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Plasma Carotenoid Levels and Cognitive Performance in an Elderly Population: Results of the EVA Study

N. Tasnime Akbaraly,[1] Henri Faure,[2] Veronique Gourlet,[3] Alain Favier,[2] and Claudine Berr[1]

[1]INSERM U888 and Université Montpellier 1, France.
[2]Département de Biologie Intégrée, CHU de Grenoble, France.
[3]INSERM U708 and Université Paris 6, France.

Background. The hypothesis of carotenoids having a preventive role in cognitive impairment is suggested by their antioxidant properties.

Methods. We examined, in a cross-sectional analysis, the relationship between cognitive performance (assessed by the Mini-Mental State Examination, Trail Making Test Part B, Digit Symbol Substitution, Finger Tapping Test, and Word Fluency Test) and different plasma carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and *trans*-β-carotene and *cis*-β-carotene) in a healthy elderly population (the EVA,"Etude du Vieillissement Artériel," study; $n = 589$, age $= 73.5 \pm 3$ years).

Results. Logistic regression showed that participants with the lowest cognitive functioning (<25th percentile) had a higher probability of having low levels of specific plasma carotenoids (<1st quartile): lycopene and zeaxanthin. For zeaxanthin, odds ratios (ORs) were as follows: $OR_{DSS} = 1.97$ (95% confidence interval [CI] = 1.21–3.20), $OR_{FTT} = 1.70$ (CI = 1.05–2.74), and $OR_{WFT} = 1.82$ (CI = 1.08–3.07); for lycopene, $OR_{DSS} = 1.93$ (CI = 1.20–3.12) and $OR_{TMTB} = 1.64$ (CI = 1.04–2.59).

Conclusion. Even if it is not possible to affirm if these low levels of carotenoids precede or are the consequence of cognitive impairment, our results suggest that low carotenoid levels could play a role in cognitive impairment. The biological significance of our findings needs further research.

AMONG the leading causes of cognitive impairment, an increase in brain oxidative stress is well documented (1). In fact, the brain is particularly prone to free radical attacks owing to its relatively low antioxidant content, a considerable amount of polyunsaturated fatty acid chains in the neuronal membrane lipids, and its high oxygen consumption rate (2). The hypothesis of carotenoids having a preventive role in cognitive impairment is suggested by their ability to trap peroxyl radicals and their singlet oxygen-quenching properties, which enables them to prevent lipid peroxidation (3,4). Epidemiological studies (5–9) and clinical trials (10,11) on cognitive impairment and plasma carotenoids mainly concern β-carotene, which is a major carotenoid. Some biological studies, however, showed that antioxidant activity of other carotenoids could be more effective than β-carotene activity (12,13). This study's aim is to examine the relationship between cognitive performance and a large variety of carotenoids including xanthophylls (lutein, zeaxanthin, β-cryptoxanthin) and carotenes (lycopene, α-carotene, *trans*-β-carotene and *cis*-β-carotene) in a healthy elderly population.

MATERIALS AND METHODS

Study Population

The EVA ("Etude du Vieillissement Artériel") study is a 9-year longitudinal study with six waves of follow-up (14). During the first 2 years (1991–1993; EVA0), 1389 volunteers (574 men and 815 women) born between 1922 and 1932 (mean age = 65 years) residing in the town of Nantes (Western France) were recruited from electoral rolls, and to a lesser extent, via information campaigns. The sixth and last follow-up of the EVA study (EVA6) was conducted between June 2000 and December 2001. During the 9-year follow-up, 101 deaths occurred. The first leading cause of death was cancer ($n = 45$, 44.5%); the second was cardiovascular diseases ($n = 22$, 21.8%). The main factors related to mortality were found to be, as reported in the literature: male gender, smoking (current and prior), alcohol intake, medication use, obesity, diabetes, hypertension, and cardiovascular diseases (15). At EVA6, blood samples after a 12-hour fast were obtained from 773 participants. Those participants who did not complete the whole study ($n = 616$, 44.3%) were significantly more frequently men (those who did not complete: 44.3% vs those who completed: 38.9%, $p = .04$), obese or overweight (57.9% vs 47.3%, $p = .0004$), or hypertensive (52.6% vs 47.2%, $p = .05$). They were statistically more frequently participants in the lowest cognitive performance class (<25th percentile of the distribution) for the Mini-Mental State Examination (MMSE) (25.2% vs 18.2%, $p = .002$), Digit Symbol Substitution (DSS) (28.3% vs 20.3%, $p = .0006$), Trail Making Test Part B (TMTB) (30.5% vs 21.4%, $p = .0002$), and Word Fluency Test (WFT) (25.1% vs 19.3%, $p = .009$), but we did not observe that for the Finger Tapping Test (FTT) ($p = .37$).

The present analysis was restricted to the 589 participants who underwent at EVA6 a cognitive evaluation and blood sampling. The study protocol was approved by the Ethical Committee of the University Center Hospital of Kremlin-

Bicêtre, Paris. Signed informed consent was obtained from all participants at enrollment.

Data Collection

Cognitive evaluation and depressive symptoms.—Trained neuropsychologists evaluated cognition with a neuropsychological battery of tests including a global test, the MMSE (16), and an assessment of a range of cognitive domains. Visual conceptual and visuomotor tracking were assessed by TMTA and TMTB (17). Involving motor speed and attention functions, the TMT is highly vulnerable to the effects of brain injury (18). Part A is considered as exploring motor speed, control, and working memory, whereas Part B assesses executive functioning such as set shifting. The variables of interest are the time in seconds (19). These tests (Parts A and B) were performed with a maximum allotted time to perform the test of 180 and 240 seconds, respectively. When participants exceeded the time allotted for each part, the maximum allotted time was imputed. The DSS from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) measured sustained attention and logical reasoning (20). Manual dexterity and psychomotor speed were evaluated with the FTT. Verbal fluency was evaluated with the WFT. Depression symptoms were assessed by the Center for Epidemiological Studies-Depression (CESD) scale using score (17 for men and 23 for women) for high risk of depression to define depressive symptomatology (21).

Carotenoid measurement.—Retetinol and carotenoids were measured with a Biotek-Kontron high-performance liquid chromatography (HPLC) system (UVK Lab, Trappes, France), which consists of a 525 dual pump, a 465 autosampler, and a 540 diode array detector. Retinol and β-carotene were purchased from Fluka (Sigma-France, L'Isle d'Abeau), and other carotenoids were provided by Hoffman-La Roche (Hoffman-La Roche, Bâle, Switzerland). The LC separation was run with an Alltech Adsorbosphere C18 column (150 × 4.5 mm ID, 3 μm particle size; Templemars, France), which was thermostated at 28°C with a 402 column oven. Carotenoids and retinol were measured by HPLC after two extractions with a hexane–tetrahydrofurane mixture. For quantification, we used the method of Steghens and colleagues (22) with minor modifications. Indeed, we used a single 150 mm-long column instead of two, and we added 10 ppm water in mobile phase A to improve the separation of retinol, lutein, and zeaxanthin. The laboratory participates in the NIST (National Institute of Standards and Technology, New York, NY) and in the French Society for Vitamins and Biofactors (SFVB) external quality assurance programs, and ChromSystems (Munchen, Germany) internal controls were analyzed in every series of measurements (one control every 10 unknown serums). The limits of detection were calculated as 5-fold the maximum baseline noise in the region of the peaks.

Hence we found limits of detection of 0.05 μM for retinol, and 0.02 μM for the carotenoids. All concentrations of retinol, lycopene, and β-carotene were above these respective limits, and only 5% of lutein, 8% of zeaxanthin, and 2% of β-cryptoxanthin were under. Total plasma carotenoid level was obtained by summing levels of lutein, zeaxanthin, β-cryptoxanthin, lycopene, and α- and β-carotenes.

Questionnaire and medical examination.—The general questionnaire allowed us to obtain information on sociodemographic factors such as sex, age, and educational achievement plus consumption habits such as smoking status, alcohol consumption (which was determined from the participant's estimated average amount of alcoholic beverages ingested weekly), and medication use. In addition, height and weight were measured. Two independent measures of systolic and diastolic blood pressure were taken with a digital electronic tensiometer after a 10-minute rest. Total plasma cholesterol and plasma glucose levels were also measured using routine methods. The apolipoprotein E genotype of participants was determined on DNA samples.

Statistical Methods

The characteristics of the 589 participants included in the analysis were described compared to the 184 participants who had blood sampling but not the cognitive evaluation at EVA6; results were expressed as percentages and means with their standard deviation (*SD*). To test the differences between these two groups, the Chi-square test and the Student *t* test were used. These characteristics comprised sex, age, educational achievement ($\leq$ primary school vs $\geq$ high school), smoking status (current or ex-smokers vs nonsmokers), alcohol consumption ($<$ 20 mL/d vs $\geq$ 20 mL/d), and medicine use ($<$ 3/d vs $\geq$ 3/d). Health characteristics were body mass index (BMI) classes [underweight: BMI < 21 kg/m^2 (23); "normal weight": $21 \geq$ BMI < 25; overweight: $25 \geq$ BMI < 30 (24); obesity ≥ 30 kg/m^2 (24)], diabetes (plasma glucose level $\geq$ 7.80 mmol/L or use of antidiabetic drugs or diabetes medical history), dyslipidemia (total cholesterol $\geq$ 6.20 mmol/L or use of lipid-lowering drugs or dyslipidemia medical history), hypertension (systolic or diastolic blood pressure $\geq$ 140 or $\geq$ 90 mmHg, respectively, or use of hypertensive drugs or hypertension medical history), history of vascular diseases (self-reported history of myocardial infarction, angina pectoris, stroke, or use of vascular drugs), depressive symptomatology (yes/no), and apolipoprotein E genotype (ε4 allele +/–). To calculate the correlation between carotenoids, we calculated Pearson correlation coefficients on log-transformed carotenoids.

The graphic representation of the percentage of participants with low cognitive functioning for the different carotenoid levels showed that the relationship between cognition and these biological variables was not linear, so we considered them to be categorical variables. We compared the characteristics of participants in the different levels of carotenoids ($<$ 25th percentile vs $\geq$ 25th percentile) by using the Student *t* test or the Chi-square test for both continuous and categorical variables.

To define participants with the lowest cognitive performances in this well-educated cohort, we chose two cutoffs: participants who had cognitive test scores below the 25th percentile (and above the 75th percentile for the TMTB), and participants who had cognitive test scores below the 10th percentile (and above the 90th percentile for the

Table 1. Demographic and Clinical Characteristics of the 589 Participants Studied

Variables	% or Mean (±*SD*)*	Range
Sex (women)	61.3	
Age	73.55 ± 2.93	68–79
Education (high school)	52.1	
Smoking status (smoker, ex-smoker)	39.9	
Alcohol consumption (≥ 2 glasses/d)*	24.0	
Medicine consumption (≥ 3/d)*	58.8	
BMI, kg/m^2*		
Underweight (< 21)	13.8	
Overweight (≥ 25 to < 30)	34.0	
Obese (≥ 30)	8.5	
Diabetes	8.1	
Dyslipidemia	65.0	
Hypertension	78.8	
Cardiovascular diseases	19.7	
APOE ε4 carrier*	20.5	
Depressive symptomatology	8.3	
Lutein, μmol/L	0.27 ± 0.15	0.05–1.29
Zeaxanthin, μmol/L	0.032 ± 0.021	0.01–0.34
β-cryptoxanthin, μmol/L	0.23 ± 0.21	0.01–2.03
Lycopene, μmol/L	0.31 ± 0.20	0.06–1.24
α-Carotene, μmol/L	0.19 ± 0.15	0.02–0.93
Trans-β-carotene, μmol/L	0.73 ± 0.52	0.04–5.48
Cis-β-carotene, μmol/L	0.10 ± 0.12	0.01–1.19
Total carotenoids, μmol/L	1.86 ± 0.99	0.15–8.35
Test scores		
MMSE, points	28 ± 2	5–30
DSS, points*	45 ± 11	12–85
TMTA, s*	48.9 ± 19.6	18–180
TMTB, s*	110.4 ± 46.3	30–240
FTT, taps*	127 ± 21	62–185
WFT, points*	27 ± 8	5–50

Notes: * For these variables, there are missing values. Alcohol consumption: $n = 10$, medicine consumption: $n = 1$, BMI class: $n = 22$, APOE ε4: $n = 39$, depressive symptomatology: $n = 23$, DSS: $n = 30$, TMTA: $n = 45$, TMTB: $n = 31$, FTT: $n = 43$, and WFT: $n = 128$.

SD = standard deviation; APOE = apolipoprotein E; BMI = body mass index; MMSE = Mini-Mental State Examination; TMTA = Trail Making Test Part A; TMTB = Trail Making Test Part B; DSS = Digit Symbol Substitution; FTT = Finger Tapping Test; WFT = Word Fluency Test.

Table 2. Percentile Distributions for Each Carotenoid and Cognitive Variable

Variables	10th Percentile	25th Percentile	50th Percentile	75th Percentile
Carotenoids, μmol/L				
Lutein		0.176	0.235	0.335
Zeaxanthin		0.020	0.029	0.042
β-Cryptoxanthin		0.098	0.167	0.288
Lycopene		0.160	0.257	0.403
α-Carotene		0.098	0.154	0.245
Trans-β-carotene		0.374	0.607	0.953
Cis-β-carotene		0.013	0.067	0.143
Total carotenoids		1.16	1.65	≥ 2.35
Cognitive variables				
MMSE, points	27	28		
DSS, points	30	37		
TMTA, s	70*	55*		
TMTB, s	178*	130*		
FTT, taps	100	114		
WFT, points	16	21		

Notes: * For TMTB and TMTA, we present values of 75th and 90th percentile of distribution of time.

MMSE = Mini-Mental State Examination; DSS = Digit Symbol Substitution; TMTA = Trail Making Test Part A; TMTB = Trail Making Test Part B; FTT = Finger Tapping Test; WFT = Word Fluency Test.

TMTB). Values for these 25th and 10th percentile cutoffs for each test are presented in Table 2.

Classical multivariate logistic regressions were performed to test associations between probability of participants to have the lowest cognitive functioning and levels of plasma carotenoids (< 25th percentile vs > 25th percentile), adjusting for all potential confounding variables. Results were expressed as odds ratios (OR) with their 95% confidence intervals (CI). All interactions between a carotenoid and each consumption habit and health variable were calculated and were not statistically significant. Statistical analyses were performed using SAS software (version 9.1; SAS Institute, Inc., Cary, NC).

RESULTS

Characteristics of the 589 EVA Participants

The 589 participants in the EVA study (361 women and 228 men, age = 73.5 ± 2.9 years), were well educated (52.1% had a high school or superior degree). Among them, 39.0% were smokers or ex-smokers, 24% consumed alcohol regularly (≥ 2 glasses/day). In dividing participants among BMI classes, we observed 13.8% underweight, 34.0% overweight, and 8.5% obese. Concerning health status, 8.3% showed depressive symptomatology, 8.1% were diabetics, 65.0 % were dyslipidemic, 78.8% had hypertension, 19.7% had a history of cardiovascular disease, and 20.5% carried at least one allele ε4 of the apolipoprotein E. For the different carotenoids and scores on neuropsychological tests, concentrations and ranges are described in Table 1, and percentile distributions in Table 2.

We also compared the characteristics of these 589 participants included in the analysis to the 184 participants for whom we obtained a blood sample but not a cognitive evaluation. Results showed that these 184 participants were significantly older (74.2 ± 3.1 years vs 73.5 ± 2.9 years), and proportions of participants with hypertension ($p = .05$) or with a history of cardiovascular disease ($p = .03$) were higher. In contrast, proportions of participants with low levels of β-cryptoxanthin and *trans*- and *cis*-β-carotene were significantly lower in this group (results not shown).

Description of Carotenoids: Correlation and Associated Factors

Plasma carotenoids were highly and significantly intercorrelated. The highest correlations were found among the following carotenes: α-carotene/*trans*-β-carotene ($r = 0.78$), *trans*-β-carotene/*cis*-β-carotene ($r = 0.61$), α-carotene/lycopene ($r = 0.60$) and among lutein/zeaxanthin ($r = 0.58$). The other correlation coefficients ranged from 0.16 for lycopene/β-cryptoxanthin to 0.50 for *trans*-β-carotene/lycopene.

In Table 3 and Table 4, we describe the characteristics of participants according to their total plasma carotenoid, the different xanthophylls, and carotenes levels (< 25th

Table 3. Characteristics of Participants According to Their Level of Plasma Total Carotenoids and Xanthophylls (<25th Percentile vs ≥25th Percentile)

	Total Carotenoids					Lutein					Zeaxanthin					Beta-Cryptoxanthin				
	<25th Percentile		≥25th Percentile			<25th Percentile		≥25th Percentile			<25th Percentile		≥25th Percentile			<25th Percentile		≥25th Percentile		
Variables	*N*	%	*N*	%	*p*	*N*	%	*N*	%	*p*	*N*	%	*N*	%	*p*	*N*	%	*N*	%	*p*
Age	144	73.5 ± 2.9	445	73.6 ± 2.9	.71	174	73.6 ± 3.0	442	73.5± 2.9	.92	143	73.8± 3.0	446	74.5 ± 2.9	.32	146	73.7 ± 2.8	443	73.5 ±3.0	.46
Sex																				
Men	80	35.1	148	64.9	$<10^{-4}$	71	31.1	157	68.9	.005	59	25.9	169	74.1	.47	89	39.0	139	61.0	$<10^{-4}$
Women	64	17.7	297	82.3		76	21.0	285	79.0		84	23.3	277	76.7		57	15.8	304	84.2	
Education																				
Primary School	71	25.2	211	74.8	.69	68	24.1	214	75.9	.65	69	24.5	213	75.5	.43	74	26.2	208	73.8	.43
High school	73	23.8	235	76.2		79	25.7	228	74.3		74	24.1	233	75.9		72	23.4	235	76.5	
Smoking status																				
Nonsmoker	67	18.7	292	81.3	$<10^{-4}$	81	22.6	278	77.4	.09	89	24.8	270	75.2	.72	68	18.9	291	81.1	$<10^{-4}$
Smoker or ex-smoker	77	33.5	153	66.5		66	28.7	164	71.3		54	23.5	176	76.5		78	33.9	152	66.1	
Alcohol																				
< 20 mL/d	97	21.8	347	78.1	.008	105	23.6	339	76.3	.18	108	24.3	336	75.7	.86	99	22.3	345	77.7	.01
≥ 20 mL/d	46	32.9	94	67.1		41	29.3	99	70.7		33	23.6	107	76.4		46	32.9	94	67.1	
Medicine use																				
< 3/d	43	17.8	199	82.2	.001	62	25.6	180	74.3	.71	52	21.5	190	78.5	.21	53	21.9	189	78.1	.19
≥ 3/d	161	29.2	245	70.81		84	24.3	262	75.7		96	26.0	256	74.0		92	26.6	254	73.4	
BMI classes, kg/m^2																				
Normal	46	18.5	202	81.4	$<10^{-4}$	50	20.2	198	79.8	$<10^{-4}$	51	20.6	197	79.4	.26	60	24.2	188	75.8	.13
Underweight	9	11.5	69	88.5		9	11.5	69	88.5		19	24.4	59	75.6		12	15.4	66	84.6	
Overweight	60	31.1	133	68.9		56	29.0	137	71.0		48	24.9	145	75.1		56	29.0	137	71.0	
Obese	21	43.7	27	56.2		21	43.7	27	56.2		16	33.3	32	66.7		12	25.0	36	75.0	
Depressive symptoms																				
No	125	24.1	394	78.1	.82	126	24.3	393	75.7	.64	124	23.9	395	76.1	.94	128	24.7	391	75.3	.89
Yes	12	25.5	35	43.7		10	21.3	37	78.7		11	23.4	36	76.6		12	25.5	35	74.5	
Diabetes																				
No	117	21.6	424	78.4	$<10^{-4}$	127	23.5	414	76.5	.005	123	22.7	418	77.3	.003	124	22.9	417	77.1	$<10^{-3}$
Yes	27	56.2	21	43.7		20	41.7	28	58.3		20	41.7	28	58.3		22	45.8	26	54.2	
Hypertension																				
No	15	12.0	110	88.0	.003	20	16.0	105	84.0	.009	29	23.2	96	76.8	.75	31	24.8	94	75.2	.99
Yes	129	27.8	335	72.2		127	27.4	337	72.6		114	24.6	350	75.4		115	24.8	349	75.2	
History of CVD																				
No	111	23.5	362	76.5	.26	115	24.3	358	75.7	.46	111	23.5	362	76.5	.35	116	24.5	357	75.5	.76
Yes	33	28.5	83	71.5		32	27.6	84	72.4		32	27.6	84	72.4		30	25.9	86	74.1	
Dyslipidemia																				
No	44	21.4	162	78.6	.21	53	25.7	153	74.3	.75	52	25.2	154	74.8	.69	57	27.7	149	72.3	.23
Yes	100	26.1	283	73.9		94	24.5	289	75.5		91	23.8	292	76.2		89	23.2	294	76.8	
APOE ε4																				
No	107	24.5	330	75.5	.90	103	23.6	334	76.4	.30	100	22.9	337	77.1	.31	110	25.2	327	74.8	.93
Yes	27	23.9	86	76.1		32	28.3	81	71.7		31	27.4	82	72.6		28	24.8	85	75.2	

Notes: For age and body mass index (BMI), data are presented as mean ± standard deviation (*SD*).
CVD = cardiovascular disease; APOE = apolipoprotein E.

Table 4. Characteristics of Participants According to Level of Plasma Carotenes (<25th Percentile vs ≥25th Percentile)

	Lycopene					α-Carotene					*Trans*-β-Carotene					*Cis*-β-Carotene				
	<25th Percentile		≥25th Percentile			<25th Percentile		≥25th Percentile			<25th Percentile		≥25th Percentile			<25th Percentile		≥25th Percentile		
Variables	*N*	%	*N*	%	*p*	*N*	%	*N*	%	*p*	*N*	%	*N*	%	*p*	*N*	%	*N*	%	*p*
Age	145	73.7 ± 2.9	444	73.5 ± 2.9	.34	144	73.7 ± 3.0	445	73.5 ± 2.9	.58	147	73.5 ± 2.9	442	73.5 ± 2.9	.99	144	73.3 ± 3.0	445	73.6 ± 2.9	.28
Sex																				
Men	66	28.9	162	71.1	.05	72	31.6	156	68.4	.001	82	36.0	146	64.0	$<10^{-4}$	79	34.6	149	65.3	$<10^{-4}$
Women	79	21.9	282	78.1		72	19.9	289	80.1		65	18.0	296	82.0		65	18.0	296	92.0	
Education																				
Primary School	73	25.9	209	74.1	.49	63	22.3	219	77.7	.25	70	24.8	212	75.2	.94	67	23.8	215	76.2	.71
High school	72	23.4	235	76.5		81	26.4	226	73.6		77	25.1	230	74.9		77	25.1	230	74.9	
Smoking status																				
Nonsmoker	83	23.1	276	76.9	.29	75	20.9	284	79.1	.01	68	18.9	291	81.1	$<10^{-4}$	67	18.7	292	81.3	$<10^{-4}$
Smoker or ex-smoker	62	27.0	168	73.0		69	30.0	161	70.0		79	34.3	151	65.6		77	33.5	153	66.5	
Alcohol																				
< 20 mL/d	106	23.9	338	76.1	.43	96	21.6	348	78.4	.002	100	22.5	344	77.5	.009	99	22.3	345	77.7	.02
≥ 20 mL/d	38	27.1	102	72.9		48	34.3	92	65.7		47	33.6	93	66.4		45	32.1	95	64.9	
Medicine use																				
< 3/d	50	20.7	192	72.3	.06	53	21.9	189	78.1	.22	54	22.3	188	77.7	.21	45	18.6	197	81.4	.007
≥ 3/d	95	27.5	251	72.5		91	26.3	255	73.7		93	26.9	253	73.1		98	28.3	248	71.7	
BMI class, kg/m^2																				
Normal	49	19.8	199	80.2	.03	48	19.3	200	80.7	$<10^{-4}$	40	16.1	208	83.9	$<10^{-4}$	51	20.6	197	79.4	.03
Underweight	20	25.6	58	74.4		7	9.0	71	91.0		12	15.4	66	84.6		12	15.4	66	84.6	
Overweight	49	25.4	144	74.6		61	31.6	132	68.4		66	34.2	127	65.8		58	30.0	135	69.9	
Obese	19	39.6	29	60.4		19	39.6	29	60.4		21	43.7	27	56.2		13	27.1	35	72.9	
Depressive symptoms																				
No	128	24.7	391	75.3	.40	130	25.0	389	75.0	.57	131	25.2	388	74.8	.35	124	23.9	395	76.1	.69
Yes	9	19.1	38	80.8		10	21.3	37	78.7		9	19.1	38	80.8		10	21.3	37	78.7	
Diabetes																				
No	123	22.7	418	77.3	.0004	121	22.4	420	77.6	$<10^{-4}$	122	22.5	419	77.5	$<10^{-4}$	122	22.5	419	77.4	$<10^{-3}$
Yes	22	45.8	26	54.2		23	47.9	25	52.1		25	52.1	23	47.9		22	45.8	26	54.2	
Hypertension																				
No	27	21.6	98	78.4	.38	20	16.0	105	84.0	.01	14	11.2	111	88.8	$<10^{-4}$	17	13.6	108	86.4	.001
Yes	118	25.4	346	74.6		124	26.7	340	73.3		133	28.7	331	71.3		127	27.4	337	72.6	
History of CVD																				
No	114	24.1	359	75.9	.55	111	23.5	362	76.5	.26	117	24.7	356	75.3	.80	114	24.1	359	75.9	.69
Yes	31	26.7	85	73.3		33	28.4	83	71.5		30	25.9	86	74.1		30	25.9	86	74.1	
Dyslipidemia																				
No	51	24.8	155	75.2	.95	50	24.3	156	75.7	.94	42	20.4	164	79.6	.06	45	21.8	161	78.2	.28
Yes	94	24.5	289	75.5		94	24.5	289	75.5		105	27.4	278	72.6		99	25.8	284	74.1	
APOE ε4																				
No	105	24.0	332	76.0	.26	115	26.3	322	73.7	.27	111	25.4	326	74.6	.60	109	24.9	328	75.1	.97
Yes	33	29.2	80	70.8		24	21.2	89	78.8		26	23.0	87	77.0		28	24.8	85	75.2	

Notes: For age and body mass index (BMI), values are mean ± standard deviation (*SD*).
CVD = cardiovascular disease; APOE = apolipoprotein E.

Table 5. Crude Risk of Low Cognitive Functioning (Score < 25th Percentile) Associated With Plasma Carotenoids for Each Cognitive Test

Plasma Antioxidant (<25th vs ≥25th Percentile)	MMSE			TMTA			TMTB		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Total carotenoids	0.88	0.55–1.40	.59	1.13	0.73–1.76	.58	1.57	1.03–2.40	.04
Lutein	1.00	0.63–1.58	.99	1.01	0.65–1.57	.96	1.50	0.98–2.30	.06
Zeaxanthin	1.37	0.88–2.13	.17	1.66	1.08–2.55	.02	1.60	1.04–2.44	.03
β-Cryptoxanthin	1.25	0.80–1.96	.32	0.95	0.61–1.47	.81	1.07	0.69–1.65	.77
Lycopene	1.03	0.65–1.62	.91	1.37	0.89–2.10	.15	1.76	1.16–2.67	.008
α-Carotene	0.83	0.51–1.33	.44	0.84	0.53–1.32	.45	1.21	0.78–1.86	.39
Trans-β-carotene	0.71	0.44–1.15	.17	0.98	0.63–1.53	.94	1.58	1.04–2.41	.03
Cis-β-carotene	0.61	0.37–1.00	.05	1.17	0.76–1.82	.47	1.02	0.65–1.58	.94
	DSS			FTT			WFT		
Total carotenoids	1.12	0.71–1.76	.62	1.22	0.78–1.89	.38	0.67	0.40–1.13	.13
Lutein	1.24	0.79–1.95	.34	1.39	0.90–2.15	.13	0.89	0.54–1.47	.65
Zeaxanthin	1.87	1.21–2.89	.005	1.70	1.10–2.62	.02	1.87	1.16–3.00	.01
β-Cryptoxanthin	1.25	0.80–1.94	.33	0.79	0.50–1.25	.31	0.83	0.50–1.36	.46
Lycopene	2.02	1.32–3.11	.01	1.43	0.93–2.21	.10	1.16	0.72–1.89	.54
α-Carotene	0.73	0.45–1.18	.20	1.11	0.71–1.73	.64	0.66	0.39–1.11	.12
Trans-β-carotene	0.93	0.58–1.47	.75	1.08	0.70–1.68	.72	0.72	0.43–1.10	.21
Cis-β-carotene	1.24	0.79–1.95	.34	0.94	0.59–1.48	.78	0.74	0.45–1.23	.25

Notes: Data are shown as odds ratios (OR) and 95% confidence intervals (CI) of probability of participants to have the lowest cognitive functioning associated with each plasma carotenoid level.

MMSE = Mini-Mental State Examination; TMTA = Trail Making Test Part A; TMTB = Trail Making Test Part B; DSS = Digit Symbol Substitution; FTT = Finger Tapping Test; WFT = Word Fluency Test.

percentile vs ≥ 25th percentile). We observe that the profiles of factors associated with α-carotene, *trans*-β-carotene, and *cis*-β-carotene were identical. Between the other carotenoids, however, the associated factors can differ. Gender was associated with all carotenoids, which (with the exception of zeaxanthin) reach higher levels in women. Smoking status and alcohol consumption were significantly associated with lower levels of total plasma carotenoids, β-cryptoxanthin, α-carotene, and *trans*- and *cis*-β-carotene. Diabetes was associated with low levels of all carotenoids, and hypertension was significantly associated with low levels of lutein, α-carotene, and *trans*- and *cis*-β-carotene. Being obesity or overweight was associated with low levels of all carotenoids except for zeaxanthin and β-cryptoxanthin. Age, education, depressive symptomatology, dyslipidemia, history of cardiovascular disease, and apolipoprotein E genotype were not associated with any plasma carotenoids.

Association Between Cognition and Carotenoids

Table 5 shows the results of crude associations between cognitive performance and carotenoid levels, obtained by univariate logistic regression analyses. Participants with the lowest cognitive performance (neuropsychological test scores < 25th percentile) had a higher probability of having low levels of some carotenoids (level < 1st quartile). Significant associations were observed between zeaxanthin and all cognitive tests except the MMSE (for the TMTA, OR = 1.66 [CI = 1.08–2.55]; for the TMTB, OR = 1.60 [CI = 1.04–2.44]; for the DSS, OR = 1.87 [CI = 1.21–2.89]; for the FTT, OR = 1.70 [CI = 1.10–2.62], and for the WFT, OR = 1.87 [CI = 1.16–3.00]). Low levels of lycopene were associated with low performance on the TMTB (OR = 1.76 [CI = 1.16–2.67]) and on the DSS (OR = 2.02 [CI = 1.32–3.11]). A significant association was found between low performance on the TMTB and low levels of total plasma carotenoids and *trans*-β-carotene (OR = 1.57 [CI = 1.03–2.40] and OR = 1.58 [CI = 1.04–2.41]). After taking into account sociodemographic factors (sex, age, education), consumption habits (tobacco, alcohol), diabetes, hypertension, and BMI class (Table 6), associations between zeaxanthin and cognitive tests remained statistically significant for the TMTA (OR = 1.67 [CI = 1.06–2.66]), the DSS (OR = 1.92 [CI = 1.18–3.14]), the FTT (OR = 1.69 [CI = 1.05–2.72]) and the WFT (OR = 1.80 [CI = 1.07–3.05]), but not for the TMTB (OR = 1.51 [CI = 0.95–2.39]; *p* = .08). Lycopene remained associated with the TMTB (OR = 1.54 [CI = 0.97–2.43]; *p* = .06) and the DSS (OR = 1.85 [CI = 1.14–2.98]). The other associations between carotenoids and cognitive performance observed in the crude analyses did not remain statistically significant after adjustment. Total plasma carotenoids, α-carotene, and β-carotene (*trans* or *cis*) levels were not statistically associated to low cognitive performance, nor were lutein and β-cryptoxanthin.

Sensitivity Analysis

The same analyses were first performed by removing participants with depressive symptomatology (*n* = 49), then by removing participants who were underweight (BMI < 21 kg/m^2) (*n* = 81), and then by adjusting for levels of plasma retinol. In the three situations, we obtained similar results. We also performed analyses on participants who had an MMSE score ≥ 25 (*n* = 570) to exclude participants with potential clinically significant cognitive impairment (*n* = 19). Results were identical (data not shown).

We also repeated analyses between cognitive performances and carotenoids levels by defining participants with

Table 6. Adjusted Risk of Low Cognitive Functioning (Score < 25th Percentile) Associated With Plasma Carotenoids for Each Cognitive Test

Plasma Antioxidant (<25th vs ≥25th Percentile)	MMSE			TMTA			TMTB		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Total carotenoids	0.93	0.55–1.58	.80	1.27	0.77–2.09	.34	1.43	0.88–2.32	.14
Lutein	1.03	0.62–1.71	.89	1.15	0.71–1.88	.57	1.51	0.94–2.42	.09
Zeaxanthin	1.24	0.77–2.00	.38	1.67	1.06–2.66	.03	1.51	0.95–2.39	.08
β-Cryptoxanthin	1.39	0.85–2.28	.19	1.05	0.64–1.71	.84	1.06	0.65–1.74	.82
Lycopene	0.91	0.55–1.50	.71	1.39	0.88–2.21	.16	1.54	0.97–2.43	.06
α-Carotene	0.89	0.53–1.49	.66	0.96	0.59–1.58	.88	1.16	0.72–1.86	.55
Trans-β-carotene	0.74	0.43–1.27	.27	1.11	0.68–1.83	.67	1.48	0.91–2.37	.11
Cis-β-carotene	0.56	0.32–0.98	.04	1.41	0.87–2.29	.16	0.96	0.58–1.57	.86
	DSS			FTT			WFT		
Total carotenoids	0.95	0.56–1.63	.87	1.53	0.91–2.55	.11	0.66	0.36–1.21	.18
Lutein	1.25	0.75–2.10	.39	1.48	0.90–2.42	.12	0.94	0.54–1.65	.84
Zeaxanthin	1.92	1.18–3.14	.008	1.69	1.05–2.72	.03	1.80	1.07–3.05	.03
β-Cryptoxanthin	1.01	0.61–1.68	.97	1.01	0.61–1.69	.95	0.72	0.41–1.27	.26
Lycopene	1.85	1.14–2.98	.01	1.44	0.89–2.32	.13	1.05	0.61–1.79	.87
α-Carotene	0.64	0.37–1.12	.12	1.24	0.76–2.03	.39	0.78	0.43–1.41	.41
Trans-β-carotene	0.75	0.43–1.29	.30	1.36	0.82–2.25	.23	0.75	0.41–1.37	.35
Cis-β-carotene	1.24	0.74–2.09	.41	1.27	0.76–2.13	.37	0.80	0.45–1.41	.44

Notes: Odds ratios (OR) with their 95% confidence intervals (CI) are shown as probability of participant to have the lowest cognitive functioning associated to each plasma carotenoid level, adjusted for sociodemographic factors (sex, age, education), consumption habits (smoking status, alcohol consumption, medicine use), and health variables (body mass index, diabetes, and hypertension).

MMSE = Mini-Mental State Examination; TMTA = Trail Making Test Part A; TMTB = Trail Making Test Part B; DSS = Digit Symbol Substitution; FTT = Finger Tapping Test; WFT = Word Fluency Test.

the lowest cognitive performances as having cognitive test scores below the 10th percentile. Only associations between zeaxanthin and FTT ($p = .003$) and TMTB ($p = .06$), and between lycopene and DSS ($p = .07$) remained marginally statistically significant (data not shown). In these analyses, the number of participants who had the lowest cognitive performance scores was 2.2 to 2.8 smaller than for the preceding analyses ($n = 54$ for the MMSE, 49 for the DSS, 55 for the TMTA, 53 for the TMTB, 52 for the FTT, and 38 for the TEL). Lack of statistical power could explain the drop in statistically significant results.

All of our analyses were conducted with dichotomized variables for both the plasma carotenoids and the cognitive outcomes; in supplemental analyses, we tested whether similar findings were observed when a continuous measure for cognition was used. We calculated Spearman correlation coefficients between cognitive variables and log-transformed carotenoids. Results showed a significant association for zeaxanthin and lycopene and DSS ($r = 0.08$, $p = .01$ and $r = 0.11$, $p = .006$ for zeaxanthin and lycopene, respectively), TMTA ($r = -0.11$, $p = .01$ and $r = -0.08$, $p = .05$), TMTB ($r = -0.08$, $p = .05$ and $r = -0.12$, $p = .004$), and FTT ($r = 0.09$, $p = .04$ and $r = 0.09$, $p = .04$). These correlations confirm that our findings were not driven by the chosen dichotomous classifications for cognition and carotenoid levels.

Discussion

To our knowledge, this study is the first that investigated, in a healthy elderly population, the relationship between cognitive performance measured by five neuropsychological tests and the different plasma carotenoids: xanthophylls (lutein, zeaxanthin, β-cryptoxanthin) and carotenes (lycopene, α-carotene, *trans*-β-carotene, and *cis*-β-carotene).

The EVA study included volunteers with higher educational status, higher incomes, and greater cognitive function than the average elderly French population. Despite this selection, plasma carotenoid concentrations in the EVA study population were in the same ranges as those in different European or American populations (4). In this present study, low levels of specific plasma carotenoids—lycopene and zeaxanthin—were associated to poor cognitive functioning in a highly educated, community-dwelling elderly population. Carotenoids are found in fruits and vegetables. Some studies have shown that plasma carotenoid levels could be related to dietary fruit and vegetable intake (25,26). More specifically, it seems that mangos, papayas, peaches, prunes, squash, oranges, and green fruits and vegetables are source of zeaxanthin, whereas tomatoes, pink grapefruit, and watermelon are sources of lycopene (3). Two cross-sectional studies (27,28) showed an association between greater intake of fruits and vegetables and better cognitive performance. In a large prospective study of older women ($n = 13{,}388$) (29), the authors reported a relationship between low vegetable intake and cognitive decline but no relationship with fruits (the strongest association being with greater intake of green leafy vegetables and cruciferous vegetables). Even if some studies show that intake of foods with high carotenoid contents are correlated with their corresponding plasma concentrations (26), fruits and vegetables are sources of many other nutrients [vitamin E (30), folates (31,32), flavonoids (33)] that have been associated with cognitive function. It is now impossible to know if the association between carotenoids and cognitive function is the result of a specific effect of carotenoids or if it is the result of combined effects of the different fruit and vegetable compounds.

For each cognitive test, we tested an eight-association hypothesis (Ho) between cognitive function and carotenoids. To ensure that significant observed associations were not hazard related (alpha risk = 5%), specific multiple test (Bonferroni, Sidak) corrections were applied to the data. Considering, however, that the tested hypotheses are not independent, submitting them to these corrections entails an overcorrection of threshold significance thus not necessarily leading to rejection of the Ho hypothesis, which we know to be false. In this exploratory analysis, the fact that one specific carotenoid was associated with more than one cognitive function, and that these associations remained statistically significant after controlling for potential confounding factors or after removing some participants (those with depressive symptomatology, those with an MMSE score < 25, or undernourished participants) seems to us more likely to ensure that our significant observed associations were not hazard related.

Although there were important correlations among all carotenoids, we observed significant associations between low cognitive performances and some (but not all) carotenoids. More specifically, we found no associations between β-carotenes (*trans* or *cis*) or α-carotene, which are the most studied carotenoids in epidemiological literature on cognitive impairment (5–11). These studies give conflicting results. Previously, in the EVA study, we showed that a low level of baseline total plasma carotenoid (< 25th percentile) was not significantly associated with a 4-year cognitive decline (5). In a cross-sectional study, Perrig and colleagues (9) showed that a higher β-carotene plasma level was associated with better memory performances (free recall, recognition, and vocabulary) in 442 healthy persons aged 65–94 years. Studying dietary intake of β-carotene, Morris and colleagues (8) found no association between carotene intake and cognitive decline, whereas the Rotterdam Study showed that a lower intake of β-carotene was associated with impaired cognitive function measured by the MMSE (7). Three studies investigated the link between cognitive performance and supplementation of antioxidants including β-carotene (6,10,11). All, except the work of the Age-Related Eye Diseases Study Research Group (11), found that use of these supplements reduced the risk of cognitive decline. In these studies with multiantioxidant supplementation it is, however, impossible to isolate the specific effect of β-carotene on cognitive impairment. Only one study, with 1769 participants, focused on the association between a large spectrum of carotenoids and cognitive performance in elderly participants without neuropsychiatric disease, but it found no association (34). Discrepancies can be explained by methodological differences (e.g., neuropsychological tests to assess cognitive performances, number of tests used, choice of modelization of carotenoids). Finally, we found two clinical epidemiological studies (35,36) that focused on the comparison of plasma carotenoids and retinol levels in elderly patients with or without Alzheimer's disease (AD). One study showed that levels of vitamin A, lutein, zeaxanthin, β-cryptoxanthin, and α-carotene were lower in AD patients ($n = 63$) than in controls ($n = 56$). However, the researchers found no difference for lycopene and β-carotene (36). In the second study, levels of carotenoids were lower in AD patients ($n = 40$) than in controls ($n = 39$) for zeaxanthin, β-cryptoxanthin, lycopene, and β-carotene but not for lutein and α-carotene (35). These conflicting results could be explained by the limited sample size of these studies. The major problem in interpreting these two studies is that they are studying AD cases for which nutritional habits, and consequently, biological status, can be modified, as a consequence of the disease progression. The different measurement methods and the bioavailability of carotenoids, which is influenced by several factors [such as characteristics of the food sources, interactions with other dietary factors, and various participant characteristics (3)] could also explain the differences between studies.

Many epidemiological studies have associated high carotenoid status with a decrease in the incidence of chronic diseases (heart diseases and cancer); the biological mechanism for such protection is, however, currently unclear (3). Multiple possibilities exist. Among them, certain carotenoids can be converted to retinoids and have a provitamin A activity. To assess if the relationship we showed could be explained partly by this hypothesis, we adjusted our models for retinol concentration, but results remained unchanged.

Our results are supported by those of a biological study, led by Woodall and colleagues (37), on oxidation of carotenoids by free radicals. The authors found that lycopene, lutein, and zeaxanthin all reacted rapidly with oxidizing agents, and must also be considered as potential dietary antioxidants. A possible explanation for low levels of plasma carotenoids in AD or cognitive impairment is that they might be consumed because of a higher rate of free radical production in the brain.

Our results on the relationship between carotenoids and cognitive performance were not modified when we excluded the participants with BMI < 21 kg/m^2 for which the hypothesis of undernutrition is probable. This finding allows us to say that this relationship could not be limited by the influence of undernourished participants. However, in this cross-sectional framework, it is not possible to affirm whether these low levels of carotenoids preceded or were the consequence of cognitive impairment in a context in which poor cognitive status may be a risk factor for poor nutrition. In this study, we used various tests to explore cognitive performance; this approach is more powerful than using only MMSE, which is not a very sensitive measurement of cognitive impairment. With the other tests, the psychometric scores ranges are large and more powerful to study cognitive impairment. In addition, because the scores were not normally distributed, a percent cut-off is appropriate. The observed association will probably have no functional significance yet, because participants had only a subtle impairment. Moreover, we have no basis to expect specific association between carotenoids and psychometric evaluation. However, it is well known that low plasma lutein and zeaxanthin concentrations were implicated in age-related macular degeneration (38). Although the retina is a puzzle (the ultimate solution of which lies on the other side of the optic nerve in its connection with the brain), a highly specific accumulation of lutein and zeaxanthin in the retina and in the macula is described (39). Could other areas of the brain have the same affinity for some specific carotenoids? The biological significance of our findings needs further research by biological studies, longitudinal epidemiological studies, and by specific clinical trials with carotenoid supplementation.

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CORRESPONDENCE

Address correspondence to N. Tasnime Akabaraly, PhD, INSERM U888, Hôpital La Colombière, 39 Avenue Charles Flahault, BP 34493, 34093 Montpellier, Cedex 5, France. E-mail: akbaraly@montp.inserm.fr

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