux study, 1999-2014 (n=1279)

	HR*	95% CI	P *for trend* †
Quintiles of n-3 PUFA (% of total fatty acids)			
EPA+DHA			*0.06*
< 2.4	Ref	-	
2.4 – 3.0	0.63	(0.43; 0.94)	
3.0 – 3.5	0.89	(0.62; 1.28)	
3.5 – 4.3	0.86	(0.60; 1.24)	
≥ 4.3	0.62	(0.42; 0.90)	
EPA			*0.07*
< 0.6	Ref	-	
0.6 – 0.8	0.84	(0.59; 1.22)	
0.8 – 1.0	0.74	(0.50; 1.09)	
1.0 – 1.4	0.87	(0.61; 1.26)	
≥ 1.4	0.66	(0.45; 0.97)	
DHA			*0.03*
< 1.7	Ref	-	
1.7 – 2.2	0.76	(0.53; 1.10)	
2.2 – 2.5	0.79	(0.53; 1.16)	
2.5 – 3.0	0.71	(0.49; 1.02)	
≥ 3.0	0.64	(0.44; 0.94)	
DHA (continuous, for a 1-SD increase)	0.86	(0.76; 0.98)	*0.02*

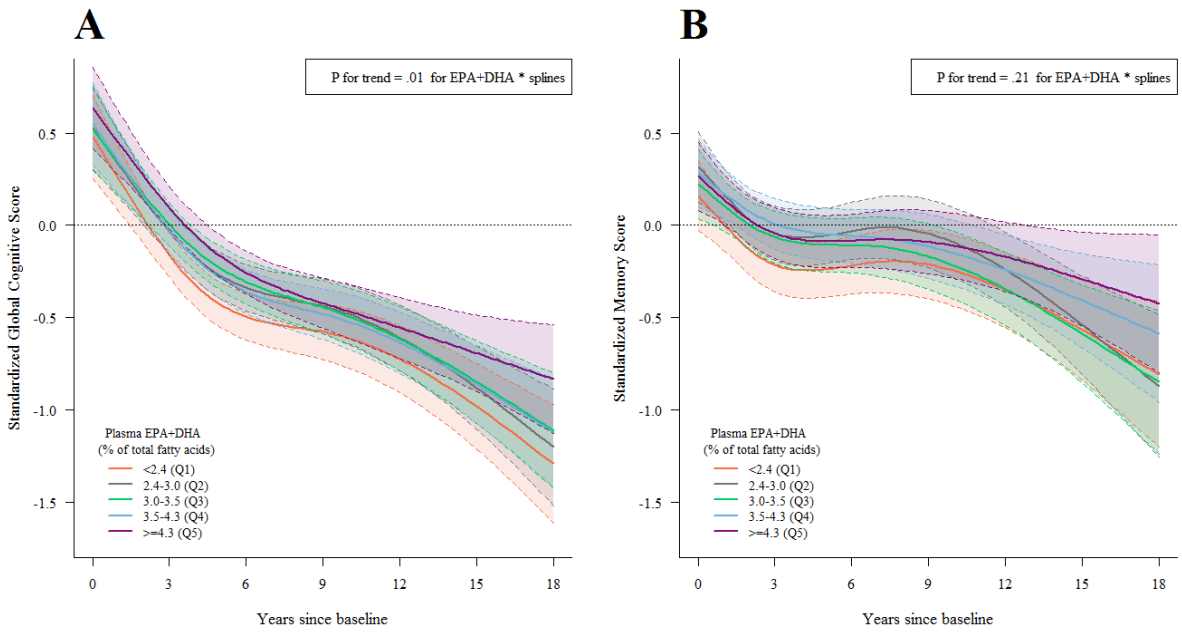
*Cox proportional hazard models with delayed entry and age as time scale. Models included: the n-3 PUFA exposure variable (categorical, by quintiles), sex, APOEε4 status, educational level, body mass index, smoking status, alcohol consumption, practice of regular physical activity, diabetes, history of cerebral and cardiovascular diseases, hypertension, hypercholesterolemia and high depressive symptoms.

† *P*-values for trend across quintiles for the categorized exposure.

NOTE. For EPA, the evident deviance from the log-linearity assumption as estimated by restricted cubic splines (Supplementary Figure 2, panel B) precluded its use as a continuous variable in a Cox proportional hazard model. In contrast, the relationship of DHA to dementia risk appeared log-linear (Supplementary Figure 2, panel C), thus DHA could be modeled as a continuous variable in the Cox model, as presented in the table.

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

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



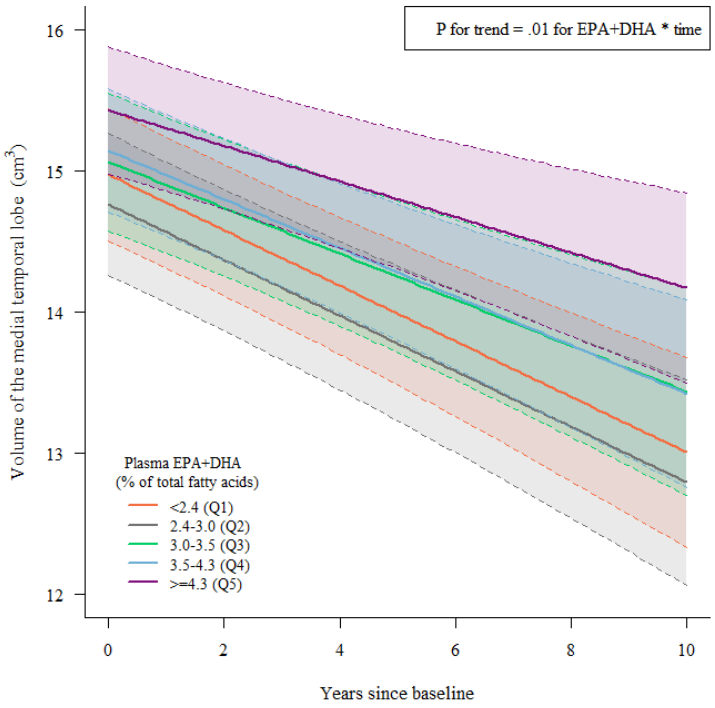
* Trajectories of change in global cognition (panel A) and memory (panel B) were estimated using linear mixed models across repeated cognitive visits for computation of the composite score of global cognition and of memory. Models considered a nonlinear trajectory with time approximated by natural cubic 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 (and corresponding random effects); an indicator for the first cognitive visit; EPA+DHA (categorical, by quintiles), 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.

NOTES: The mean predicted trajectories (solid lines) with 95% Confidence Intervals (indicated with shading) in each quintile of EPA+DHA were plotted for 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).

P-values for the parameter "EPA+DHA * splines" refer to the test for interaction between EPA+DHA and spline functions of time in the linear mixed model, trend across quintiles.

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

Supplementary Figure 4. Mean trajectories of medial temporal lobe volume estimated by a multivariable linear mixed model*, by quintiles of baseline plasma EPA+DHA, the 3C Bordeaux study, 1999-2011 (n=467)



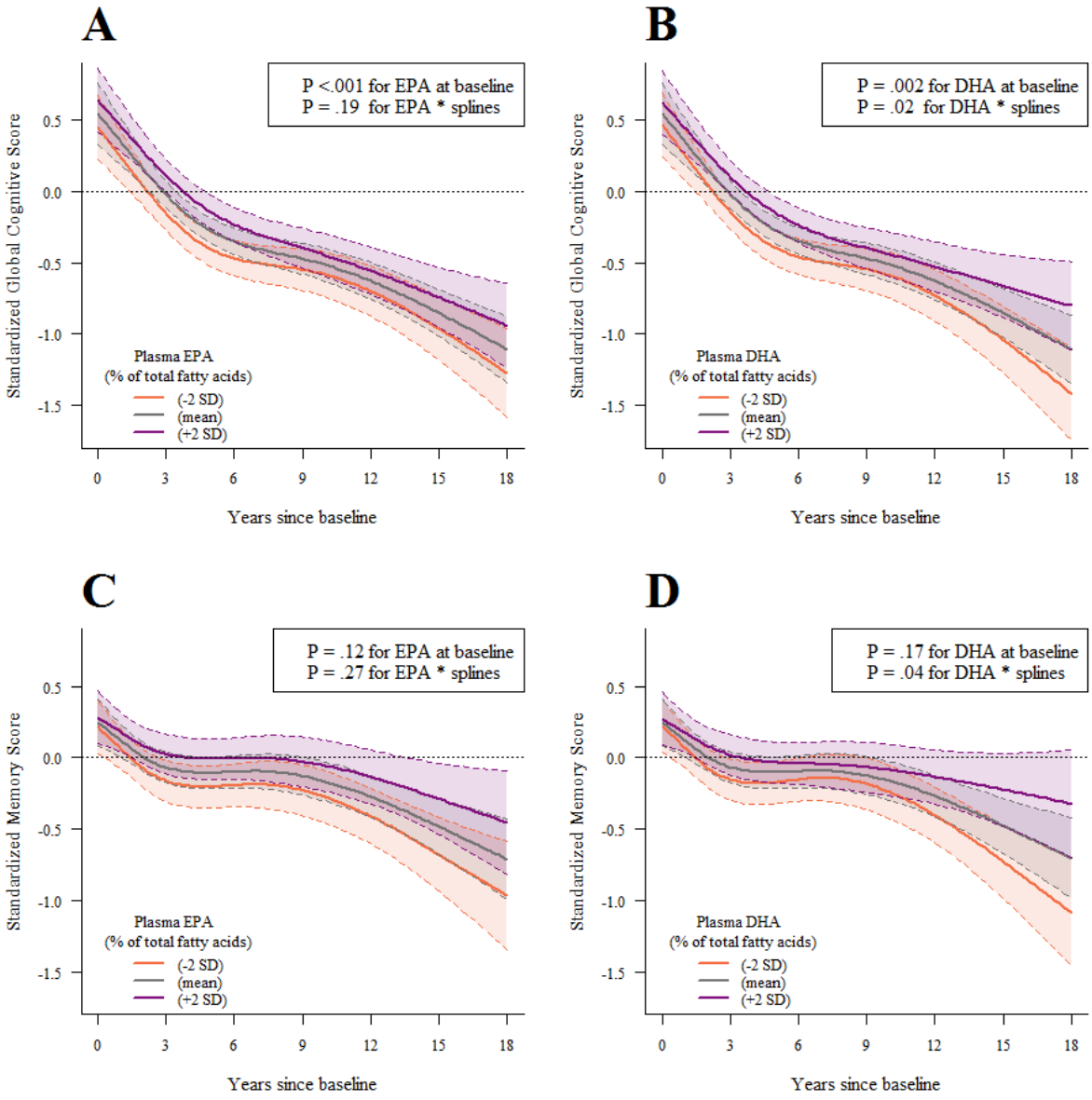
* Trajectories of change in medial temporal lobe (MTL) volume were estimated using a linear mixed model across three repeated MRI examinations. The model considered a linear function of time, with corresponding random effects; it also included: an intercept representing the MTL volume at baseline, and corresponding random effect; EPA+DHA (categorical, by quintiles), 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.

NOTES. The mean predicted trajectories (solid lines) with 95% Confidence Intervals (indicated with shading) in each quintile of EPA+DHA were plotted for 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).

P-value for the parameter "EPA+DHA * time" refers to the test for interaction between EPA+DHA and the linear function of time in the linear mixed model, trend across quintiles.

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

Supplementary Figure 5. Mean trajectories of global cognition (panels A and B, n=1185) and memory (panels C and D, n=1245) estimated by multivariable linear mixed models*, according to increasing levels of baseline plasma EPA and DHA, the 3C Bordeaux study, 1999-2018



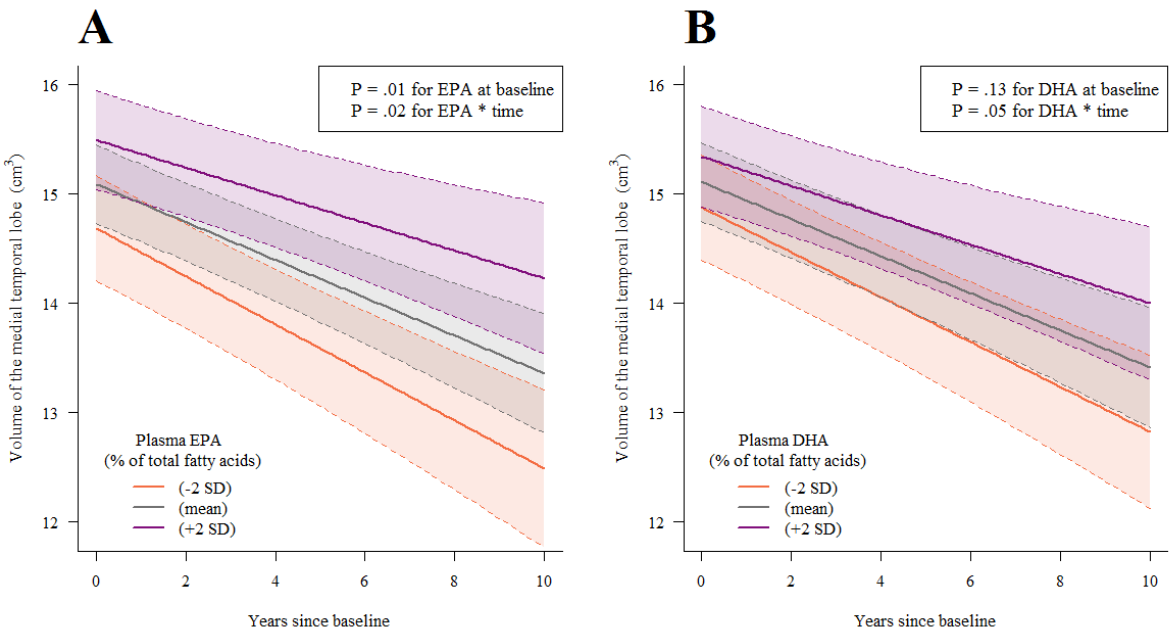
* Trajectories of change in global cognition and memory were estimated using linear mixed models across repeated cognitive visits for computation of the composite score of global cognition and of memory. Models considered a nonlinear trajectory with time approximated by natural cubic splines (two internal knots placed at tertiles of measurement times), with corresponding random effects; they also included: an intercept representing the cognitive score at baseline (and corresponding random effect); an indicator for the first cognitive visit; EPA (panels A and C) or DHA (panels B and D) (continuous, standardized), covariates (age, sex, APOEε4 status, 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% Confident Intervals (indicated with shading) were plotted for a chosen profile of covariates; we chose three representative levels of continuous plasma EPA or 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, APOEε4 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).

P-values for "EPA or DHA at baseline" refer to the mean difference of composite cognitive scores estimated at baseline for an increase of 1SD of EPA or DHA. "EPA or DHA * splines" refers to the interactions of EPA or DHA levels with splines functions of time in the linear mixed models; the associated p-values give an indication on the differences in trajectories of composite cognitive scores for each increase of 1SD of EPA or DHA.

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

Supplementary Figure 6. Mean trajectories of medial temporal lobe volumes estimated by multivariable linear mixed models*, according to increasing levels of baseline plasma EPA (panel A) and DHA (panel B), the 3C Bordeaux study, 1999-2011 (n=467)



* Trajectories of change in medial temporal lobe (MTL) volume were estimated using linear mixed models across three repeated MRI examinations. Models considered a linear function of time, with corresponding random effects; it also included: an intercept representing the MTL volume at baseline, and corresponding random effect; EPA (panel A) or DHA (panel B) (continuous, standardized), covariates (age, sex, APOEε4 status, 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 or 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, APOEε4 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).

P-values for "EPA or DHA at baseline" refer to the mean difference of MTL volumes estimated at baseline for an increase of 1SD of EPA or DHA. "EPA or DHA * time" refers to the interactions of EPA or DHA levels with linear functions of time in the linear mixed models; the associated p-values give an indication on the differences in trajectories of MLT volumes for each increase of 1SD of EPA or DHA.

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