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Relationships of Macular Pigment Optical Density With Plasma Lutein, Zeaxanthin, and Diet in an Elderly Population: The Montrachet Study

Seydou Alassane,¹⁻⁴ Christine Binquet,¹ Vanessa Cottet,^{1,5} Olivier Fleck,⁶ Niyazi Acar,²⁻⁴ Sandrine Daniel,¹ Cécile Delcourt,^{7,8} Lionel Bretillon,²⁻⁴ Alain M. Bron,^{2-4,6} and Catherine Creuzot-Garcher^{2-4,6}

¹INSERM, CIC 1432, Clinical Epidemiology Unit, Dijon, France; University Hospital, Clinical Center Investigation, Clinical Epidemiology/Clinical trials Unit, Dijon, France

²INRA, UMR1324 Centre des Sciences du Goût et de l'Alimentation, Dijon, France

³CNRS, UMR6265 Centre des Sciences du Goût et de l'Alimentation, Dijon, France

⁴Université Bourgogne Franche-Comté, Centre des Sciences du Goût et de l'Alimentation, Dijon, France

⁵INSERM, U866, Epidemiology and Clinical Research in Digestive Oncology Team, Dijon, France; Burgundy and Franche-Comté University, UMR866, Dijon, France

⁶Ophthalmology Department, Dijon University Hospital, Dijon, France

⁷University Bordeaux, ISPED, F-33000 Bordeaux, France

⁸INSERM, Centre INSERM U897-Epidemiologie-Biostatistique, F-33000 Bordeaux, France

Correspondence: Catherine Creuzot-Garcher, Service d'Ophthalmologie, CHU Dijon, 14 Rue Gaffarel, 21000 Dijon, France; catherine.creuzot-garcher@chu-dijon.fr

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PURPOSE. To investigate the association of macular pigment optical density (MPOD) with plasma lutein, zeaxanthin, and diet in an elderly population.

METHODS. We conducted a population-based study, the Montrachet (Maculopathy Optic Nerve, nuTRition neurovAsCular, and HEArT disease) study, in subjects older than 75 years. The MPOD was measured by means of the two-wavelength autofluorescence technique. Plasma lutein and zeaxanthin were measured in fasting blood samples using HPLC. Food frequency consumption was assessed with self-reported food frequency questionnaires.

RESULTS. Overall, 433 healthy participants (62.1% females) were included. Mean age was 82.0 ± 3.6 years. Mean MPOD at 0.5° eccentricity was 0.57 ± 0.25 density units (DU) and was higher in women than in men (0.59 ± 0.25 vs. 0.53 ± 0.25, $P = 0.017$). The MPOD was lower in alcohol consumers than in non-alcohol consumers (0.55 ± 0.25 vs. 0.61 ± 0.25, $P = 0.016$). Median plasma lutein and zeaxanthin levels were 281.4 µg/L and 20.0 µg/L, respectively, and were higher in women ($P = 0.010$ and $P = 0.003$, respectively). The MPOD was positively correlated with plasma lutein and zeaxanthin ($r = 0.10$, $P = 0.030$ and $r = 0.11$, $P = 0.027$, respectively). A higher consumption of squash was associated with higher plasma lutein and zeaxanthin. Adjusting for confounders and diet revealed that MPOD was weakly associated with plasma lutein in nonsmokers ($\beta = 0.11$, $P = 0.021$).

CONCLUSIONS. This study suggests that plasma lutein is associated with MPOD after taking into account potential confounding factors in an elderly population.

Keywords: macular pigment optical density, lutein, zeaxanthin, diet, elderly, population-based study

Increased life expectancy is a major characteristic of modern populations.¹ However, aging has long been recognized as a risk factor for life-threatening cardiovascular and neurologic diseases; for example, and diseases impacting quality of life such as osteoporosis, dementia, and eye diseases.² Age-related eye diseases are a burden for the economy and a major cause of moderate to severe visual impairment and blindness.³ Age-related macular degeneration (AMD) has a high prevalence, up to 36% after 85 years of age for early AMD,⁴ and could affect 196 million people by 2020 and 288 million by 2040.⁵ Several risk factors have already been identified, both genetic and environmental.⁶ Oxidative stress is involved in the pathogenesis of AMD related to the retina's high consumption of nutrients and oxygen and its exposure to light. To counteract

the deleterious effects of free radicals to the retina, macular pigment (MP) made up of three main carotenoids, lutein (L), zeaxanthin (Z), and meso-zeaxanthin, constitutes a barrier to blue-light damage and has antioxidant properties.⁷ Many epidemiological and interventional studies have assessed carotenoid intake and content through dietary questionnaires or plasma L and Z measurements, finding that the consumption and plasma levels of L are inversely correlated with the risk of AMD.⁸ Macular pigment optical density (MPOD) is a measurement of MP and can be assessed with different techniques.⁹ However, the associations between MPOD measured in the retina and L and Z found in the diet and plasma are weak.¹⁰ The imprecision of food questionnaires and the variations of the techniques used to assess MP are partly responsible for this



weak relation. Although controversial,¹¹ it has been reported that higher MPOD is associated with lower risk of AMD.¹²⁻¹⁴ Macular pigment optical density is dependent on the nutritional intake of the two main macular xanthophylls (L and Z) but also on many influencing factors. The MPOD measurement technique can be influenced by lens status, and higher MPOD levels have been found in pseudophakic than in phakic individuals.¹⁵ In addition, various confounding factors are associated with carotenoid metabolism such as adipose tissue that can compete with the retina to store carotenoids¹⁶ and polyunsaturated fatty acids (PUFAs) also interacting with L and Z pathways.¹⁷

To clarify the relationships between MP and dietary and plasma L and Z, we investigated these parameters in the participants of a population-based study undertaken in people older than 75 years.

MATERIALS AND METHODS

Study Design and Population Study

The Montrachet study (Maculopathy Optic Nerve and nuTri-tion neurovAscular and HEArT) is an ancillary study of the population-based Three Cities (3C) study, which has been previously described.¹⁸ Briefly, the 3C cohort study examined the relationships between vascular risk factors and the onset of aging disorders. Overall, 9294 persons aged 65 years and older, selected from the electoral rolls of three French urban cities (Bordeaux, Dijon, and Montpellier), were included ($n = 4931$ living in Dijon). Ten years later, a subgroup of participants from Dijon were invited to participate in the Montrachet study investigating the relationships between age-related eye diseases and neurologic and heart diseases in the elderly.

The methodology of the Montrachet study and baseline characteristics of volunteers have been detailed elsewhere.¹⁹ Briefly, from October 2009 to March 2013, 1153 volunteers underwent a comprehensive eye examination in the Department of Ophthalmology of the Dijon University Hospital, France. This examination included the collection of self-reported eye diseases and treatment history, visual acuity measurement, refractive error identification, IOP measurement, visual field examination, optical coherence tomography imaging, retinal photographs, and MPOD measurement in participants with macula images of sufficient quality. In addition, fasting blood samples were drawn to measure plasma carotenoids and fatty acids. Finally, all participants were asked to complete a questionnaire about lifestyle (alcohol consumption and smoking status), environment (sun exposure), and nutrition (food frequency questionnaire). All participants gave their written informed consent. The study followed the tenets of the Declaration of Helsinki and was approved by the regional ethics committee (Number 2009-A00448-49).

Main Parameter Measurements

Macular pigment optical density was measured with the two-wavelength autofluorescence method using a modified Heidelberg Retinal Angiograph (Spectralis; Heidelberg Engineering Co., Heidelberg, Germany).^{20,21} Each participant was positioned in front of the modified scanning laser ophthalmoscope (SLO) and instructed to look straight ahead. After focusing the SLO on the macular region, sequences of 20° images at 30-second intervals, after retinal bleaching, were captured at two wavelengths: 488 nm (well absorbed by the MP) and 514 nm (minimally absorbed by the macular pigment).²² Macular pigment optical density maps were generated by automatic subtraction of the log AF images. Macular pigment optical

density was recorded at 0.5°, 1.0°, 2.0°, and 6.0° eccentricities, using the software provided by the manufacturer. We recorded the average optical density volume for analysis. Macular pigment optical density was expressed in optical density units (DU). The measurements between the two eyes were highly correlated ($r = 0.93$) and only one eye per participant was retained for analysis. We selected the best image quality²¹ and we chose the MPOD at a 0.5° retinal eccentricity or the average MPOD in the central 1-degree diameter circle for analysis.²³ Age-related macular degeneration was classified with the same methodology as used in the Alienor study, another French population-based study in the elderly undertaken in southwestern France.²⁴ It should be noted that it was impossible to obtain good images for MPOD analysis in late AMD stages, as already reported.²⁵

Xanthophylls and fatty acids were analyzed in plasma samples from fasted volunteers and stored at -80°C before analysis (CSGA, Dijon, France). Plasma fatty acids were analyzed by gas chromatography, as described elsewhere.²⁶ Lutein and zeaxanthin were extracted in yellow light conditions from 200 μL plasma with 200 μL absolute ethanol after the addition of 40 ng echinenone (dissolved in ethanol) as internal standard. Carotenoids were further extracted twice with 500 μL hexane. Both hexane phases were pooled and evaporated to dryness under nitrogen stream without heating. The dry extract was suspended in 200 μL of the mobile phase for HPLC analysis, made with acetonitrile/50 mM ammonium acetate in methanol/water/dichloromethane (75:15:5:10, vol/vol/vol/vol). The samples (80 μL) were injected via an autosampler (maintained at 15°C) onto two HPLC columns used in tandem (Nucleosil C18 [Thermo Finnigan, Villebon Sur Yvette, France], 250×4.6 mm ID, 5 μm and VIDAK C18 [Altech France, Epernon, France], 250×4.6 mm ID, 5 μm) thermostated at 37°C at a flow rate of the mobile phase (see composition above) of $2 \text{ mL}\cdot\text{min}^{-1}$, and connected to a diode array detector set from 250 nm to 600 nm (Jasco MD1510; Bouguenais, France). Lutein and Z were identified by their absorption spectra (max absorption at 450 nm) and retention times (see Supplementary Fig. S1) along the 50-minute long run. The area under the curve was used for quantification. A calibration curve for each molecule was obtained by analyzing an increasing range of standards of each analyte (kind gift from Roche, Basel, Switzerland) and using a fixed concentration of the internal standard, equivalent to that used for sample analysis. Dietary intake was assessed with a standardized self-reported food frequency questionnaire validated in the Bordeaux participants of the 3C study.²⁷ The questionnaire referred to the frequency of consumption during the year before the eye examination and thus took into account seasonal variation. In the present study, 10 major food items rich in L and Z were selected (see Supplementary Table S1). The consumption of oral supplements containing xanthophylls was documented on self-declaration.

Statistical Analysis

Categorical variables were expressed as number (percentage), and continuous variables as mean \pm SD or median (interquartile ratio), depending on their distribution. The Kolmogorov-Smirnov test was used to detect a significant deviation from normal distribution. Bivariate comparisons were performed with χ^2 tests for categorical variables and with Student's *t*-tests, Mann-Whitney, ANOVA, or Kruskal-Wallis tests for continuous variables according to the variables' distribution. Correlations between continuous variables were tested using Pearson or Spearman correlation coefficients when appropriate. Missing data for body mass index (BMI) was observed in 16.2%, and a multiple imputation method was applied for this variable. Multivariate analyses were performed using multiple regression linear models. Models were systematically adjusted for age and

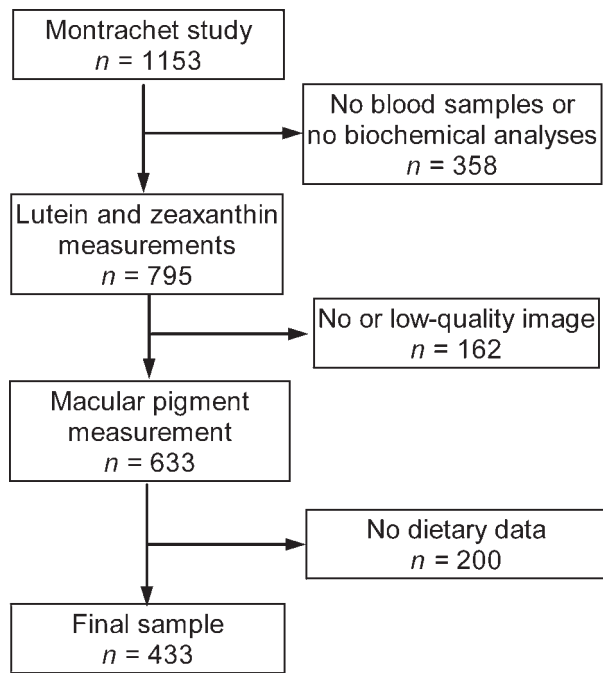


FIGURE. Flowchart of the Montrachet study and macular pigment study.

sex. In the first step, we investigated associations between plasma L (model 1a), plasma Z (model 1b), or plasma L and Z (model 1c) and MPOD. All variables associated with the MPOD value with a P value less than 0.20 in bivariate analyses were introduced in these models and selected through a backward procedure. When covariates were highly correlated ($r > 0.7$), the covariate providing the lowest Akaike criterion was selected. Deviation from linearity of the relationship between the continuous covariates retained and MPOD was systematically tested using χ^2 tests for linear trend after having categorized the covariates according to the quintiles of their distribution. First-order interaction terms were systematically tested. The adequacy of each final model was checked by a visual examination of the residual distributions. In the second step, the relationships between each of the selected food items and plasma L or plasma Z level were explored after log transformation of L and Z. Each food item associated with L or Z levels with a P value less than 0.1 in bivariate analyses was included in a multiple linear regression model. These models were adjusted on the other potential confounders associated with L or Z levels with a P value less than 0.2 in bivariate analyses. In the last steps, food items having a significant impact on plasma L or Z levels were included in the final models obtained at the first step (models 1a, 1b, and 1c) to determine whether L or Z levels as well as food items had an independent impact on MPOD. All statistical analyses were performed with SAS software, version 9.3 (SAS Institute, Inc., Cary, NC, USA). The tests were two-tailed and a P -value less than 0.05 was considered statistically significant.

RESULTS

Of the 1153 participants of the Montrachet study, 433 had complete data and were considered for analysis (Fig.). Baseline characteristics of these 433 participants did not differ from the nonparticipants of the Montrachet population, except for the percentage of pseudophakics (Table 1). This number of subjects allowed identifying, with a power of 80% and an

alpha level of 0.05, a 0.11 or 1.99 DU increase in MPOD for each additional 1000 $\mu\text{g/L}$ of L or Z in the plasma, respectively. Table 2 presents the MPOD, L, and Z levels according to the participants' main characteristics. In bivariate analysis, MPOD was significantly higher in women, in individuals who never drank alcohol, had normal weight, or were pseudophakic or suffering from AMD. Plasma levels of L were significantly higher in women, in those with a normal weight, and in pseudophakics. Plasma Z was higher only in women. The use of oral supplements containing L and Z was associated with high plasma carotenoid levels ($P < 0.0001$) but not with MPOD ($P = 0.192$). The correlations between MPOD and total cholesterol or its fractions (high-density lipoprotein [HDL], low-density lipoprotein), or omega-3 as well as PUFAs were not statistically significant (minimum P value = 0.110) (see Supplementary Table S2). Conversely, total cholesterol or its fractions and omega-3 as well as PUFAs were all positively correlated with L or Z plasma levels (maximum P value = 0.030), but the correlation appeared less pronounced for Z (see Supplementary Table S2). Plasma L and Z were highly correlated ($r = 0.62$, $P < 0.0001$), but poorly correlated with MPOD at 0.5° ($r = 0.10$, $P = 0.030$ and $r = 0.11$, $P = 0.027$, respectively).

After adjusting for confounders, higher L level became significantly associated with higher MPOD ($P = 0.015$). A significant interaction was found between L and smoking status ($P < 0.001$) (see Table 3). The MPOD at 0.5° increased by 0.11 ± 0.04 DU for each additional 1000 $\mu\text{g/L}$ of plasma L in nonsmokers ($P = 0.021$) but decreased marginally by 0.11 ± 0.06 ($P = 0.071$) in smokers. Adding Z to these models did not change the results.

These findings were not modified when adjusting for AMD (data not shown). Higher squash (more than once a week) or intermediate broccoli (1–3 times per month) consumptions were significantly associated with higher plasma L levels in bivariate analyses ($P = 0.009$ and $P = 0.049$, respectively) (see Supplementary Table S1). When adjusting for confounders, only higher squash consumption remained associated with a higher plasma L level ($\beta = 0.082$, $P = 0.029$) (Table 4). In addition, participants with intermediate green bean consumption (1–3 times per month) had a marginally higher plasma L level than those with the lower consumption frequency ($P = 0.063$). Plasma Z levels were also higher with high consumption of squash ($P = 0.007$) or maize ($P = 0.027$) in bivariate analyses (see Supplementary Table S1). However, only high consumption of squash remained associated with higher plasma Z levels after adjustment for confounders ($\beta = 0.075$, $P = 0.045$) (Table 4). Estimates were similar when adjusted for PUFAs or omega-3s (data not shown).

Finally, the associations between MPOD and plasma L and Z described in Table 3 were not modified by further adjustment on foods rich in carotenoids. In these models, food consumption was not related to the MPOD level (minimum P value = 0.114). As previously found, plasma Z was still not associated with MPOD (model 1b) after further adjustment for food consumption model 1b ($P > 0.682$).

DISCUSSION

This study determined the impact of several parameters on MPOD in a relatively large sample of urban Caucasians older than 75 years in the setting of a population-based study. Mean MPOD value in this study was in accordance with the MPOD at 0.5° found with the same technique in nonsupplemented patients (0.61 ± 0.21).²⁸ Median plasma L and Z were 281.4 $\mu\text{g/L}$ (0.49 μM) and 20.0 $\mu\text{g/L}$ (0.03 μM), which is higher than some recently published series, especially in the elderly.^{17,29} In

TABLE 1. Comparison of the Main Characteristics of Nonparticipants and Participants in the Evaluation of the Relationships Among MPOD, Plasma Xanthophylls, and Diet

	Participants, <i>n</i> = 433	Nonparticipants, <i>n</i> = 720	<i>P</i>
Age, y	82.0 ± 3.6	82.4 ± 3.8	0.08
Females	269 (62.1)	454 (63.1)	0.75
Iris color			
Blue/gray	183 (42.3)	282 (39.2)	0.16
Green/brown	141 (32.5)	219 (30.4)	
Dark brown	109 (25.2)	219 (30.4)	
Lens status			
Phakic	192 (44.3)	389 (54.5)	<0.001
Pseudophakic	241 (55.7)	235 (45.5)	
Former or current smokers	156 (36.0)	238 (33.7)	0.52
Alcohol consumption	302 (69.8)	503 (69.9)	0.97
BMI, kg/m ²			
≤25	193 (44.6)	286 (53.6)	0.09
>25	240 (55.4)	334 (46.4)	
Sun protection			
Never	183 (42.3)	67 (9.4)	0.26
Occasionally	109 (25.2)	171 (23.9)	
Often	141 (32.5)	478 (66.8)	
Retina status*			
No AMD	235 (56.4)	364 (58.0)	0.61
LZ supplementation	21 (4.8)	44 (6.1)	0.43
MPOD at 0.5°, DU	0.57 ± 0.25		
MPOD at 1°, DU	0.49 ± 0.21		
MPOD at 2°, DU	0.31 ± 0.14		
MPOD at 6°, DU	0.07 ± 0.03		
L, µg/L	281.4 [180.0–453.4]	263.0 [177.5–453.9]	0.76
Z, µg/L	20.0 [11.6–25.6]	18.2 [10.5–26.0]	0.99
L + Z, µg/L	296.6 [194.9–471.1]	288.8 [190.1–473.3]	0.76
Plasma triglycerides, mM	1.06 [0.83–1.44]	1.06 [0.8–1.4]	0.98
Plasma total cholesterol, mM	5.80 ± 0.92	5.79 ± 0.96	0.67
Plasma LDL-cholesterol, mM	3.60 ± 0.83	3.59 ± 0.83	0.85
Plasma HDL-cholesterol, mM	1.67 ± 0.40	1.65 ± 0.40	0.57
Plasma PUFAs, %†	40.55 ± 4.13	40.20 ± 4.24	0.22
Plasma omega-3 PUFAs, %†	4.6 [3.9–5.4]		
Plasma omega-6 PUFAs, %†	35.3 [33.1–37.5]		

The results are displayed as *n* (%) for categorical variables and mean ± SD or median [interquartile range] for continuous variables depending on their distribution.

* Missing data (*n* = 16 in participants and *n* = 92 in nonparticipants).

† Missing data (*n* = 9 in participants and *n* = 330 in nonparticipants).

a 40- to 60-year-old population, Delyfer et al.¹⁷ found a mean L and Z value of 161.8 µg/L and 41.3 µg/L, respectively. In the AREDS2, the median of L + Z varied from 177 to 191 µg/L within the four arms of the study.²⁹ In our population, we found that higher plasma L was associated with higher MPOD in nonsmokers. Previous studies found stronger associations between plasma L or Z and MPOD, measured using the same technique, but they did not adjust for confounders.³⁰ Conversely, a slight lower MPOD was found in former or current smokers. This is in agreement with one published study,³¹ although no relation was found in another report.³² This modest relation can be explained by the low number of current smokers and a lack of precision on the number of cigarettes smoked per day in our study. Smoking is a well-known risk factor for AMD.³³ However, the mechanisms by which smoking can influence AMD are not fully elucidated. In our population, alcohol drinkers had lower MPOD than nondrinkers, in accordance with the literature,³⁴ whereas in the CAREDS study, alcohol consumption did not influence

MPOD.¹¹ A modest relation was found between alcohol consumption and increased AMD risk in a recent epidemiological study.³⁵ Age was not associated with MPOD in our population, as found in some studies but not others.^{31,36,37} This result may be related to the narrow distribution of age in our study. Macular pigment optical density and plasma xanthophylls were significantly higher in women in our population, which has already been reported,³⁸ but other authors observed higher MPOD in men.³⁹ Both results can be explained by higher intake of dietary carotenoids in women and by sex differences in adipose tissue.⁴⁰ We found that BMI was inversely associated with MPOD. Although some authors have found a positive association between BMI and MPOD,³² an inverse relation has already been reported.³¹ Adipose tissue can store 80% of the total carotenoids found in the body and thus limit their bioavailability.⁴⁰ As already mentioned by Sasamoto et al.,¹⁵ MPOD in phakic eyes was lower than in pseudophakic eyes in our population. This is mainly due to the absorption of blue light by the cataractous lens and results in

TABLE 2. Macular Pigment Optical Density, Plasma L and Z According to Participant Characteristics

	MPOD 0.5°, DU	P	L, µg/L	P	Z, µg/L	P
Age, y						
≤80	0.55 ± 0.25	0.12	289.9 [193.6–454.7]	0.053	17.8 [11.2–27.4]	0.63
80–85	0.56 ± 0.36		252.8 [156.8–417.0]		16.7 [11.3–24.5]	
>85	0.62 ± 0.24		321.5 [179.3–486.7]		17.3 [12.5–27.5]	
Sex						
Male	0.53 ± 0.25	0.017	238.3 [173.0–405.0]	0.010	15.5 [10.5–23.8]	0.003
Female	0.59 ± 0.25		298.4 [192.4–469.7]		18.4 [12.3–27.5]	
Smoking status						
Never	0.58 ± 0.25	0.19	293.8 [183.0–463.6]	0.16	17.6 [11.8–26.5]	0.18
Current or past	0.55 ± 0.25		253.5 [174.9–404.3]		15.8 [11.2–24.8]	
Alcohol consumption						
Never	0.61 ± 0.25	0.016	296.7 [193.0–438.1]	0.51	16.2 [12.1–25.5]	0.76
Current or past	0.55 ± 0.25		266.1 [173.7–460.7]		17.8 [11.3–26.1]	
BMI, kg/m ²						
≤25	0.59 ± 0.24	0.049	329.3 [207.4–479.0]	<0.001	18.3 [11.9–27.7]	0.06
>25	0.55 ± 0.26		250.7 [166.6–429.2]		16.2 [11.4–25.1]	
Sun protection						
Never	0.57 ± 0.26	0.99	211.2 [131.4–428.2]	0.08	13.4 [8.9–25.5]	0.25
Occasionally	0.57 ± 0.25		294.3 [183.0–464.8]		17.2 [11.6–26.5]	
Often	0.57 ± 0.25		253.2 [180.0–392.2]		18.4 [12.2–25.0]	
Lens status						
Phakic	0.45 ± 0.22	<0.001	249.4 [172.4–429.0]	0.006	15.8 [11.3–23.6]	0.06
Pseudophakic	0.66 ± 0.23		305.5 [191.8–481.4]		18.2 [11.7–28.1]	
Retina status						
No AMD	0.54 ± 0.24	0.002	261.4 [182.6–431.9]	0.29	16.6 [11.6–25.1]	0.30
AMD	0.61 ± 0.26		286.4 [173.4–476.2]		17.9 [11.3–29.1]	
LZ supplementation						
No	0.56 ± 0.25	0.19	266.1 [175.1–433.7]	<0.0001	17.2 [11.5–25.1]	<0.0001
Yes	0.64 ± 0.25		569.2 [428.2–1190.9]		35.0 [19.4–45.2]	

Values in bold indicate statistical significance.

misinterpretation of MPOD measurements, at least with two-wavelength fundus autofluorescence. Therefore, the lens status must be taken into account in multivariate analysis to provide accurate estimates, a crucial point when studying older subjects with blurred media due to cataract.

In the present study, higher plasma L was associated with higher MPOD in nonsmokers after adjustment for potential confounders. This association persisted after taking into

account squash and green bean consumption, associated with higher plasma L. Frequent squash consumption was associated with higher plasma Z. However, we found no association between these food items or plasma Z with MPOD. These results may stem from the difficulty of assessing carotenoid intake using food frequency questionnaires, which remain only semiquantitative and not designed to precisely measure intake

TABLE 3. Association of MPOD With Plasma L, Z, and Other Factors

	Model 1a		Model 1b		Model 1c	
	β (SE)	P	β (SE)	P	β (SE)	P
Age, for each 10-y increase	0.088 (0.003)	0.77	0.010 (0.003)	0.74	0.088 (0.003)	0.78
Sex, female vs. male	0.001 (0.026)	0.96	0.008 (0.025)	0.73	0.001 (0.027)	0.97
Smoking status, current or past smokers vs. nonsmokers	−0.079 (0.035)	0.028	–	–	−0.078 (0.027)	0.036
Alcohol consumption, yes vs. no	−0.052 (0.025)	0.039	−0.049 (0.025)	0.056	−0.052 (0.025)	0.040
BMI, for each kg/m ² increase	−0.007 (0.002)	0.010	−0.006 (0.003)	0.016	−0.007 (0.003)	0.010
Lens status, pseudophakic vs. phakic	0.200 (0.023)	<0.001	0.192 (0.023)	<0.001	0.200 (0.023)	<0.001
LZ supplementation, yes vs. no	0.071 (0.056)	0.20	0.057 (0.052)	0.28	0.071 (0.056)	0.20
L, for every 1000-µg/L increase	0.121 (0.049)	0.015	–	–	0.120 (0.057)	0.036
Interaction term L and smoking status	−0.253 (0.069)	<0.001	–	–	−0.253 (0.070)	<0.001
Zeaxanthin, for every 1000-µg/L increase	–	–	0.235 (0.681)	0.73	0.035 (0.810)	0.95

Model 1a refers to the associations between plasma L, model 1b refers to the associations between plasma Z, and model 1c refers to the associations between L and Z. Dashes indicate variables not included in the model.

TABLE 4. Association Between Vegetables With Plasma L and Z

	L*			Z†	
	n (%)	β (SE)	P	β (SE)	P
Broccoli					
Never or < once/mo	160 (36.9)				
1–3 times/mo‡	191 (44.1)	0.042 (0.030)	0.16	0.044 (0.031)	0.14
1–3 times/wk‡	82 (18.9)	0.026 (0.037)	0.49	0.064 (0.044)	0.09
Squash					
Never or < once/mo	84 (19.4)				
1–3 times/mo‡	159 (36.7)	0.022 (0.038)	0.39	0.036 (0.038)	0.34
1–3 time/wk‡	190 (43.9)	0.082 (0.037)	0.029	0.075 (0.037)	0.045
Green beans					
Never or < once/mo	15 (3.5)				
1–3 times/mo‡	159 (36.7)	0.138 (0.075)	0.06	-	-
1–3 times/wk‡	259 (59.8)	0.120 (0.074)	0.10	-	-
Maize					
Never or < once/mo	370 (85.5)	-			
1–3 times/mo‡	43 (9.9)	-	-	0.003 (0.045)	0.94
1–3 times/wk‡	20 (4.6)	-	-	0.109 (0.066)	0.09
Leafy greens					
Never or < once/mo	350 (80.3)				
1–3 times/mo‡	60 (13.9)	-	-	-0.042 (0.040)	0.28
1–3 times/wk‡	23 (5.3)	-	-	0.107 (0.060)	0.08

Dashes indicate variables not included in the model.

* Model adjusted for age, sex, smoking status, BMI, lens status, retina status, supplementation, sun protection, and HDL-cholesterol.

† Model adjusted for age, sex, smoking status, BMI, lens status, AMD, supplementation, and HDL-cholesterol.

‡ Versus never or < once per month.

of these nutrients. Moreover, minimum carotenoid intake seems to be necessary to observe MPOD changes.²³

No statistically significant relation was found between MPOD and plasma PUFAs (see Supplementary Table S1), unlike the PIMAVOSA study.¹⁷ However, we found that plasma xanthophylls were associated with plasma cholesterol, PUFAs as a whole, and omega-3 and omega-6 PUFAs; the highest correlation was between L and omega-3 PUFAs. The relation of plasma xanthophylls and HDL is supported by the fact that HDL lipoproteins transport xanthophylls in the blood stream. The literature does not report a consistent association between PUFAs and plasma xanthophylls,¹⁷ or the possibility to intervene on AMD with PUFAs through interventional studies.²⁹

These results highlight the difficulty of assessing not only carotenoid intake or plasma xanthophylls, but also the final incorporation in the retina. These results showed that there is probably no ideal marker for MP and that all three methods (i.e., diet, plasma level, or MPOD) have advantages and disadvantages. These weaknesses probably explain the discrepancy between the results obtained in the different studies on the relationship between MP and age-related eye diseases.

We acknowledge several limitations to this study. First, the number of participants with complete data on MPOD, plasma xanthophylls, and diet was relatively low despite the large Montrachet population. However, the characteristics between participants and nonparticipants were similar except for lens status. Second, the technique used for MPOD measurement in this study has been challenged, but, as pointed out by some authors, we still lack a gold standard for MPOD measurement.⁹ Third, self-reported food frequency questionnaires have their own limitations in evaluating the different food items and were not designed for precise carotenoid intake measurement. Fourth, we must acknowledge that our methodology for

carotenoid measurement in plasma shows some limitations with respect to the chromatographic resolution between L and Z. One other method using C30 column has a better capacity to separate L from Z and has been developed meanwhile. Although it was been available to us before the end of the present study (March 2013), we decided to analyze all samples with the same methodology so as to obtain a set of comparable data. Fifth, we did not mention the distribution of MP in our study, as it was described elsewhere.⁴¹ This will be the focus of a later report based on the same population. Sixth, these findings in an elderly urban, healthy, Caucasian population living in a country with specific dietary habits cannot be extended to other age groups, ethnicities, groups, or countries. The first results with the Montrachet population have shown that these participants are overall in good health with a low number of patients suffering from dementia or low visual acuity and with a low AMD rate.¹⁹

In conclusion, in this elderly population, we demonstrated that plasma L was weakly associated with MPOD after taking into account confounders, mainly in nonsmokers. The results suggest that a higher consumption of squash may increase plasma L and Z³⁵ in the elderly. Considering the cross-sectional design of our population-based study, these data should be completed with a longitudinal follow-up of this cohort to investigate the relationship with subsequent occurrence of AMD.

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