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Title: Plasma carotenoids and medial temporal lobe atrophy in older adults

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ABSTRACT

Background & aims: Carotenoids are vegetable pigments with neuroprotective properties. Clinical studies found efficacy of specific carotenoids on improving brain perfusion and functioning with aging. However, evidence of an effect on neurodegeneration, which may require longer follow-up period to observe, is more limited. Leveraging biomarkers from a large population-based cohort study of older adults, we investigated whether blood carotenoids were associated with atrophy of the medial temporal lobe (a biomarker of neurodegeneration in aging) over 10 years.

Methods: This study included 461 dementia-free participants from the Three-City Bordeaux study (aged ≥65) who had plasma carotenoids measured at baseline and up to three repeated brain imaging exams in the subsequent 10 years.

Results: In adjusted linear mixed models, each increase of 1 SD in plasma level of total carotenoids and of β-carotene was associated with 0.02 cm^3 (95% CI, 0.001–0.04; $P = 0.04$) and 0.02 cm^3 (95% CI, 0.01–0.04; $P = 0.008$) smaller medial temporal lobe volume loss per year, respectively.

Conclusions: Our results based on a unique long-term prospective evaluation of a neuroimaging biomarker suggest a beneficial role of carotenoids for the prevention of age-related neurodegeneration.

Key words: Carotenoids; Biomarkers; Magnetic Resonance Imaging; Medial Temporal Lobe; Prospective studies; Risk factors in Epidemiology

Abbreviations: 3C, Three-City; *APOEε4*, ε4 allele of the apolipoprotein E gene; BMI, Body Mass Index; CI, confidence Interval; MRI, Magnetic Resonance Imaging; MTL, Medial Temporal Lobe; SD, Standard Deviation.

TEXT

1. Introduction

Carotenoids are vegetable pigments uniquely provided by diet with antioxidant, immunomodulatory and specific neuroprotective properties [1,2]. Carotenes (found in orange-yellow fruits and vegetables) are precursors of retinoids, key signaling molecules for synaptic plasticity [1]. The xanthophylls, lutein and zeaxanthin (found in green vegetables, orange-yellow fruits and corn) cross the blood-brain barrier, may decrease lipid peroxidation and stabilize lipid-protein structures in neuronal membranes [1]. Serum carotenoid levels have been correlated to their concentration in the human brain [3].

Observational and clinical research has demonstrated associations of carotenoids with brain health [2,4]. For example, clinical studies demonstrated efficacy of lutein and/or zeaxanthin supplementation on cognitive decline [5] and on preservation of brain functional connectivity [6] and perfusion [7] with aging. However, evidence of an effect of carotenoids on brain structure and neurodegeneration, which may require longer follow-up period to observe, is more limited.

In a study, within the large population-based Three-City (3C) cohort, using biomarkers of carotenoid intake, we found associations between circulating carotenoid levels and a lower risk of dementia over the subsequent 10 years [8]. Here, we further investigated whether blood carotenoids were also associated with atrophy of the medial temporal lobe (MTL), a biomarker of neurodegeneration in aging [9].

2. Materials and methods

The 3C study is a prospective cohort of non-institutionalized community dwellers (≥65 years), initiated in 1999-2000 in three French cities (Bordeaux, Dijon, Montpellier). All participants

provided written consent and the study protocol was approved by the ethical committee of the Kremlin-Bicêtre University-Hospital (Paris, France). In-person baseline data collection included sociodemographic and lifestyle information, medical history, blood pressure, anthropomorphic measurements, and neuropsychological testing. Plasma carotenoids (α and β-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin) were measured in blood samples collected at baseline [8]. In Bordeaux, an ancillary brain imaging study was conducted, with up to three repeated Magnetic Resonance Imaging (MRI) exams over 10 years (2000-2001, 2004-2006 and 2010-2011) allowing estimation of cerebral atrophy (using Freesurfer 5.1 software; see **Supplementary Methods** for details). MTL volume was defined as the sum of amygdalar, parahippocampal and hippocampal volumes of both hemispheres. The present study included 461 participants non-demented at baseline with plasma carotenoids measured at baseline and at least one analyzable anatomical scan (i.e. exclusion of major brain pathologies or major acquisition artefacts on MRI scans and post-processing failure; 61% had more than one MRI) (**Supplementary Figure**).

Plasma carotenoid levels were modeled as a continuous, standardized variables (i.e. for 1 SD increase), to account for the entire continuum of exposure. For descriptive analyses, we categorized carotenoid levels in four categories around mean ± 1 SD.

The associations of blood carotenoids with MTL volume change over the three repeated MRI exams were estimated using linear mixed models; which 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). Models were adjusted for age, sex, educational level, status for ε4 allele of the apolipoprotein E gene (*APOE*ε4; carrying at least one ε4 allele versus no ε4 allele), plasma triglycerides and total cholesterol concentrations, body mass index (BMI), smoking status (never, former, current), alcohol consumption, practice of regular physical activity, diabetes (fasting blood glucose ≥7.0 mmol/L, or treated), history of cerebral and cardiovascular diseases, hypertension (blood pressure ≥140/90 mmHg, or treated), hypercholesterolemia (plasma total cholesterol ≥6.2 mmol/L, or treated), high depressive symptoms (Center for Epidemiologic Studies-Depression scale score ≥17 for men and ≥23 for women, or being too depressed to answer), and total intracranial volume; and their interactions with time.

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 $\alpha = 0.05$ threshold for statistical significance.

3. Results

The participants, aged 72.5 (±3.9) on average at baseline (**Table**), were followed for a median time of 5.1 years (maximum 11.8). Individuals with higher blood carotenoid levels at baseline were more often female ($P < 0.001$) and *APOE*ε4 carriers ($P = 0.01$), tended to practice more regular physical activity ($P = 0.09$), were less often smokers ($P < 0.001$), had higher fruits and vegetables intakes ($P < 0.001$), and a lower BMI ($P < 0.001$), were less often diabetics ($P = 0.06$), and hypertension ($P = 0.005$) but more hypercholesterolemia ($P < 0.001$) (**Table**). In contrast, no difference was observed for educational level, cognitive performances, alcohol consumption, depressive symptoms, and history of cardiovascular diseases (all $P > 0.05$).

Table. Baseline characteristics of participants, the 3C Bordeaux study, 1999-2011 (n=461)

Characteristics	Total population	Categories of plasma total carotenoids (µg/L)				P-values[a]
		<574 (<mean -1 SD; N=59)	[574; 1168[([mean -1 SD; mean[; N=214)	[1168; 1762[([mean; mean +1 SD[; N=120)	≥1762 (≥mean +1 SD; N=68)	
Age (year)	72.5 ± 3.9	72.4 ± 3.8	72.7 ± 4.1	72.4 ± 3.7	72.3 ± 3.9	0.66
Female	269 (58.4)	20 (33.9)	117 (54.7)	78 (65.0)	54 (79.4)	<0.001
Educational level (≤ secondary)	243 (52.8)	39 (66.1)	106 (49.8)	63 (52.5)	35 (51.5)	0.85
*APOE*ε4	92 (20.2)	10 (16.9)	40 (19.0)	20 (16.9)	22 (32.4)	0.01
MMSE score (range, 0-30)	27.9 ± 1.8	27.9 ± 1.6	27.8 ± 2.0	27.9 ± 1.6	27.9 ± 1.9	0.58
Regular exercise	175 (41.2)	21 (37.5)	78 (40.0)	45 (40.5)	31 (49.2)	0.09
Smoking						<0.001
Never	276 (59.9)	26 (44.1)	118 (55.1)	80 (66.7)	52 (76.5)	
Ex-smoker	162 (35.1)	28 (47.5)	87 (40.7)	34 (28.3)	13 (19.1)	
Current smoker	23 (5.0)	5 (8.5)	9 (4.2)	6 (5.0)	3 (4.4)	
Alcohol consumption						0.54
Never	63 (13.7)	8 (13.6)	27 (12.6)	16 (13.3)	12 (17.6)	
Former	11 (2.4)	1 (1.7)	5 (2.3)	2 (1.7)	3 (4.4)	
Current	387 (83.9)	50 (84.7)	182 (85.0)	102 (85.0)	53 (77.9)	
Fruits and vegetables intake (serving/week)	15.8 ± 3.3	14.3 ± 3.8	15.7 ± 3.5	16.5 ± 2.8	16.6 ± 2.6	<0.001
Body Mass Index (kg/m²)	26.0 ± 3.7	27.4 ± 4.3	26.3 ± 3.3	25.8 ± 3.7	24.5 ± 3.4	<0.001
Diabetes	36 (7.9)	7 (11.9)	20 (9.4)	6 (5.0)	3 (4.5)	0.06
History of cardiovascular diseases	117 (25.4)	13 (22.0)	59 (27.6)	32 (26.7)	13 (19.1)	0.43
High depressive symptoms	22 (4.8)	3 (5.1)	12 (5.6)	5 (4.2)	2 (2.9)	0.44
Hypertension	338 (73.3)	45 (76.3)	165 (77.1)	84 (70.0)	44 (64.7)	0.005
Hypercholesterolemia	260 (56.4)	24 (40.7)	113 (52.8)	81 (67.5)	42 (61.8)	<0.001
Plasma lipids (mmol/L)						
Total cholesterol	5.7 ± 0.9	5.4 ± 0.8	5.6 ± 0.9	6.0 ± 1.0	6.2 ± 1.0	<0.001
Triglycerides	1.2 ± 0.5	1.2 ± 0.6	1.2 ± 0.5	1.2 ± 0.5	1.1 ± 0.5	0.08
MTL volume (cm³)	15.7 ± 1.7	15.7 ± 1.8	15.8 ± 1.8	15.8 ± 1.5	15.4 ± 1.6	0.11
Plasma carotenoids (µg/L)						
Total carotenoids	1168 ± 594	432 ± 98	885 ± 165	1410 ± 156	2274 ± 454	
β-carotene	435 ± 331	109 ± 49	288 ± 103	521 ± 150	1028 ± 378	
α-carotene	103 ± 93	39 ± 16	72 ± 37	118 ± 64	231 ± 152	
Lycopene	253 ± 175	110 ± 59	197 ± 109	309 ± 156	451 ± 232	
Lutein	166 ± 83	95 ± 41	150 ± 64	205 ± 95	210 ± 86	
Zeaxanthin	40 ± 22	23 ± 12	37 ± 18	48 ± 26	50 ± 22	
β-cryptoxanthin	171 ± 124	57 ± 37	140 ± 79	209 ± 123	303 ± 150	

3C, Three-City; *APOE*ε4, ε4 allele of the apolipoprotein E gene; MMSE, Mini-Mental State Examination; MTL, medial temporal lobe.

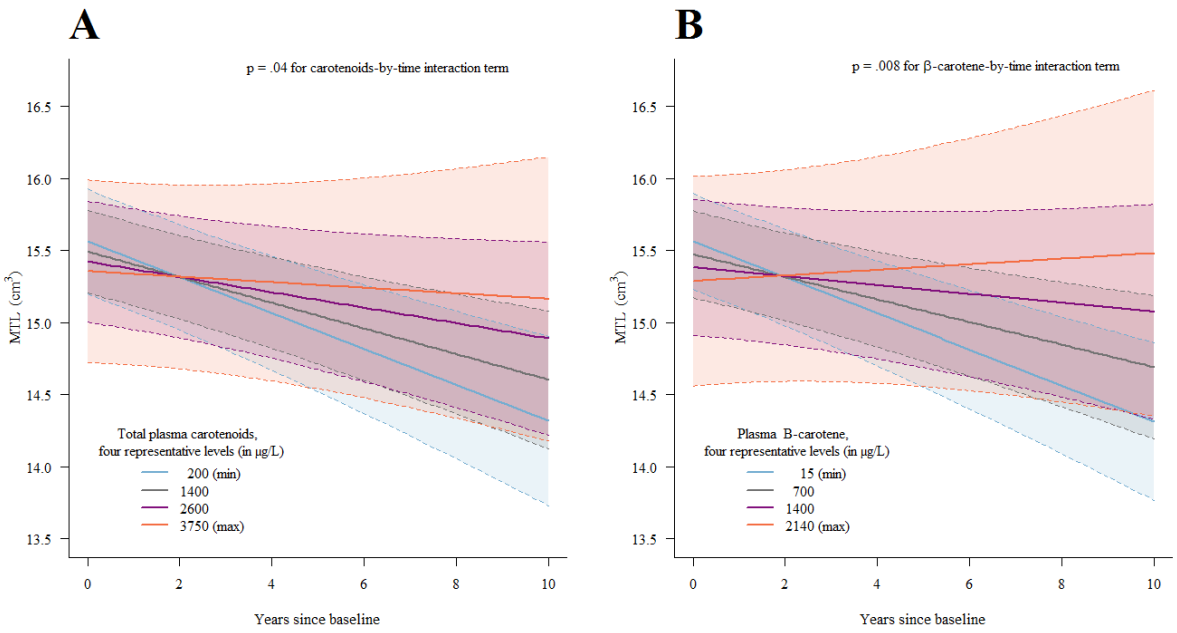
Data are given as mean ± SD and *n* (%) and are for non-missing values. Missing baseline values: 0.2% for educational level and MMSE score, 0.9% for diabetes, 1.1% for *APOE*ε4 status, 7.8% for regular exercise, 10.4% for MTL volume.

[a] *P*-values estimated using unadjusted linear regressions of the continuous measure of plasma total carotenoids with each covariate.

In multivariable models, higher blood levels of total carotenoids and of β-carotene were associated with lower rates of subsequent MTL atrophy (**Fig**). The mean differences in MTL volume change for each increase of 1 SD were 0.02 (95% CI, 0.001–0.04; $P = 0.04$) cm^3/year and 0.02 (95% CI, 0.01–0.04; $P = 0.008$) cm^3/year for total carotenoids and β-carotene, respectively. For comparison, the difference in MTL volume change for each 1 year increase in age at baseline was -0.007 (95% CI, -0.01 to -0.003) cm^3/year; thus, the effect estimates for 1 SD of carotenoids was approximately equivalent to a 3-year-delay in brain atrophy (see **Supplementary Table** for details).

Other carotenoids were not significantly associated with MTL atrophy. We found similar results when: (i) correcting carotenoid values by plasma lipids (cholesterol + triglycerides), which influence their bioavailability, (ii) modeling MTL relative to total intracranial volume, (iii) further adjusting for diet quality (using a Mediterranean diet score), (iv) running complete case analysis (n = 417), and (v) after exclusion of incident cases of cardiovascular diseases during follow-up (n = 428).

Figure. Mean trajectory of medial temporal lobe volume predicted by multivariable linear mixed models according to increasing levels of baseline plasma total carotenoids (panel A) and β-carotene (panel B), the 3C Bordeaux study, 1999-2011 (n = 461)



3C, Three-City; MTL, medial temporal lobe.

Trajectories of change in MTL volume were estimated using linear mixed models across three repeated MRI examinations. Models considered a linear function of time, with corresponding random effect; they also included: an intercept representing MTL volume at baseline (and corresponding individual random effect); total carotenoids (panel A) or β-carotene (panel B) (continuous), covariates, 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.

The mean predicted trajectories (solid lines) with 95% Confidence Intervals (indicated with shading) were plotted for a chosen profile of covariates; we chose four regularly increasing representative levels of total carotenoids or β-carotene (every 1200 µg/L from min [200 µg/L] to max [3750 µg/L] values, for total carotenoids; every 700 µg/L from min [15 µg/L] to max [2140 µg/L], for β-carotene), of an average study participant profile (a woman aged 72 years at study baseline, with no higher than primary education level, *APOEε*4 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, triglycerides and total cholesterol concentrations of 1.2 mmol/L and 5.7 mmol/L respectively, without history of cerebral or cardiovascular diseases, diabetes or high depressive symptoms, with hypertension and hypercholesterolemia, and with a total intracranial volume of 900 cm^3). 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 plasma carotenoid level taken as a continuous variable). Linearity of the carotenoid association was assessed in sensitivity analyses.

4. Discussion

In this large cohort, we found associations of plasma total carotenoids and β-carotene with MTL atrophy suggesting that dietary carotenoids may lower neurodegeneration with aging. These results are in agreement with previous studies reporting associations between higher carotenoids intake and lower cognitive decline or risk of dementia; although most observational studies examined carotenoids through dietary questionnaires and epidemiological findings have been mixed overall [2,4,8].

We did not find any specific association of blood lutein and zeaxanthin with MTL atrophy, which contrasts with the reported associations of serum lutein with volume of the right parahippocampal cortex [10] and with the clinical benefits of both xanthophylls on brain functional connectivity [6] and perfusion [7]. However, in an observational study like ours, blood status reflects both dietary intakes and internal metabolism, and comparison across observational and clinical studies is always difficult in nutrition science. The possibility that β-carotene, a precursor of retinoids involved in synaptic plasticity, is particularly potent to slower age-related neurodegeneration, as suggested by our findings, has biological plausibility and deserves further clinical evaluation. Indeed, preclinical studies showed that β-carotene and retinoids inhibits the deposition of amyloid beta in the brain and might slower neurodegeneration in Alzheimer's disease [1,2]. Moreover, results from observational studies indicate that β-carotene levels are lower in patients with Alzheimer's disease as compared to healthy controls [2]. Prospective studies on β-carotene and cognitive aging yielded more conflicting results, but they were limited to studies using dietary questionnaires (prone to measurement error) to assess exposure, and cognitive change (and not specific biomarkers of neurodegeneration) as an outcome [2,4].

Our epidemiological results bring mechanistic insights into the potential beneficial role of carotenoids in brain aging. The MTL is primarily affected in Alzheimer's disease [9], and

preclinical studies suggested that carotenoids may reduce the accumulation of brain amyloid (a core Alzheimer lesion) [2]. Besides, the MTL is one of the structures most vulnerable to aging, and its integrity appears critical for maintaining cognitive functions and mental health with aging [9]. Carotenoids may thus contribute to preserve vulnerable brain structures such as the MTL from aging and neurodegenerative processes, thereby delaying dementia.

Our study has several major strengths, including a large population-based sample with three repeated MRI exams over 10 years, and the evaluation of carotenoids exposure through blood biomarkers, limiting measurement errors in diet assessment. Moreover, our analyses were controlled for a large number of potential confounders, including lifestyle and diet quality. A limitation of our study is the use of a single blood measurement, which might have caused misclassification in exposure assessment. However, baseline plasma carotenoids were significantly correlated with repeated assessments of fruit and vegetable intakes over the subsequent 10 years; and using a repeated blood measure in a subsample, we found acceptable stability of individual rankings between the two measurements a decade apart.

In summary, our findings based on blood biomarkers of dietary carotenoids and a unique long-term prospective evaluation of a MRI biomarker of neurodegeneration, suggest a beneficial role of carotenoids for the preservation of brain tissue integrity with aging that certainly deserves investigation in large clinical trials.

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