Plasma Concentrations of Lutein and Zeaxanthin, Macular Pigment Optical Density, and Their Associations With Cognitive Performances Among Older Adults

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Purpose. We investigated the cross-sectional associations between macular pigment optical density (MPOD), plasma lutein (L), and zeaxanthin (Z) concentrations and cognitive function in 184 older adults of the 3-City-Bordeaux cohort.

Methods. MPOD was measured using the two-wavelength autofluorescence method with a modified scanning laser ophthalmoscope. Plasma L and Z (L+Z) concentrations were determined by high-performance liquid chromatography and were considered either crude or expressed as a ratio of the concentration of plasma lipids (total cholesterol [TC] + triglycerides [TG]). Cognitive performances were assessed using the following four separate neuropsychological tests: the Mini-Mental State Examination (MMSE), the Isaacs Set Test (IST), the Benton Visual Retention Test (BVRT), and the sum of the three free recalls of the Free and Cued Selective Reminding Test (FCSRT). These test results were summarized by a composite global cognitive *z*-score.

Results. Higher MPOD at 0.5° was significantly associated with a higher composite *z*-score ($\beta = 0.15$, 95% confidence interval [CI] 0.04–0.26), higher BVRT ($\beta = 0.39$, 95%CI 0.08–0.70), and higher IST ($\beta = 1.16$, 95%CI 0.11–2.22) performances. Higher plasma L+Z concentrations were significantly associated with higher IST scores ($\beta = 0.97$, 95%CI 0.01–1.94). Furthermore, a higher L+Z/TC+TG ratio was associated with a higher composite *z*-score ($\beta = 0.12$, 95%CI 0.01–0.23), along with higher IST ($\beta = 1.02$, 95%CI 0.002–2.04) and FCSRT ($\beta = 1.55$, 95%CI 0.41–2.69) performances.

Conclusions. This analysis suggested that both higher MPOD and L+Z concentrations were significantly associated with higher cognitive performances. However, MPOD measurements have the advantage of being a fast and representative measure of long-term carotenoid intake.

Keywords: macular pigment, lutein, zeaxanthin, cognition

Located in the back of the eye (macula), the macular pigment (MP) is a yellow pigmented area composed of lutein (L) and its isomers zeaxanthin (Z) and meso-zeaxanthin.[1] The only carotenoids selected by the macula are L and Z (L+Z).[2] Additionally, L+Z are also detected in at least four brain areas (cerebellum, frontal cortices, occipital cortices, and pons).[3,4] Hence, interest in L+Z has expanded beyond the retina to determine their possible contributions to brain function.[5]

It has been observed that L+Z concentrations in the brains of centenarians were positively correlated with better cognitive function, as assessed by the Mini-Mental State Examination (MMSE); the Fuld Object Memory Evaluation; the Wechsler Adult Intelligence Scale III (WAIS-III) Similarities; the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) verbal fluency, naming, and constructional praxis subtests; and the Geriatric Deterioration Rating Scale (antemortem cognitive measures).[6] Additionally, several lines of evidence suggest a positive relationship between both L+Z circulating concentrations and intake, and cognitive functions.[7–10]

Furthermore, recent research has identified that better cognitive performances were also significantly associated with higher macular pigment optical density (MPOD).[11,12]

This accruing body of evidence suggests that L+Z concentrations in the plasma and MPOD could both be associated with cognitive function. However, unlike L+Z plasma concentrations, which mirror only recent dietary intake, MPOD could be considered as a stable and representative measure of long-term L+Z intake.[13,14] Moreover, Vishwanathan et al.[15,16] showed that L+Z concentrations in the brain were positively correlated with macular L+Z concentrations (i.e., MPOD) in both nonhuman primates and humans. This suggests that MPOD could act as a surrogate to measure brain L+Z concentrations.[11]

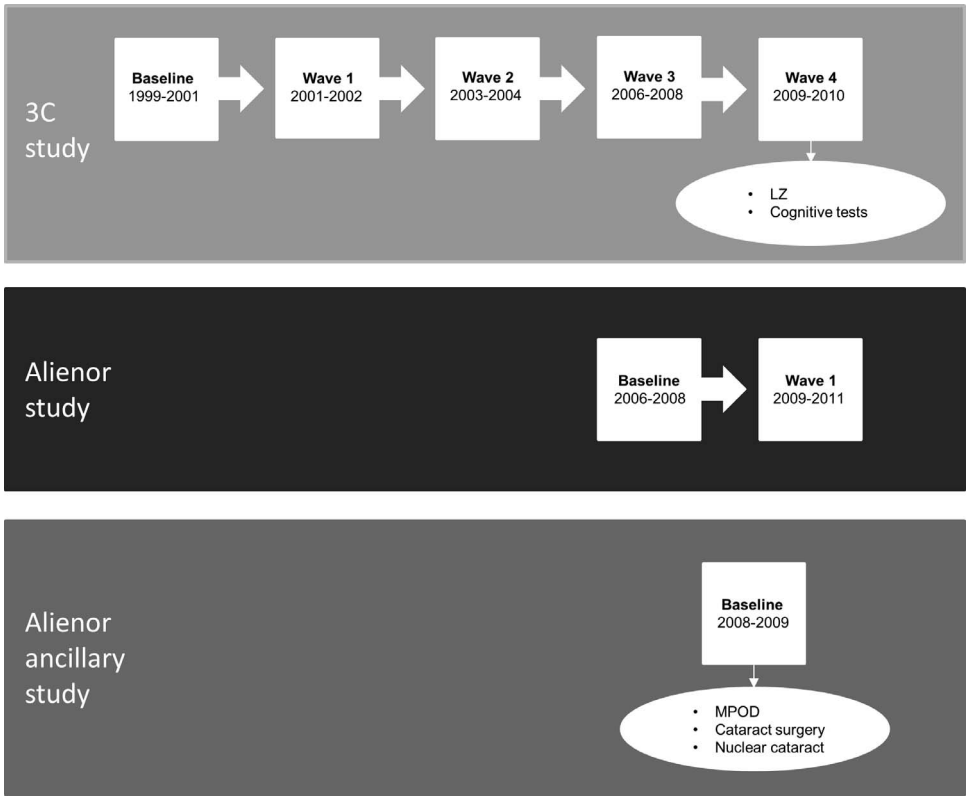

FIGURE. The timelines of 3C, ALIENOR, and ALIENOR ancillary studies.

In this cross-sectional study, we investigated the associations of both plasma carotenoid (L+Z) concentrations and MPOD with cognitive performances in older adults from the general population.

METHODS

Study Design and Participants

The ALIENOR (Antioxydants, LIpids Essentiels, Nutrition et maladies OculaiRes) study is a population-based prospective study focused on the associations of nutritional factors (particularly antioxidants, MP, and fatty acids) with age-related eye diseases such as glaucoma, age-related macular degeneration (AMD), dry eye syndrome, and cataract. The complete methodology of the ALIENOR study has been detailed previously.[17]

Participants of the ALIENOR study were recruited from an ongoing population-based study (3-City Study, 3C) on the vascular risk factors for dementia.[18] The 3C study included 9294 community-dwelling persons aged 65 years and older from three French cities (Bordeaux, Dijon, and Montpellier), among whom 2104 were recruited in Bordeaux. Subjects were initially recruited in 1999 to 2001 and were followed up approximately every 2 years after baseline.

The ALIENOR study consisted of an eye examination, which was proposed to all participants after the third follow-up (2006–2008) of the 3C cohort in Bordeaux (Figure). Participants in this study received follow-up communication approximately every 2 years following the examination. Of the 1450 individuals reexamined between 2006 and 2008, 963 participants (aged 73 years or higher) were enrolled in the ALIENOR study baseline eye examination. From these participants, 395 were reexamined between May 2008 and June 2009 in the framework of an ancillary study on MP. A complementary eye examination was proposed to all subjects diagnosed with early AMD during the baseline examination and an equal number of subjects without early AMD. This clinical exam included measurement of the MPOD.

This research followed the tenets of the Declaration of Helsinki. Participants gave written consent. The design of the ALIENOR study was approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in May 2006. The protocol for the 3C study was approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research at Kremlin-Bicetre University Hospital.

Among the 395 included participants, 4 participants developed late AMD in the eye with the best visual acuity or in both eyes and therefore were excluded from the present analyses. One additional participant was also discarded as the information about his intermediate AMD status was missing. We also excluded 152 participants who had missing information for MPOD or plasma L+Z concentrations. We next excluded 23 participants who had incomplete data for cognitive tests and 31 others who had missing information on potential confounders to obtain a final sample size of $n = 184$ subjects.

Macular Pigment Measurement

The eye examination was performed at the Department of Ophthalmology, University Hospital of Bordeaux. All subjects underwent complete ocular examination. The examination included a measurement of best-corrected visual acuity and MPOD in both eyes as previously described.[17] MPOD measurements were collected using the modified confocal scanning laser ophthalmoscope (mpHRA; Heidelberg Engineering, Heidelberg, Germany).[19] Autofluorescence images

were obtained at two wavelengths, based on the pioneering work of Delori et al.[20,21] Participants were positioned in front of the tabletop and instructed to look straight ahead and to remain steady; 20° autofluorescence images were then obtained at excitation wavelengths of 488 and 514 nm of the posterior pole, with a high-pass filter transmitting at a wavelength greater than 530 nm. The MPOD was quantified by calculating an MPOD map and comparing foveal and parafoveal autofluorescence at 488 and 514 nm. Density maps were processed to estimate the MPOD within a circle centered on the fovea at different degrees of eccentricities (0.5°, 1°, 2°, and 6°), using the software provided by the device manufacturer. Higher MPOD measurements were due to higher concentrations of L+Z in the macula. The correlation of MPOD values between each eye was greater than 0.8 for all degrees of eccentricities measured. For each participant, the obtained MPOD, expressed in optical density units, was the MPOD measurement in the eye with the best visual acuity according to the Parinaud scale. In this study, the MP at 0.5° and 1° are reported.

Plasma Lutein and Zeaxanthin Determination

Fasting blood samples were obtained at the 10-year follow-up (2009–2010) to the 3C study. Blood was collected in heparinized vacutainers, centrifuged at 1000*g* for 15 minutes, and stored (–80°C) until plasma carotenoid determination.

Extraction and HPLC analysis of plasma carotenoids were performed as previously described[22,23] with some modifications. Briefly, plasma (40 μL) was extracted with an ethanol/n-butanol mixture containing β-apo-8′-carotenal-methyloxime as an internal standard. After centrifugation, the clear supernatant was analyzed using a Shimadzu Prominence HPLC (LC-20A; Kyoto, Kansai, Japan) equipped with an UV-Vis detector (set at 450 nm); the carotenoids α- and β-carotene, lycopene, β-cryptoxanthin, and lutein and zeaxanthin were separated using a ReproSil 80 ODS-2 column (3 μm, 250 × 4.6 mm; Dr. Maisch GmbH, Ammerbuch, Germany) and an eluent as described previously[24] at a flow rate of 1.5 mL/min. L+Z were not baseline separated and are therefore reported as the sum of L+Z. The present analyses were restricted to those circulating carotenoids identified in the MP (i.e., L+Z concentrations measured in μM).

Assessment of Cognitive Function

At the time of the MPOD assessment, four cognitive tests were administered by trained psychologists to assess cognitive functions as follows[25]: the MMSE,[26] the Isaacs Set Test (IST15),[27] the Benton Visual Retention Test (BVRT),[28] and the Free and Cued Selective Reminding Test (FCSRT).[29]

1. The MMSE is a sum-score evaluating various dimensions of cognition. It provides a brief and objective measure of global cognitive functioning. The score ranges from 0 to 30 with higher values denoting better cognitive functioning.
2. The IST15 is a test that evaluates verbal fluency abilities and speed of verbal production. Subjects must give a list of words that belong to a specific semantic category (cities, color, animals, and fruits). The IST score was obtained after 15 seconds, ranging from 0 to 40, with higher values denoting better verbal fluency abilities.
3. The BVRT measures visual memory and visual perception. A stimulus card that displayed a geometric figure was presented to the subjects for 10 seconds. Subjects were then asked to choose the initial figure among four possibilities. Fifteen figures were presented one at a time, so the total score ranged from 0 to 15, with higher values denoting better visual function.
4. The FCSRT evaluates memory performance and verbal learning. In this analysis, we used the sum of three free recalls, as this subscore is more sensitive to cognitive function than total recall.[30] The total score ranged from 0 to 48, with higher values denoting better cognitive function.

These tests were chosen as they have been shown to be clinically relevant (i.e., performances on these tests were demonstrated to have been involved in the successive emergence of cognitive deficits in the prodromal phase of Alzheimer's disease).[31]

To combine the results of all four individual cognitive tests, a global composite measure was developed. The four individual cognitive tests were standardized and averaged to derive a cognitive *z*-score, which was considered the primary outcome in the present analysis.

Other Variables

The interview conducted at the 10-year follow-up (2009–2010) of the 3C study (Figure) included (1) demographic characteristics such as sex, age, and educational level (no education or primary school only, secondary school or higher), and (2) clinical characteristics such as body mass index (BMI in kg/m^2), smoking (pack-year), Center for Epidemiological Studies-Depression (CES-D) scale, hypertension, alcohol consumption (number of glasses per week), and diabetes. The season was defined according to the date of the blood drawing. Apolipoprotein E ε4 (*ApoE4*) alleles and intermediate AMD status were also considered in the present analyses. For participants who had not undergone cataract surgery, nuclear cataract was defined as nuclear opalescence (NO) >3 and/or nuclear color (NC) >3 on the LOCSIII classification.[32] Baseline cases of all-cause dementia were also reported (Table 1). Of note, the diagnosis of dementia was based on a two-step procedure.[18]

Moreover, total cholesterol (TC) and triglyceride (TG) concentrations were measured by routine enzymatic methods (mM). Measurement of TC and TG allows the ability to determine the effect of concurrent levels of lipids on the bioavailability of L+Z[33] by calculating the L+Z/(TC+TG) ratio.

Statistical Analyses

Multiple linear regression models were used to examine the relationship between MPOD, crude L+Z or L+Z/(TC+TG), and cognitive scores.

Each cognitive test and the *z*-score (primary outcome) were considered as separate outcomes in the multivariate linear models. The variation in the cognitive tests (β and 95% confidence interval [CI]) for 1-SD increase in plasma crude L+Z or L+Z/(TC+TG), or MPOD was reported.

The MMSE score suffers from poor metrological properties, mainly floor/ceiling effects and curvilinearity (i.e., varying sensitivity to change), and a normalizing transformation of the crude score was performed to ensure the normality assumption, as previously suggested.[34,35] Results are presented as "MMSE" in Tables 1, 3, 4, and 5 for clarity reasons, and higher scores corresponded to higher global cognitive performances. All other cognitive scores were standardized to compare the obtained results.

In the multivariate analysis, we first adjusted for age, sex, educational level, cataract surgery, nuclear cataract, and intermediate AMD (model 1). Then adjustments for additional clinical factors (smoking, BMI, *ApoE4*, CES-D, hypertension,

Table 1. Sociodemographic, Lifestyle, Plasma, and Macular Pigment Optical Density Characteristics ($N = 184$)

Characteristics	Mean (SD)	*n* (%)	Median (Range)
Demographic characteristics			
Sex			
Women		126 (68.5)	
Men		58 (31.5)	
Age, y	82.3 (4.3)		81.7 (75.6–93.4)
Educational level			
No education or primary school only		52 (28.3)	
Secondary school or higher		132 (71.8)	
Clinical characteristics			
BMI, kg/m^2			
<23		40 (21.7)	
23–27		81 (44.0)	
>27		63 (34.2)	
CES-D score		13 (7.1)	
Hypertension		155 (84.2)	
Diabetes		20 (10.9)	
ApoE4		26 (14.1)	
Smoking, pack-year	8.5 (18.3)		0 (0–102)
Alcohol, glasses/wk	6.1 (7.3)		7 (0–43)
All-cause dementia		7 (3.8)	
MMSE	27.7 (1.9)		28 (20–30)
FCSRT	25.4 (7.5)		26 (0–40)
IST15	27.9 (6.2)		27.5 (14–43)
BVRT	11.5 (1.9)		12 (6–15)
Lutein + zeaxanthin, μM	0.4 (0.3)		0.4 (0.03–2)
Lutein + zeaxanthin/(TG+TC) ratio, μmol/mmol	0.06 (0.04)		0.06 (0.01–0.26)
MPOD 1°, optical density unit	0.6 (0.2)		0.6 (0.1–1.2)
MPOD 0.5°, optical density unit	0.7 (0.2)		0.7 (0.2–1.2)
Cataract surgery		103 (56.0)	
Nuclear cataract		40 (21.7)	
Intermediate AMD		89 (48.4)	

alcohol consumption, and diabetes) were performed (model 2).

To study the association between cognitive tests and crude L+Z or the ratio of L+Z/(TC+TG), multivariate analyses were also adjusted for the season of the blood draw.

Multivariate analyses investigating the relationship between cognitive tests and MPOD were adjusted for cataract surgery and nuclear cataract but not for the season of the blood draw. Indeed, recent studies have observed significant relationships between cataract surgery, nuclear cataract, and MPOD concentrations.[36,37] Statistical analyses were conducted using SAS Statistical package release 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

The study sample consisted of 184 persons aged 82.3 years on average (SD 4.3 years) (Table 1). In our study, 68.5% of the participants were women, and 71.8% had secondary or higher educational level. Among all participants, 7.1% were depressed, 10.9% were diabetic, 14.1% were carriers of the *ApoE4* allele, and 84.2% had hypertension. The mean (SD) cognitive scores were 27.7 (1.9) for MMSE, 25.4 (7.5) for FCSRT, 27.9 (6.2) for IST15, and 11.5 (1.9) for BVRT.

To summarize the ophthalmic characteristics: 56.0% of participants had previous cataract surgery and 21.7% suffered from nuclear cataract. The mean (SD) values of MPOD measured at 0.5° and 1° were 0.7 (0.2) and 0.6 (0.2), respectively. The mean (SD) values of crude L+Z concentrations and of the ratio L+Z/(TC+TG) were 0.4 (0.3) μM and 0.06 (0.04) μmol/mmol, respectively. Additionally, the differences in cognitive functions related to MPOD versus serum L+Z/TC+TG might relate to the different characteristics of individuals who have high (above median) versus low (below median) levels in MPOD and serum as shown in Table 2.

In Table 3, higher MPOD values at 0.5° were significantly associated with higher global cognitive performance; 1-SD increase in MPOD at 0.5° was associated with a higher *z*-score ($\beta = 0.12$, 95%CI 0.01–0.23) in the model adjusted for age, sex, cataract surgery, nuclear cataract, educational level, and intermediate AMD. However, there was no significant association between MPOD at 0.5° and each of the cognitive tests considered separately. After additional adjustments for smoking, BMI, *ApoE4*, CES-D, hypertension, alcohol consumption, and diabetes (model 2), higher MPOD values at 0.5° were significantly associated with a higher composite global *z*-score ($\beta = 0.15$, 95%CI 0.04–0.26). Moreover, associations between MPOD at 0.5° and IST ($\beta = 1.16$, 95%CI 0.11–2.22) and BVRT ($\beta = 0.39$, 95%CI 0.08–0.70) performances became statistically significant.

Similar results were obtained for MPOD at 1°; in the partly adjusted model 1 and in the fully adjusted model 2, higher MPOD values at 1° were significantly associated with a higher *z*-score ($\beta = 0.11$ 95%CI 0.01–0.22 and $\beta = 0.14$, 95%CI 0.03–0.25, respectively). Moreover, higher MPOD values at 1° were significantly associated with higher IST and BVRT scores in the fully adjusted models.

The association between plasma L+Z concentrations and cognitive performances was firstly controlled for age, sex, educational level, season of the blood draw, cataract surgery, nuclear cataract, and intermediate AMD (Table 4, model 1). Higher plasma L+Z concentrations were significantly associat-

Table 2. Description of the Relationship of Covariates to MPOD, L+Z, and L+Z/TC+TG Ratios

Covariates	MPOD 0.5°*		MPOD 1°*		Lutein + Zeaxanthin*		Lutein + Zeaxanthin/(TG+TC) Ratio*	
	Below Median, $N = 95$	Above Median, $N = 89$	Below Median, $N = 96$	Above Median, $N = 88$	Below Median, $N = 92$	Above Median, $N = 92$	Below Median, $N = 91$	Above Median, $N = 93$
Demographic characteristics								
Sex, %								
Men	35.8	27.0	37.5	25.0	37.0	26.1	30.8	32.3
Women	64.2	73.0	62.5	75.0	63.0	73.9	69.2	67.7
Age, y, mean (SD)	81.9 (4.0)	82.8 (4.5)	82.0 (4.1)	82.7 (4.4)	82.1 (4.2)	82.6 (4.3)	82.2 (4.3)	82.4 (4.2)
Educational level, %								
No education or primary school only	24.2	32.6	25.0	31.8	29.4	27.2	31.9	24.7
Secondary school or higher	75.8	67.4	75.0	68.2	70.7	72.8	68.1	75.3
Clinical characteristics								
BMI, kg/m^2								
<23	18.9	24.7	18.8	25.0	17.4	26.1	15.4	28.0
23–27	38.9	49.4	39.6	48.9	41.3	46.7	46.2	41.9
>27	42.1	25.8	41.7	26.1	41.3	27.2	38.5	30.1
CES-D score, %	7.4	6.7	7.3	6.8	7.6	6.5	6.6	7.5
Hypertension, %	82.1	86.5	82.3	86.4	90.2	78.3	90.1	78.5
Diabetes, %	13.7	7.9	14.6	6.8	14.1	7.6	12.1	9.7
ApoE4, %	12.6	15.7	11.5	17.0	14.1	14.1	14.3	14.0
Smoking, pack-year, mean (SD)	8.4 (17.0)	8.5 (19.6)	8.8 (17.0)	8.1 (19.6)	10.1 (20.8)	6.9 (15.3)	7.8 (17.0)	9.1 (19.5)
Alcohol, glasses/wk, mean (SD)	7.2 (8.6)	4.9 (5.5)	7.2 (8.6)	4.9 (5.4)	6.7 (7.5)	5.5 (7.1)	6.4 (7.7)	5.8 (7.0)
Cataract surgery, %	42.1	70.8	41.7	71.6	55.4	56.5	56.0	55.9
Nuclear cataract, %	40.0	2.2	39.6	2.3	22.8	20.7	23.1	20.4
Intermediate AMD, %	51.6	44.9	51.0	45.5	43.5	53.3	44.0	52.7

* MPOD, L+Z, and L+Z/TC+TG ratios are expressed in quantiles (below median versus above median).

ed with higher IST scores but not with the composite *z*-score or the MMSE, BVRT, or FCSRT performances.

In the fully adjusted models, the association between plasma L+Z concentrations and IST scores remained statistically significant. Higher concentrations of plasma L+Z were only associated with higher IST performances.

Additionally, higher L+Z/TG+TC ratios were significantly associated with a higher composite *z*-score and with higher FCSRT performances (Table 5, model 1). After full adjustment, these associations remained significant; furthermore, higher L+Z/TG+TC ratios were significantly associated with higher IST performances.

Discussion

Among 3C participants, our previous work has shown that higher values of plasma crude L concentrations and the L/(TG+TC) ratio were both significantly associated with a decreased risk of incident dementia.[38] In the present analysis, higher MPOD was significantly associated with higher cognitive function (considered as a composite *z*-score) and specifically, with higher verbal fluency abilities and visual memory. This relation was independent of major confounders including cataract surgery, nuclear cataract, and intermediate AMD status. Regarding circulating MPs, a positive association was observed only between crude concentrations of L+Z and verbal fluency abilities, while a higher plasma L+Z/TG+TC ratio was significantly associated with a higher global cognitive *z*-score, episodic memory, and verbal fluency.

To our knowledge, this study is the first to consider both MPOD and circulating L+Z concentrations (as a function of plasma lipids). The significant associations between MPOD and cognitive performances reported in this study are consistent with previous reports of relationships of MPOD (measured by a

Table 3. Multivariate Association Between Macular Pigment Optical Density, Considering Two Eccentricities and Cognitive Performances, ALIENOR Study ($N = 184$)

Cognitive Performances	Model 1				Model 2			
	MPOD 0.5°		MPOD 1°		MPOD 0.5°		MPOD 1°	
	β (95%CI)	*P* Value	β (95%CI)	*P* Value	β (95%CI)	*P* Value	β (95%CI)	*P* Value
z-score*	0.12 (0.01, 0.23)	0.03	0.11 (0.01, 0.22)	0.04	0.15 (0.04, 0.26)	0.01	0.14 (0.03, 0.25)	0.02
FCSRT	1.12 (−0.05, 2.28)	0.06	1.10 (−0.05, 2.25)	0.06	1.17 (−0.03, 2.37)	0.06	1.12 (−0.06, 2.30)	0.06
IST	0.93 (−0.09, 1.94)	0.07	0.90 (−0.10, 1.90)	0.08	1.16 (0.11, 2.22)	0.03	1.12 (0.08, 2.16)	0.03
MMSE	1.34 (−0.95, 3.63)	0.25	1.26 (−1.00, 3.52)	0.27	1.48 (−0.93, 3.89)	0.23	1.32 (−1.04, 3.69)	0.27
BVRT	0.29 (−0.02, 0.59)	0.06	0.23 (−0.07, 0.53)	0.13	0.39 (0.08, 0.70)	0.02	0.32 (0.02, 0.63)	0.04

* Composite global cognitive *z*-score calculated by averaging the *z*-scores of MMSE, IST, FCSRT, and BVRT. Model 1: adjusted for age, sex, cataract surgery, nuclear cataract, educational level, and intermediate AMD. Model 2: model 1 + additional adjustment for smoking, body mass index, apolipoprotein E ε4 alleles, Center for Epidemiological Studies-Depression score, hypertension, alcohol consumption, and diabetes.

Table 4. Multivariate Regression Analyses of the Association Between Cognitive Test Performance and Plasma Lutein + Zeaxanthin Concentrations ($N = 184$)

	Lutein + Zeaxanthin			
	Model 1		Model 2	
Cognitive Test	β (95%CI)	*P* Value	β (95%CI)	*P* Value
z-score*	0.09 (−0.01, 0.19)	0.07	0.09 (−0.01, 0.19)	0.09
FCSRT	0.91 (−0.15, 1.97)	0.09	0.98 (−0.12, 2.07)	0.08
IST	0.97 (0.04, 1.89)	0.04	0.97 (0.01, 1.94)	0.047
MMSE	1.73 (−0.35, 3.81)	0.10	1.38 (−0.81, 3.56)	0.21
BVRT	−0.03 (−0.31, 0.25)	0.82	−0.02 (−0.30, 0.27)	0.91

* Composite global cognitive *z*-score calculated by averaging the *z*-scores of MMSE, IST, FCSRT, and BVRT. Model 1: adjusted for age, sex, season, cataract surgery, nuclear cataract, educational level, and intermediate AMD. Model 2: model 1 + additional adjustment for pack-year of cigarettes, body mass index, apolipoprotein E ε4 alleles, Center for Epidemiological Studies-Depression score, hypertension, alcohol consumption, and diabetes.

Table 5. Multivariate Regression Analyses of the Association Between Cognitive Test Performance and Plasma Lutein + Zeaxanthin/(Triglycerides + Total Cholesterol) Ratio ($N = 184$)

	Lutein + Zeaxanthin/(TG+TC) Ratio			
	Model 1		Model 2	
Cognitive Test	β (95%CI)	*P* Value	β (95%CI)	*P* Value
z-score*	0.12 (0.01, 0.22)	0.03	0.12 (0.01, 0.23)	0.03
FCSRT	1.40 (0.29, 2.52)	0.01	1.55 (0.41, 2.69)	0.008
IST	0.97 (−0.01, 1.95)	0.053	1.02 (0.002, 2.04)	0.049
MMSE	1.85 (−0.35, 4.05)	0.10	1.49 (−0.82, 3.80)	0.20
BVRT	0.007 (−0.29, 0.30)	0.96	0.03 (−0.27, 0.33)	0.86

* Composite global cognitive *z*-score calculated by averaging the *z*-scores of the four tests. Model 1: adjusted for age, sex, season, cataract surgery, nuclear cataract, educational level, and intermediate AMD. Model 2: model 1 + additional adjustment for pack-year of cigarettes, body mass index, apolipoprotein E ε4 alleles, Center for Epidemiological Studies-Depression score, hypertension, alcohol consumption, and diabetes.

variety of techniques) to cognitive function. In a cross-sectional study of 108 elderly participants, cognitive performances showed significant associations with MPOD, whereas associations with serum L+Z concentrations were less consistent.[12] Kelly et al.[10] compared two groups of participants (subjects free of retinal disease with low MPOD and subjects with early AMD) in a cross-sectional study. Higher MPOD was significantly associated with better cognition in both groups, while serum L+Z concentrations were not associated with cognitive performances. Additionally, in a large cross-sectional study of older adults aged ≥50 years ($n = 4453$), lower values of MPOD were significantly associated with poorer cognitive performances.[39] The present study is the first to adjust serum L+Z for serum lipids, as previously suggested for the analysis of vitamin E concentrations.[40] Brady et al.[41] observed that serum L+Z concentrations were significantly and positively related to HDL and non-HDL cholesterol. Indeed, carotenoids are not distributed uniformly between lipoproteins.[42] Particularly, L+Z are carried predominantly by HDL (53%) and in lower proportions by LDL (31%) followed by VLDL (16%).[43] These findings suggest that the correlation of absolute plasma carotenoids to concurrent plasma lipids cannot be neglected,[33] and not standardizing for lipids adds random misclassification, thus biasing the estimates toward the null. Thus, one should consider the L+Z/TC+TG ratio as a more accurate indicator of the true L+Z plasma concentrations.

Altogether, the available literature to date, mainly cross-sectional, underlines a consistent relationship between higher MP constituents, as measured by circulating concentrations of L+Z and MPOD, and higher cognitive performances via assessment of several cognitive domains by different neuropsychological tests. Interestingly, as in the present work, it appears that MPOD is likely more sensitive than circulating concentrations of L+Z to cognitive performances. Indeed, higher MPOD was usually associated with several cognitive domains, while L+Z concentrations, somewhat more fluctuating and dependent on recent dietary intake, were only associated with some particular cognitive tests (i.e., mainly verbal fluency test). As L+Z circulating levels and MPOD are mainly determined by dietary habits, our results would encourage adoption of a healthier dietary lifestyle, such as following a Mediterranean diet[25,38] or a MIND diet,[44,45] in which green leafy vegetables (providers of L/Z) are highly represented.

Furthermore, data from our group suggested that during the prodromal phase of dementia, a decrease in IST and BVRT scores occurs more than a decade before the diagnosis of dementia.[31] More specifically, the first measurable decline in cognitive function was detected on the IST. In other words, the IST might be more sensitive to cognitive decline than other cognitive tests, as suggested by its significant association with MPOD, plasma L+Z concentration, and the L+Z/TG+TC ratio. Altogether, these arguments provide a comprehensive insight into the nonsignificant (borderline) association between crude L+Z plasma concentrations and the global cognitive test.

However, our results must be interpreted with caution, first because of the cross-sectional design of our study, which cannot exclude reverse causality. Second, taking into account other dietary factors known to influence cognition such as omega-3 fatty acids[46] would have improved and strengthened our results. Third, a selection bias cannot be dismissed; participants not included ($n = 211$) in the present analysis were more depressed, were more diabetic, were more demented, and had higher alcohol consumption than those included (Supplementary Table S1). Moreover, the small sample size may have decreased the statistical power of the present study and could thus limit the generalizability of the results.

However, this analysis was conducted on a subsample of a population-based cohort of elderly people, which, as a whole, was representative of elderly people aged >75 years living in Bordeaux in 2009 and 2010.[47] Furthermore, this study is the first to relate the concurrent levels of lipids on the bioavailability of L+Z to cognitive performances. Finally, multiple potential confounders were considered, including cataract status (presence of nuclear cataract, cataract surgery). Indeed, cataract, and especially its nuclear subtype, may lead to an underestimation of MPOD values. For instance, in a study by Sasamoto et al.,[37] in 40 subjects affected by nuclear cataract, preoperative MPOD correlated negatively with preoperative nuclear color score and MPOD increased from 0.35 DU preoperatively to 0.60 DU postoperatively. It is noteworthy that serum L+Z concentrations are inversely related to the risk of cataract.[48] Moreover, cataract surgery may be related to better cognitive function,[49] although this latter relationship is not fully understood.[50,51] Cataract status was thus carefully taken into account in all our models.

In conclusion, higher MPOD and plasma L+Z concentrations (expressed as a function of plasma lipids) were both significantly associated with higher cognitive performances among French elderly community dwellers. The advantages of using MPOD over plasma L+Z measurements to assess the relationship between these MP components and cognitive functions are clear. Notably, MPOD is a fast and stable indicator of long-term carotenoid status since it is less impacted by short-term dietary variations compared to plasma L+Z concentrations. Additionally, MPOD can also be a noninvasive tool to assess MP constituents (e.g., when measured via heterochromatic flicker photometry). Moreover, many authors claim that the retina can be considered as a window to the brain, the eye being an extension to the central nervous system.[52,53]

Future work will investigate the evolution of cognitive performances through subsequent waves of the 3C study in relation to baseline MPOD and plasma L+Z concentrations.

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