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

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Plasma carotenoids levels and cognitive performances in an elderly population

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Short running head: Carotenoids and Cognition

Abstract

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 performances (assessed by the Mini Mental Status Examination: MMSE, Trail Making Test part B: TMTB, Digit Symbol Substitution: DSS, Finger Tapping Test: FTT and Word Fluency Test: WFT) and different plasma carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, trans and cisβ-carotene), in a healthy elderly population: the EVA study "Etude du Vieillissement Artériel" (n=589, 73.5 ± 3 years).

Results: Logistic regression showed that subjects 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 OR_{DSS}=1.97 [1.21; 3.20], OR_{FTT}=1.70 [1.05; 2.74] and OR_{WFT} =1.82 [1.08; 3.07]), for lycopene OR_{DSS} =1.93 [1.20; 3.12] and OR_{TMTB}=1.64 [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.

Keywords:

Plasma carotenoids

Cognitive performances

Elderly population

Cross sectional epidemiological study

1. Introduction

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 antioxidants 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. But some biological studies 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 performances and a large variety of carotenoids including xanthophylls (lutein, zeaxanthin, β-cryptoxanthin) and carotenes (lycopene, α-carotene, transβ-carotene and cisβ-carotene) in a healthy elderly population.

2. Materiel and methods

1. Study population

The EVA study ("Etude du Vieillissement Artériel") is a nine-year longitudinal study with 6 waves of follow-up (14). During the first two years 1991-1993 (EVA0), 1389 volunteers (574 men and 815 women) born between 1922 and 1932 (mean age = 65) 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, one hundred and one deaths occurred. Cancer was the first leading cause of death (n=45, 44.5%), the second one 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 ex-), alcohol intake, medication use, obesity, diabetes, hypertension and cardiovascular diseases (15). At EVA6, the blood samplings after a 12-hour fast were obtained for 773 subjects. Subjects who did not completed the whole study (n=616, 44.3%) were significantly more frequently men (those who not completed: 44.3% vs. those who completed: 38.9%, p=0.04)), obese or overweight subjects (57.9% vs. 47.3%, p=0.0004) and those who had hypertension (52.6% vs. 47.2%, p=0.05). They were statistically more frequently subjects in the lowest cognitive performances class (<25th percentile of the distribution) for MMSE (25.2% vs. 18.2%, p=0.002), DSS (28.3% vs. 20.3%, p=0.0006), TMT B (30.5% vs. 21.4%, p=0.0002) and WFT (25.1% vs. 19.3%, p=0.009), but we did not observe that for FTT (p=0.37).

The present analysis was restricted to the 589 subjects 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 enrolment.

2. Data collection

Cognitive evaluation and depressive symptoms

Trained neuropsychologists evaluated cognition with a neuropsychological battery of tests including a global test, the Mini-Mental State Examination (MMSE) (16), and an assessment of a range of cognitive domains. Visual conceptual and visuomotor tracking were assessed by the Trail Making Test part A (TMTA) and B (TMTB) (17). Involving motor speed and attention functions, the trail making test is highly vulnerable to the effects of brain injury (18). The A form is considered as exploring motor speed and control and working memory whereas the B form assesses executive function such as set shifting. The variables of interest are the time in seconds (19). These tests (part A and B) were performed with a maximum allotted time to perform the test of 180 and 240 seconds respectively. When subjects exceeded the time allotted for each Part, the maximum allotted time was imputed. The Digit Symbol Substitution (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 Finger Tapping Test (FTT). Verbal fluency was evaluated with the Word fluency Test (WFT). Depression symptoms were assessed by the Centre of the Epidemiological Studies-Depression (CESD) scale using score (score=17 for men and score=23 for women) for high risk of depression to define depressive symptomatology (21).

Carotenoids measurements

Retinol and carotenoids were measured with a Biotek-Krontron HPLC system (UVK Lab – Trappes France), which consists in a 525 dual pump, a 465 auto-sampler 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-Laroche (Hoffman-Laroche – Bâle Switzerland) as a generous gift. The LC separation was run with an Alltech Adsorbosphere C18 column 150 x 4.5 mm ID, 3 µ particle size (Alltech-France), which was thermostated at 28° C with a 402 column oven. Carotenoids and retinol were measured by HPLC after 2 extractions with a hexane/tetrahydrofurane mixture. For the quantization we used the Steghens method (22) with minor modifications. Indeed, we used a single 150 mm long column instead of 2, and we added 10 ppm water in the mobile phase A to improve the separation of retinol, lutein and zeaxanthin. The laboratory participates in the NIST (National Institute for Standards and Technology – New York) external quality assurance program, and ChromSystems internal controls are analyzed in every series of measurements (one control every 10 unknown serums) and in the SFVB quality assurance programmes. 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 carotenoid. All concentrations of retinol, lycopene and β-carotene were above these respective limits, while only 5% of lutein, 8% of zeaxanthin, 2% of β-cryptoxanthin were under.

Total plasma carotenoids levels were obtained by summing levels of lutein, zeaxanthin, β-cryptoxanthin, lycopene, α and β-carotenes.

Questionnaire and medical examination

The general questionnaire allowed us to obtain information on socio-demographic factors such as sex, age, educational achievement, plus consumption habits like tobacco status, alcohol consumption (which was determined from the subject'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 made 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 subjects was determined on DNA samples.

3. Statistical Methods

The characteristics of the 589 subjects included in the analysis were described compared to the 184 subjects who had blood sampling but not the cognitive evaluation at EVA6, results were expressed by percentage and means with their standard deviation (SD). To test the differences between these two groups, Chi square test and the Student T test were used. These characteristics comprised sex, age, educational achievement (≤ primary school / ≥high school), smoking status (current, ex-smokers /non-smokers), alcohol consumption (< 20ml / ≥ 20ml per day) and medicine use <3/ ≥ 3 per day). Health characteristics were body mass index (BMI) classes (underweight: BMI<21kg/m² (23);"normal weight": 21≥BMI<25; overweight: 25≥BMI<30 (24); obesity≥30 kg/m² (24)), diabetes (plasma glucose level ≥ 7.80 mmol/L or use of anti-diabetic drugs or diabetes medical history), dyslipidemia (total cholesterol ≥ 6.20 mmol/L or use of lipid-lowering drugs or dyslipidemia medical history), hypertension (systolic or diastolic blood pressure ≥ 140 or ≥ 90 mm Hg 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), the 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 subjects with low cognitive functioning for the different carotenoids levels showed that the relation between cognition and these biological variables were not linear. It brought us to consider them as categorical variables. We compared the characteristic of subjects for the different levels in carotenoids ($<25^{th}$ percentile versus $\geq 25^{th}$ percentile) by using the Student T-Test or Chi square test for both continuous and categorical variables.

To define subjects with the lowest cognitive performances in this well-educated cohort, we chose two cut-offs: subjects who had cognitive test scores below the 25^{th} percentile (and above 75^{th} for TMTB), and subjects who had cognitive test scores below the 10^{th} percentile (and above 90^{th} for TMTB). Values for these 25^{th} and 10^{th} percentile cut-offs for each test are presented in Table 2.

Classical multivariate logistic regressions were performed to test associations between probability of subjects to have the lowest cognitive functioning and levels of plasma carotenoids ($<25^{th}$ percentile vs. $>25^{th}$ percentile) adjusting on all potential confounding variables. Results were expressed by odds ratios (OR) with their 95% confidence intervals (CI). All interactions between each carotenoid and each consumption habits and health variables were calculated and were not statistically significant. Statistical analyses were performed using SAS software version 9.1 (SAS Institute, Inc. Cary, North Carolina).

Results

Characteristics of the 589 EVA subjects (Table1)

The 589 subjects of EVA study (361 women and 228 men, aged 73.5 ±2.9), on which, we carried the analysis, 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 (≥2glass/day). For the repartition of subjects between BMI classes, we observed 13.8% of underweight, 34.0% of overweight and 8.5% of 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 diseases and 20.5% carried at least one allele ε4 of the apolipoprotein E. For the different carotenoids and scores of neuropsychologic tests, concentrations and ranges are described in table 1, and percentile distributions in table 2.

We also compared the characteristics of these 589 subjects included in the analysis to the 184 subjects for which we only obtained a blood sample but not a cognitive evaluation. Results showed that these 184 subjects were significantly older (74.2±3.1 vs. 73.5± 2.9), proportions of subjects with hypertension (p=0.05) or with a history of cardiovascular diseases (p=0.03) were higher. On the other hand, proportions of subjects with low levels in β-cryptoxanthin, trans and cis β-carotene were significantly lower in this group (results not showed).

Description of carotenoids: correlation and associated factors

Plasma carotenoids were highly and significantly inter-correlated. The highest correlations were found among carotenes: α-carotene/ transβ-carotene (r=0.78), transβ-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 transβ-carotene/ lycopene.

In Table 3a and 3b, we describe the characteristics of subjects according to their total plasma carotenoid, the different xanthophylls and carotenes levels (<25th percentile vs. ≥ 25th percentile). We observe that the profile of factors associated to α-carotene, transβ-carotene and cis β-carotene were identical. Between the other carotenoids, however, the associated factors can differ. Gender was associated to all carotenoids, which reach higher levels in women with the exception of zeaxanthin. Tobacco status and alcohol consumption were significantly associated to lower levels for total plasma carotenoids, β-cryptoxanthin, α-carotene, trans and cis β-carotene. Diabetes was associated to low levels of all carotenoids, while hypertension were significantly associated to low levels of lutein, α-carotene, trans and cis β-carotene. Obesity and overweight were associated to low levels of all carotenoids excepted for zeaxanthin and β-cryptoxanthin. Age, education, depressive symptomatology, dylipidemia, history of cardio-vascular diseases and apolipoprotein E genotype were not associated with any plasma carotenoids.

Association between cognition and carotenoids

Table 4 showed results of crude association between cognitive performances and carotenoid levels, obtained by univariate logistic regression analyses. Subjects with the lowest cognitive performances (neuropsychologic tests 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 excepted MMSE (for TMTA OR= 1.66 [1.08; 2.55], for TMTB OR=1.60 [1.04; 2.44], for DSS OR=1.87 [1.21; 2.89], for FTT OR=1.70 [1.10; 2.62] and for the WFT OR=1.87 [1.16; 3.00]). Low levels of lycopene were associated to low performances in TMTB (OR=1.76 [1.16; 2.67]) and in DSS

(OR=2.02 [1.32; 3.11]). A significant association was found between low performances in TMTB and low levels of total plasma carotenoids and transβ-carotene (OR=1.57 [1.03; 2.40] and OR=1.58 [1.04; 2.41]). After taking into account socio-demographic factors (sex, age, education), consumption habits (tobacco, alcohol), diabetes, hypertension and BMI classes (Table5), associations between zeaxanthin and cognitive tests remained statistically significant for TMTA (OR=1.67 [1.06; 2.66]), DSS (OR=1.92 [1.18; 3.14]), FTT (OR= .69 [1.05; 2.72]) and WFT (OR=1.80 [1.07; 3.05]) but not for TMTB (OR=1.51 [0.95; 2.39], p=0.08). Lycopene remained associated to TMTB (OR=1.54 [0.97; 2.43], p=0.06) and DSS (OR=1.85 [1.14; 2.98]). The other associations between carotenoids and cognitive performances observed in the crude analyses did not remained statistically significant after adjustment. Total plasma carotenoids, α-carotene and β-carotene (trans or cis) levels were not statistically associated to low cognitive performances, neither were lutein and β-cryptoxanthin.

Sensitivity analysis

The same analyses were first performed, by removing subjects with depressive symptomatology (n=49), then by removing subjects who were underweight (BMI<21kg/m²) (n=81) and thirdly by adjusting on levels of plasma retinol. In the three situations, we obtained similar results. We also performed analyses on subjects who had a MMSE score ≥ 25 (n=570), to exclude subjects with potential clinically significant cognitive impairment (n=19). Results were identical (data not showed).

On the other hand, we performed models which explain the probability to have cognitive tests scores <10th percentile of the distribution scores for the different plasma carotenoids levels. Only associations between zeaxanthin and FTT (p=0.003) and TMTB (p=0.06), and lycopene and DSS (p=0.07) remained marginally statistically significant (data

not showed). In these analyses, the number of subjects, which had the lowest cognitive performances was 2.2 to 2.8 smaller than for the precedent analyses (n=54 for MMSE, 49 for DSS, 55 for TMTA, 53 for TMTB, 52 for FTT and 38 for the TEL). It comes from a lack of statistical power, which could explain the drop in statistically significant results.

All our analyses were conducted with dichotomized variables for both the plasma carotenoids and the cognitive outcomes; in supplemental analyses, we tested if 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=0.01 and r=0.11, p=0.006 for zeaxanthin and lycopene respectively), TMTA (r=-0.11, p=0.01 and r=-0.08, p=0.05), TMTB (r=-0.08, p=0.05 and r=-0.12, p=0.004) and FTT (r=0.09 p=0.04 and r=0.09 p=0.04). These correlations confirm that our findings were not driven by the chosen dichotomous classifications for cognition and carotenoids levels.

Discussion

To our knowledge, this study is the first one, which investigated, in a healthy elderly population, the relation between cognitive performances 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 carotenoids 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 free-living elderly community.

Carotenoids are found in coloured fruits and vegetables. Some studies showed that plasma carotenoids levels could be related to dietary fruits and vegetables intake (25, 26). More specifically, it seems that mango, papaya, peaches, prune, squash, orange, green fruits and vegetables were source of zeaxanthin, while 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 performances. In a large prospective study of older women (n=13388) (29), the authors reported relation between low vegetables intake and cognitive decline but no relation with fruits, the strongest association being with greater intake of green leafy vegetables and cruciferous vegetables. Even if some studies show that intakes of foods with high carotenoid contents were correlated with their corresponding plasma concentrations (26), intake of fruits and vegetables are sources of many other nutrients which have been associated to cognitive functions : vitamin E (30), folates (31, 32), flavonoids (33). It is now impossible to know if the association between carotenoids and

cognitive functions is the results of a specific effect of carotenoids or if it is the resultant of combined effects of the different fruits and vegetables compounds.

For each cognitive test, we tested eight-association hypothesis (Ho) between cognitive functions and carotenoids. In order to ensure that significant observed associations were not hazard related (alpha risk=5%), specific multiple test (Bonferroni, Sidak) corrections were applied to data. Considering, however, that the tested hypothesis 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 to more than one cognitive function, and that these associations remained statistically significant after controlling on potential confounding factors, or after removing some subjects (subjects with depressive symptomatology, subjects with a MMSE score <25 or undernourished subjects) 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 of low cognitive performances with 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 β-carotene (<25^{th} percentile) was not significantly associated to a 4-year cognitive decline (5). In a cross-sectional study, Perrig et al. (9) showed that a higher β-carotene plasma level was associated to better memory performances (free recall, recognition and vocabulary) in 442 healthy persons aged 65 to 94. Studying dietary intake of β-carotene, Morris et al. (8) found no association between carotene intake and cognitive decline, while the Rotterdam Study showed that a lower intake of β-carotene was

associated with impaired cognitive function measured by MMSE (7). Three studies investigated the link between cognitive performances 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 multi-antioxidant supplementation it is, however, impossible to isolate the specific effect of β-carotene on cognitive impairment. Only one study, concerning 1769 subjects, focused on the association of a large spectrum of carotenoids and cognitive performances in elderly subjects without neuropsychiatric disease, but it found no association (34). Discrepancies can be explained by methodological differences (neuropsychological tests to assess cognitive performances, number of tests used, choice of modelling of carotenoids…). Finally we found two clinical epidemiological studies (35, 36) which focused on the comparison of plasma carotenoids and retinol levels in elderly patients with or without Alzheimer disease (AD). One showed that levels of vitamin A, lutein, zeaxanthin, β-cryptoxanthin and α-carotene were lower in AD patients (n=63) than controls (n=56). However, they 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 for 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 subject 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, amongst them, certain carotenoids can be converted to retinoid and have a pro-vitamin A activity. To assess if the relation we showed could be explained partly by this hypothesis, we adjusted our models on retinol concentration, but results remained unchanged.

Our results are supported by those of a biological study on oxidation of carotenoids by free radicals led by Woodall et al (37), which found that lycopene, lutein and zeaxanthin all reacted rapidly with oxidising 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 higher rate of free radical production in the brain.

Our results on the relation between carotenoids and cognitive performances were not modified when we excluded the subjects with BMI <21 kg/m² for which the hypothesis of under nutrition is probable. This allows us to say that this relation could not be limited to the influence of undernourished subjects. However, in this cross sectional frame-work, it is not possible to affirm if these low levels of carotenoids preceded or were the consequence of cognitive impairment in a context where poor cognitive status may be a risk factor for poor nutrition. In this study, we used various tests to explore cognitive performances and 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. Beside, because the scores were not normally distributed, a percentual 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 the age-related macular degeneration (38). While retina is a puzzle whose ultimate solution 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 area of the brain have the same affinity for some specific carotenoids? The biological significance of our finding needs further research by biological studies, longitudinal epidemiological studies and by specific clinical trials with carotenoid supplementation.

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Tables

Table1: Demographic and clinical characteristics of the 589 subjects studied

		% or mean (± SD)*	Range
Sex (women)		61.3	
Age		73.55 ± 2.93	68-79
Education (High school)		52.1	
Smoking Status (S, Ex-S)		39.9	
Alcohol consumption (≥2 glasses/day) *		24.0	
Medicine consumption (≥3/day)*		58.8	
BMI classes * (kg/m²)	Underweight (<21)	13.8	
	Overweight (≥25-<30)	34.0	
	Obese (≥30)	8.5	
Diabetes		8.1	
Dyslipidemia		65.0	
Hypertension		78.8	
Cardio-vascular diseases		19.7	
APOE ε 4 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
Transβ-carotene (µmol/L)		0.73 ± 0.52	0.04; 5.48
Cisβ-carotene (µmol/L)		0.10 ± 0.12	0.01-1.19
Total Carotenoids (µmol/L)		1.86 ± 0.99	0.15-8.35
MMSE (points)		28 ± 2	5-30
DSS* (points)		45 ± 11	12-85
TMTA* (seconds)		48.9 ± 19.6	18-180
TMTB* (seconds)		110.4 ± 46.3	30-240
FTT* (taps)		127 ± 21	62-185
WFT* (points)		27 ± 8	5-50

Table 2: Percentile distributions for each carotenoid and cognitive variables

	10^{th} percentile	25^{th} percentile	50^{th} percentile	75^{th} percentile
Carotenoids variables				
Lutein (µmol/L)		0.176	0.235	0.335
Zeaxanthin (µmol/L)		0.020	0.029	0.042
β-cryptoxanthin (µmol/L)		0.098	0.167	0.288
Lycopene (µmol/L)		0.160	0.257	0.403
α-carotene (µmol/L)		0.098	0.154	0.245
Transβ-carotene (µmol/L)		0.374	0.607	0.953
Cisβ-carotene (µmol/L)		0.013	0.067	0.143
Total Carotenoids (µmol/L)		1.16	1.65	≥2.35
Cognitive variables				
MMSE (points)	27	28		
DSS (points)	30	37		
TMTA (seconds)	70*	55*		
TMTB (seconds)	178*	130*		
FTT (taps)	100	114		
WFT (points)	16	21		

Table 3a: Characteristics of subjects according to their level of plasma total carotenoids and xanthophylls (<25th perc. versus ≥25th percentile)

		Total carotenoids					Lutein					Zeaxanthin					Beta-cryptoxanthin				
		<25th perc.		≥25th perc.			<25th perc.		≥25th perc.			<25th perc.		≥25th perc.			<25th perc.		≥25th perc.		
		n	%*	n	%*	p	n	%*	n	%*	p	n	%*	n	%*	p	n	%*	N	%*	p
Age*		144	73.5 ± 2.9	445	73.6 ± 2.9	0.71	174	73.6 ± 3.0	442	73.5 ± 2.9	0.92	143	73.8± 3.0	446	74.5 ± 2.9	0.32	146	73.7 ± 2.8	443	73.5 ±3.0	0.46
Sex	Men	80	35.1	148	64.9	<10-4	71	31.1	157	68.9	0.005	59	25.9	169	74.1	0.47	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 Sch.	71	25.2	211	74.8	0.69	68	24.1	214	75.9	0.65	69	24.5	213	75.5	0.43	74	26.2	208	73.8	0.43
	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	
Tobacco	No Smoker	67	18.7	292	81.3	<10-4	81	22.6	278	77.4	0.09	89	24.8	270	75.2	0.72	68	18.9	291	81.1	<10-4
status	Smoker or ex	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 /day	97	21.8	347	78.1	0.008	105	23.6	339	76.3	0.18	108	24.3	336	75.7	0.86	99	22.3	345	77.7	0.01
	≥20 ml /day	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 / day	43	17.8	199	82.2	0.001	62	25.6	180	74.3	0.71	52	21.5	190	78.5	0.21	53	21.9	189	78.1	0.19
	≥3 / day	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	Normal	46	18.5	202	81.4	<10-4	50	20.2	198	79.8	<10-4	51	20.6	197	79.4	0.26	60	24.2	188	75.8	0.13
classes	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	
Kg/m²	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	No	125	24.1	394	78.1	0.82	126	24.3	393	75.7	0.64	124	23.9	395	76.1	0.94	128	24.7	391	75.3	0.89
symptoms	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	0.005	123	22.7	418	77.3	0.003	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	0.003	20	16.0	105	84.0	0.009	29	23.2	96	76.8	0.75	31	24.8	94	75.2	0.99
	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	No	111	23.5	362	76.5	0.26	115	24.3	358	75.7	0.46	111	23.5	362	76.5	0.35	116	24.5	357	75.5	0.76
CVD	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	0.21	53	25.7	153	74.3	0.75	52	25.2	154	74.8	0.69	57	27.7	149	72.3	0.23
	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	
Apoe4	No	107	24.5	330	75.5	0.90	103	23.6	334	76.4	0.30	100	22.9	337	77.1	0.31	110	25.2	327	74.8	0.93
	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	

Table3b : Characteristics of subjects according to their level of plasma carotenes (<25th percentile versus ≥25th percentile)

		Lycopene					α-carotene					Transβ-carotene					Cisβ-carotene				
		<25th perc.		≥25th perc.			<25th perc.		≥25th perc.			<25th perc.		≥25th perc.			<25th perc.		≥25th perc.		
		n	%*	n	%*	p	n	%*	n	%*	p	n	%*	n	%*	p	n	%*	N	%*	p
Age*		145	73.7 ±2.9	444	73.5 ±2.9	0.34	144	73.7 ±3.0	445	73.5 ±2.9	0.58	147	73.5 ±2.9	442	73.5 ±2.9	0.99	144	73.3 ±3.0	445	73.6 ±2.9	0.28
Sex	Men	66	28.9	162	71.1	0.05	72	31.6	156	68.4	0.001	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 Sch.	73	25.9	209	74.1	0.49	63	22.3	219	77.7	0.25	70	24.8	212	75.2	0.94	67	23.8	215	76.2	0.71
	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	
Tobacco	No Smoker	83	23.1	276	76.9	0.29	75	20.9	284	79.1	0.01	68	18.9	291	81.1	<10-4	67	18.7	292	81.3	<10-4
status	Smoker or ex	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 /day	106	23.9	338	76.1	0.43	96	21.6	348	78.4	0.002	100	22.5	344	77.5	0.009	99	22.3	345	77.7	0.02
	≥20 ml /day	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 / day	50	20.7	192	72.3	0.06	53	21.9	189	78.1	0.22	54	22.3	188	77.7	0.21	45	18.6	197	81.4	0.007
	≥3 / day	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	Normal	49	19.8	199	80.2	0.03	48	19.3	200	80.7	<10-4	40	16.1	208	83.9	<10-4	51	20.6	197	79.4	0.03
classes	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	
Kg/m²	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	No	128	24.7	391	75.3	0.40	130	25.0	389	75.0	0.57	131	25.2	388	74.8	0.35	124	23.9	395	76.1	0.69
symptoms	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	0.0004	121	22.4	420	77.6	<10-4	122	22.5	419	77.5	<10-4	122	22.5	419	77.4	0.000
	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	3
Hypertension	No	27	21.6	98	78.4	0.38	20	16.0	105	84.0	0.01	14	11.2	111	88.8	<10-4	17	13.6	108	86.4	0.001
	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	No	114	24.1	359	75.9	0.55	111	23.5	362	76.5	0.26	117	24.7	356	75.3	0.80	114	24.1	359	75.9	0.69
CVD	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	0.95	50	24.3	156	75.7	0.94	42	20.4	164	79.6	0.06	45	21.8	161	78.2	0.28
	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	
Apoe4	No	105	24.0	332	76.0	0.26	115	26.3	322	73.7	0.27	111	25.4	326	74.6	0.60	109	24.9	328	75.1	0.97
	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	

Table 4: Crude risk of low cognitive functioning (score <25th) associated to plasma carotenoids for each cognitive test.

Plasma antioxidant <25th vs. ≥25 th	MMSE			TMTA			TMTB		
	OR*	[95%] CI*	p	OR*	[95%] CI*	p	OR*	[95%] CI*	p
Total carotenoids	0.88	0.55 ; 1.40	0.59	1.13	0.73 ; 1.76	0.58	1.57	1.03 ; 2.40	0.04
Lutein	1.00	0.63 ; 1.58	0.99	1.01	0.65 ; 1.57	0.96	1.50	0.98 ; 2.30	0.06
Zeaxanthin	1.37	0.88 ; 2.13	0.17	1.66	1.08 ; 2.55	0.02	1.60	1.04 ; 2.44	0.03
β-cryptoxanthin	1.25	0.80 ; 1.96	0.32	0.95	0.61 ; 1.47	0.81	1.07	0.69 ; 1.65	0.77
Lycopene	1.03	0.65 ; 1.62	0.91	1.37	0.89 ; 2.10	0.15	1.76	1.16 ; 2.67	0.008
α-carotene	0.83	0.51 ; 1.33	0.44	0.84	0.53 ; 1.32	0.45	1.21	0.78 ; 1.86	0.39
transβ-carotene	0.71	0.44 ; 1.15	0.17	0.98	0.63 ; 1.53	0.94	1.58	1.04 ; 2.41	0.03
cisβ-carotene	0.61	0.37 ; 1.00	0.05	1.17	0.76 ; 1.82	0.47	1.02	0.65 ; 1.58	0.94
	DSS			FTT			TEL		
Total carotenoids	1.12	0.71 ; 1.76	0.62	1.22	0.78 ; 1.89	0.38	0.67	0.40 ; 1.13	0.13
Lutein	1.24	0.79 ; 1.95	0.34	1.39	0.90 ; 2.15	0.13	0.89	0.54 ; 1.47	0.65
Zeaxanthin	1.87	1.21 ; 2.89	0.005	1.70	1.10 ; 2.62	0.02	1.87	1.16; 3.00	0.01
β-cryptoxanthin	1.25	0.80 ; 1.94	0.33	0.79	0.50 ; 1.25	0.31	0.83	0.50 ; 1.36	0.46
Lycopene	2.02	1.32 ; 3.11	0.01	1.43	0.93 ; 2.21	0.10	1.16	0.72 ; 1.11	0.54
α-carotene	0.73	0.45 ; 1.18	0.20	1.11	0.71 ; 1.73	0.64	0.66	0.39 ; 1.11	0.12
transβ-carotene	0.93	0.58 ; 1.47	0.75	1.08	0.70 ; 1.68	0.72	0.72	0.43 ; 1.10	0.21
cisβ-carotene	1.24	0.79 ; 1.95	0.34	0.94	0.59 ; 1.48	0.78	0.74	0.45 ; 1.23	0.25

Table 5: Adjusted risk of low cognitive functioning (score <25th) associated to plasma carotenoids for each cognitive test.

Plasma antioxidant <25th vs. ≥25th	MMSE			TMTA			TMTB		
	OR*	[95%] CI*	p	OR*	[95%] CI*	p	OR*	[95%] CI*	p
Total carotenoids	0.93	0.55 ; 1.58	0.80	1.27	0.77 ; 2.09	0.34	1.43	0.88 ;2.32	0.14
Lutein	1.03	0.62 ; 1.71	0.89	1.15	0.71 ; 1.88	0.57	1.51	0.94 ; 2.42	0.09
Zeaxanthin	1.24	0.77 ; 2.00	0.38	1.67	1.06 ; 2.66	0.03	1.51	0.95 ; 2.39	0.08
β-cryptoxanthin	1.39	0.85 ; 2.28	0.19	1.05	0.64 ; 1.71	0.84	1.06	0.65 ;1.737	0.82
Lycopene	0.91	0.55 ; 1.50	0.71	1.39	0.88 ; 2.21	0.16	1.54	0.97 ; 2.43	0.06
α-carotene	0.89	0.53 ; 1.49	0.66	0.96	0.59 ; 1.58	0.88	1.16	0.72 ; 1.86	0.55
transβ-carotene	0.74	0.43 ; 1.27	0.27	1.11	0.68 ; 1.83	0.67	1.48	0.91 ; 2.37	0.11
cisβ-carotene	0.56	0.32 ; 0.98	0.04	1.41	0.87 ; 2.29	0.16	0.96	0.58 ; 1.57	0.86
	DSS			FTT			TEL		
Total carotenoids	0.95	0.56 ; 1.63	0.87	1.53	0.91 ; 2.55	0.11	0.66	0.36 ; 1.21	0.18
Lutein	1.25	0.75 ; 2.10	0.39	1.48	0.90 ; 2.42	0.12	0.94	0.54 ; 1.65	0.84
Zeaxanthin	1.92	1.18 ; 3.14	0.008	1.69	1.05 ; 2.72	0.03	1.80	1.07 ; 3.05	0.03
β-cryptoxanthin	1.01	0.61 ; 1.68	0.97	1.01	0.61 ; 1.69	0.95	0.72	0.41 ; 1.27	0.26
Lycopene	1.85	1.14 ; 2.98	0.01	1.44	0.89 ; 2.32	0.13	1.05	0.61 ; 1.79	0.87
α-carotene	0.64	0.37 ; 1.12	0.12	1.24	0.76 ; 2.03	0.39	0.78	0.43 ; 1.41	0.41
transβ-carotene	0.75	0.43 ; 1.29	0.30	1.36	0.82 ; 2.25	0.23	0.75	0.41 ; 1.37	0.35
cisβ-carotene	1.24	0.74 ; 2.09	0.41	1.27	0.76 ; 2.13	0.37	0.80	0.45 ; 1.41	0.44

Captions for tables

Table1: Demographic and clinical characteristics of the 589 subjects studied
MMSE= Mini Mental Status 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, APOE = apolipoprotein E
* For these variables, there are missing values. Alcohol consumption: n=10, medicine consumption: n=1, BMI classes: n=22,APOE ε4: n=39, depressive symptomatology: n=23, DSS :n=30, TMTA :n= 45, TMTB: n=31, FTT: n=43 and TEL: n=128.

Table 2: Percentile distributions for each carotenoid and cognitive variables
*For TMTB and TMTA, we presented values of 75^{th} and 90^{th} percentile of distribution of time.

Table3a: Characteristics of subjects according to their level of total plasma carotenoids and xanthophylls ($<25^{th}$ percentile versus $\geq 25^{th}$ percentile)
*Mean ± SD for age and BMI

Table3b: Characteristics of subjects according to their level of plasma carotenes ($<25^{th}$ percentile versus $\geq 25^{th}$ percentile)
*Mean ± SD for age and BMI

Table 4: Crude risk of low cognitive functioning (score $<25^{th}$) associated to plasma carotenoids for each cognitive test.
* Odds Ratio, with their confident interval at 95 %, of probability of subject to have the lowest cognitive functioning associated to each plasma carotenoid level.

Table 5: Adjusted risk of low cognitive functioning (score $<25^{th}$) associated to plasma carotenoids for each cognitive test.
* Odds Ratio, with their confident interval at 95 %, of probability of subject to have the lowest cognitive functioning associated to each plasma carotenoid level, adjusted on socio-demographic factors (sex, age and education, consumption habits (tobacco status, alcohol consumption, medicine use) and health variables (BMI classes, diabetes and hypertension).