Supplementary Methods

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 [1]. Hippocampal and amygdalar parcellations were done using fMRIB's Integrated Registration and Segmentation Tool [2], part of FSL [3]. 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.

Statistical analyses

Associations between blood carotenoids and the trajectories of medial temporal lobe (MTL) volume change over the three repeated MRI exams were estimated using a linear mixed model. The linear mixed models included an intercept representing the MTL volume at baseline and a linear function of time representing the annual change in volume over time, with corresponding random intercept and slope to account for inter individual variability. 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 [4]).

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.

Covariates were derived from baseline evaluation and included: age, sex, educational level, smoking status (never, former, current), alcohol consumption (never, former, current), 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), *APOE*ε4 allele carrier status (carrying at least one ε4 allele versus no ε4 allele), 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), body mass index (BMI, body weight/height² in kg/m²), and epressive symptoms (recorded using the Center for Epidemiologic Studies-Depression (CES-D) scale [5]; high depressive symptoms were defined as a CES-D score ≥17 for men and ≥23 for women, or being too depressed to answer [6]).

Missing data for covariates were imputed by multiple imputations (using chained equations with fully conditional specification method; M=5 imputations). Statistical analyses were performed using R version 3.6.0 (R Foundation). Two-sided p-values were used with an α=.05 threshold for statistical significance.

References

[1] Destrieux C, Fischl B, Dale A, Halgren E. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. NeuroImage 2010;53:1–15.

[2] Patenaude B, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. NeuroImage 2011;56:907–22.

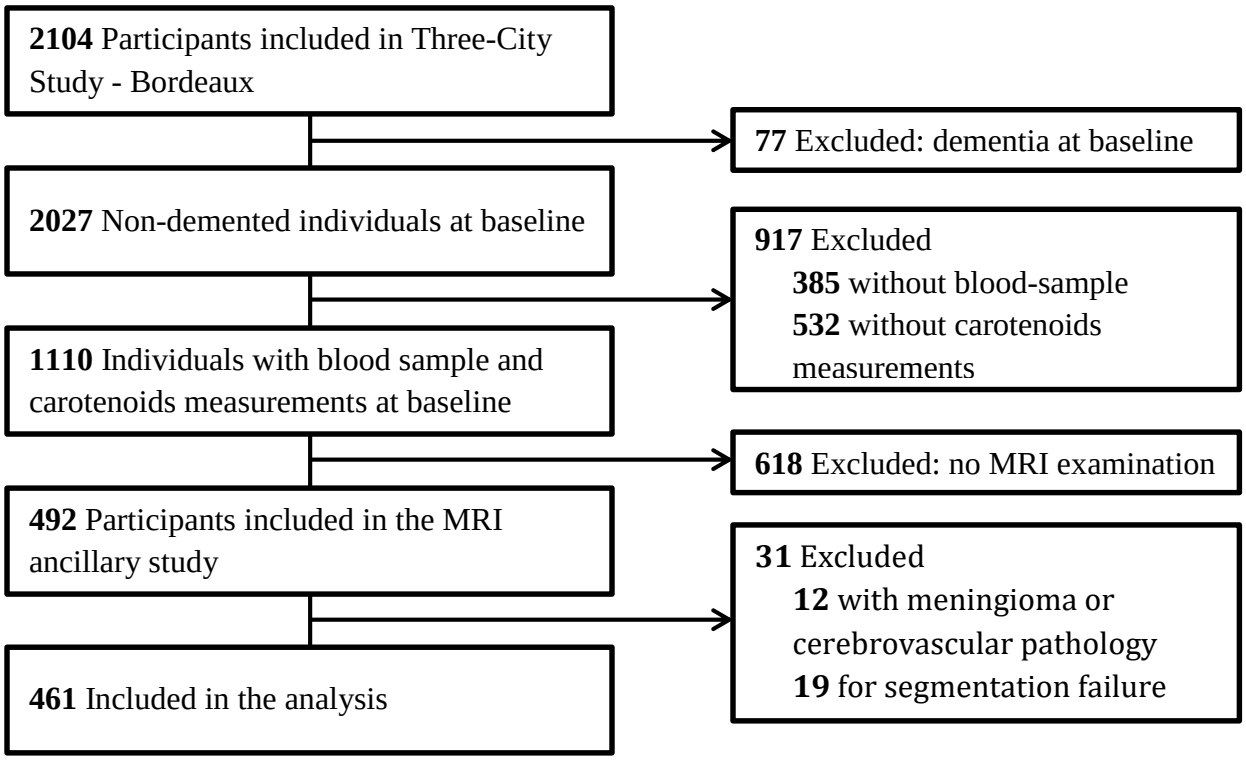
[3] Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 2004;23 Suppl 1:S208-219.

[4] Wagner M, Dartigues J-F, Samieri C, Proust-Lima C. Modeling Risk-Factor Trajectories When Measurement Tools Change Sequentially During Follow-up in Cohort Studies: Application to Dietary Habits in Prodromal Dementia. Am J Epidemiol 2018;187:845–54.

[5] Radloff LS. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. Appl Psychol Meas 1977;1:385–401.

[6] Fuhrer R, Rouillon F. La version française de l'échelle CES-D (Center for Epidemiologic Studies-Depression Scale) : description et traduction de l'échelle d'autoévaluation. Psychiatr Psychobiol 1989;3:163–6.

Supplementary Figure. Flow chart of participants included in the study, the Three-City Bordeaux study, 1999-2011



Supplementary Table. Multivariable association of baseline plasma total carotenoids with medial temporal lobe volume change, estimated by a linear mixed model, the 3C Bordeaux study, 1999-2011 (n=461)

	β*	95% CI	p-value
Total carotenoids (for + 1 SD)	0.02	[0.001; 0.04]	0.04
Age (for + 1 year)	-0.007	[-0.01; -0.003]	0.002
Female (vs male)	-0.003	[-0.04; 0.04]	0.89
Educational level (> secondary vs ≤ secondary)	0.03	[-0.002; 0.06]	0.06
*APOE*ε4 (vs no)	-0.04	[-0.08 ; -0.001]	0.04
Regular exercise (vs no)	0.01	[-0.02 ; 0.04]	0.53
Smoking			
Never	Ref		
Ex-smoker	0.03	[-0.01 ; 0.07]	0.14
Current smoker	-0.005	[-0.08 ; 0.07]	0.89
Alcohol consumption			
Current	Ref		
Never	-0.008	[-0.05 ; 0.04]	0.73
Former	0.13	[0.01 ; 0.24]	0.03
Body Mass Index (for an increase of 1 kg/m²)	0.005	[0.001; 0.01]	0.03
Diabetes (vs no)	-0.03	[-0.10 ; 0.04]	0.36
History of cardiovascular diseases (vs no)	0.03	[-0.01; 0.06]	0.18
High depressive symptoms (vs no)	-0.04	[-0.12; 0.03]	0.28
Hypertension (vs no)	-0.007	[-0.04 ; 0.03]	0.68
Hypercholesterolemia (vs no)	-0.02	[-0.05 ; 0.02]	0.30
Plasma lipids (for + 1 mmol/L)			
Total cholesterol	-0.004	[-0.02 ; 0.02]	0.68
Triglycerides	0.009	[-0.02 ; 0.04]	0.59
Total intracranial volume (for + 1 cm^3)	-0.0002	[-0.0004; 0.00001]	0.07

3C, Three-City; *APOE*ε4, ε4 allele of the apolipoprotein E gene; CI, confidence interval; SD, Standard Deviation

Trajectories of change in 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 MTL volume at baseline (and corresponding individual random effect); total carotenoids (continuous); each covariate listed in the Table; and their interactions with 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 and a scanner-specific variance for the measurement error.

*β coefficient is for the variable-by-time interaction term in the linear mixed model, that reflects the annual change in medial temporal lobe volume (in cm^3/year) per each increase of 1 unit of variable (for continuous variables), or compared to the reference category (for categorical variable).