Plasma Lutein and Zeaxanthin and Other Carotenoids as Modifiable Risk Factors for Age-Related Maculopathy and Cataract: The POLA Study

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Purpose. To assess the associations of plasma lutein and zeaxanthin and other carotenoids with the risk of age-related maculopathy (ARM) and cataract in the population-based Pathologies Oculaires Liées à l'Age (POLA) Study.

Methods. Retinal photographs were graded according to the international classification. ARM was defined by the presence of late ARM (neovascular ARM, geographic atrophy) and/or soft indistinct drusen (>125 μm) and/or soft distinct drusen (>125 μm) associated with pigmentary abnormalities. Cataract classification was based on a direct standardized lens examination at the slit lamp, according to Lens Opacities Classification System III. Plasma carotenoids were measured by high-performance liquid chromatography (HPLC), in 899 subjects of the cohort.

Results. After multivariate adjustment, the highest quintile of plasma zeaxanthin was significantly associated with reduced risk of ARM (OR = 0.07; 95% CI: 0.01–0.58; *P* for trend = 0.005), nuclear cataract (OR = 0.23; 95% CI: 0.08–0.68; *P* for trend = 0.003) and any cataract (OR = 0.53; 95% CI: 0.31–0.89; *P* for trend = 0.01). ARM was significantly associated with combined plasma lutein and zeaxanthin (OR = 0.21; 95% CI: 0.05–0.79; *P* for trend = 0.01), and tended to be associated with plasma lutein (OR = 0.31; 95% CI: 0.09–1.07; *P* for trend = 0.04), whereas cataract showed no such associations. Among other carotenoids, only β-carotene showed a significant negative association with nuclear cataract, but not ARM.

Conclusions. These results are strongly suggestive of a protective role of the xanthophylls, in particular zeaxanthin, for the protection against ARM and cataract. (*Invest Ophthalmol Vis Sci.* 2006;47:2329–2335) DOI:10.1167/iovs.05-1235

Although cataract and age-related maculopathy (ARM) are leading causes of blindness in the elderly,[1–3] their pathogenesis is not clearly understood. In recent years, there has been increasing interest in the potential role of the xanthophyll carotenoids lutein and zeaxanthin in the pathogenesis of these diseases. Lutein and zeaxanthin accumulate selectively in the retina and are particularly dense in the macula, where they are referred to as macular pigment.[4] Macular pigment is thought to protect against retinal damage by filtering out phototoxic short-wavelength visible light and by defending rod outer segment membranes from oxidative stress.[5] In three case–control studies, subjects with high macular pigment density had a reduced risk of age-related macular degeneration.[6–8] Some epidemiologic studies have also suggested that subjects with high dietary intakes and/or high plasma concentrations of lutein and zeaxanthin have a reduced risk of ARM,[9–12] although other studies did not evidence significant associations.[13–17] Lutein and zeaxanthin are the only carotenoids present in the lens, where they probably have similar functions (phototoxic blue light filtering and neutralization of reactive oxygen species).[18,19] Some epidemiologic data are suggestive of a negative association of cataract with lutein and zeaxanthin status,[20–23] whereas other studies found no association[24] or even a positive association.[25]

Epidemiologic data on the association of xanthophylls with the risk of ARM and cataract remain scarce, and partly inconsistent. Moreover, until recently, lutein and zeaxanthin in plasma could not be separated easily. Most studies have therefore assessed the associations of ARM or cataract with the combined plasma concentration of lutein and zeaxanthin, thereby limiting the chances of finding specific associations of lutein or zeaxanthin with these diseases.

In the present report, we separately assessed the associations of plasma lutein and zeaxanthin as well as that of other carotenoids with the risk of ARM and cataract, in a Mediterranean population-based study.

Materials and Methods

Study Population

The POLA (Pathologies Oculaires Liées à l'Age) Study is a prospective study, designed to identify the risk factors of age-related eye diseases (cataract, age-related macular degeneration). The methods used in the study this study have been published elsewhere.[26] Briefly, inclusion criteria were (1) being a resident of Sète (in southern France); and (2) being aged ≥60 years. According to the 1990 population census, there were almost 12,000 eligible residents, from which our objective was to recruit 3000 participants. The population was informed of the study through the local media (television, radio, and newspapers). We also contacted 4543 residents individually by mail and telephone, using the

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electoral roll. Between June 1995 and July 1997, we recruited 2584 participants.

The present study uses data from this baseline examination, which included a standardized ophthalmic examination, an interviewer-based questionnaire, and fasting blood samples collected at home on the morning of the examination. After measurements were made on plasma (lipids, glucose, and antioxidants) and on red blood cells (superoxide dismutase), aliquots of plasma samples collected during the baseline examination were kept frozen at −80°C. In 2002 to 2004, plasma carotenoids were measured in these aliquots (which had never been thawed), for all participants recruited before April 1996 (n = 899).

This research adhered to the tenets of the Declaration of Helsinki. Participants gave written consent for participation in the study. The design of this study has been approved by the Ethics Committee of Montpellier's University Hospital.

Ophthalmic Examination

Four ophthalmologists (Louis Balmelle, Jacques Costeau, Jean-Luc Diaz, and Fabienne Robert) performed the ophthalmic examinations. This examination included a record of ophthalmic history (in particular, lens extraction and year of the extraction); a measure of best corrected distance visual acuity in the right and left eyes; after pupil dilation, a standardized assessment of nuclear, cortical, and posterior subcapsular lens opacities at slit lamp examination according to the Lens Opacities Classification System III (LOCS)[27] and one 50° color photograph (Gold 100 ASA; Eastman-Kodak Company, Rochester, NY) centered on the macular area in each eye.

Photographic Grading

After the film was processed, the retinal photographs were scanned, digitized, and recorded on compact discs (Kodak procedure). The digitized images were recorded in TIFF format, with 768 × 512 pixels. Finally, for evaluation, photographs were examined on a 17-in. (43-cm) computer screen. The total magnification was approximately ×31.5.

For grading the photographs, we used the definitions and grids of an international classification.[28] We also used the standard photographs of the Wisconsin Age-Related Maculopathy Grading System,[29] to train the ophthalmologist and the technician in charge of the evaluation. Two levels of grading were then applied to the fundus photographs. A preliminary grading was performed by an ophthalmologist; for subjects in whom soft drusen or pigmentary abnormalities were present anywhere on the photograph, a detailed grading was performed by a specially trained technician who used the international classification system.[28]

All lesions that were classified as geographic atrophy or neovascular macular degeneration by the ophthalmologist were discussed and adjudicated by two of the authors. In four cases of probable late ARM, we also asked the ophthalmologists in charge of the patients for additional information on the history of the lesion.

Classification of ARM

Early and late ARM was defined according to the international classification,[28] on the basis of 50° color photographs centered on the macular area in each eye. Late ARM was defined by the presence of neovascular ARM or geographic atrophy within the grid (3000 μm from the foveola). Neovascular ARM included serous or hemorrhagic detachment of the retinal pigment epithelium (RPE) or sensory retina, subretinal or sub-RPE hemorrhages, and fibrous scar tissue. Geographic atrophy was defined as a discrete area of retinal depigmentation, 175 μm in diameter or larger, characterized by a sharp border and the presence of visible choroidal vessels.

Soft, distinct drusen were larger than 125 μm in diameter, with uniform density and sharp edges, whereas indistinct drusen were of the same size with decreasing density from the center outward and fuzzy edges. Pigmentary abnormalities were defined as areas of hyperpigmentation and/or hypopigmentation (without visibility of choroidal vessels). Similar to the definitions used in the Blue Mountains Eye Study[30] and the Rotterdam Study,[31] early ARM was defined by the presence in at least one eye of (1) soft indistinct drusen (>125 μm) and/or (2) soft distinct drusen (>125 μm) associated with pigmentary abnormalities (hyper- or hypopigmentation), in the absence of late ARM. This definition of early ARM has high predictive value for incident AMD in the POLA Study,[32] as in the Blue Mountains Eye Study and the Rotterdam Study.

Definition of Cataract

As in the other publications from the POLA Study,[33–35] the presence of cataract was defined as: NC or NO grades ≥4 for nuclear opacities, C grade ≥4 for cortical opacities, and P grade ≥ 2 for posterior subcapsular (PSC) opacities. This level of opacification corresponded to significant visual impairment in most participants.

Eyes were classified as having a single type of cataract (nuclear, cortical, or posterior subcapsular) when only one type of opacity was present. The mixed cataract group consisted of eyes with various combinations of nuclear, cortical, and posterior subcapsular cataracts. Eyes that already had lens extraction formed a separate group (cataract surgery). All other eyes were considered to be free of cataract (NO, NC, and C <4; P <2 in both eyes).

Interview Data

Data were collected by trained study personnel who were unaware of ARM or cataract status. A standardized interview was performed to assess, in particular, sociodemographic variables (e.g., marital status, educational level, major lifetime occupation); medical history (e.g., treated hypertension, cardiovascular diseases, diabetes, knee or hip osteoarthritis); recording of all medications currently used; and history of smoking and light exposure.

The interviewer then measured height, weight, waist, and hip circumferences and systolic and diastolic blood pressure. Body mass index was defined as: weight (in kilograms)/height squared (in meters).

Biochemical Data

Plasma samples were analyzed for lutein, zeaxanthin, and 3′-dehydrolutein as well as for other carotenoids, tocopherols, total cholesterol, and triglycerides using dedicated analytical methods.[28] These measurements were performed at DSM Nutritional Products (Kaiseraugst, Switzerland; headquarters, Heerlen, The Netherlands). None of the people involved in plasma carotenoid determination at DSM, had any access to eye diseases classifications or any other clinical finding, at any time of the study.

Previous biochemical data included measurements in plasma (cholesterol, triglycerides; vitamins A, E, and C; and glutathione peroxidase) and in red blood cells (superoxide dismutase). Measurement of plasma glutathione peroxidase concentration (plGPx) was performed by enzyme-linked immunoassay (Bioxytech pl-Gpx-EIA; Oxis International SA, Portland, OR). Red blood cell superoxide dismutase activity (SOD) was measured by a spectrophotometric assay (Bioxytech SOD-525; Oxis International SA). Lipid-standardized plasma α-tocopherol was defined as: millimoles of α-tocopherol/(millimoles cholesterol + millimoles triglycerides).

Missing Data

Among the 899 subjects with plasma carotenoid measurements, photographs gradable for ARM were available in one eye at least of 644 (72%) subjects. In the majority (82%) of cases, the absence of gradable photographs was due to technical failure (in particular, to problems with the flash system at the beginning of the study). Of those, 640 had complete data for all potential confounders and were used for the estimation of associations between ARM and plasma carotenoids.

Table 1. Correlation Coefficients among Plasma Carotenoids after Logarithmic Transformation in the POLA Study

	Lutein	Zeaxanthin	Dehydro-lutein	β-Carotene	α-Carotene	β-Cryptoxanthin	Lycopene
Lutein	1	0.74	0.86	0.54	0.53	0.45	0.32
Zeaxanthin		1	0.80	0.43	0.33	0.56	0.32
Dehydro-lutein			1	0.45	0.43	0.45	0.33
β-carotene				1	0.77	0.62	0.48
α-Carotene					1	0.43	0.31
β-cryptoxanthin						1	0.43
Lycopene							1

$n = 899$.

Similarly, cataract status was available in 881 (98%) of the 899 participants with plasma carotenoid measurements. Of those, 815 subjects had complete data for all potential confounders.

Statistical Analyses

For each biochemical variable of interest, we determined the 20th and 80th percentile values, which formed three groups (low quintile, middle quintiles, high quintile). To take into account data from both eyes and their correlation, we used logistic generalized estimating equation (GEE) models for all analyses.[36] Age- and gender-adjusted odds ratio and 95% confidence interval (CI) were first obtained with the eye disease as the dependent variable, and age, gender, and the two nonreference quintile groups of the plasma carotenoid as the independent variables (A, adjusted analyses). Potential confounders were then added to the models to obtain multivariate odds-ratios (M, multivariate analyses)—that is, variables that had been identified in the POLA Study as significant risk factors for early or late ARM[26,37,38] or for each type of cataract.[33–35,39] For ARM, the potential confounders were therefore smoking, lipid-standardized α-tocopherol, plasma HDL cholesterol and BMI. For nuclear cataract, they were educational level, brown iris, smoking, plasma glutathione peroxidase ($\log_{10}$), annual ambient solar radiation, and plasma transthyretin. For cortical cataract, they were educational level, cardiovascular disease, diabetes, plasma glutathione peroxidase ($\log_{10}$), brown iris, annual ambient solar radiation, and leisure exposure to sunlight. For PSCs, they were educational level, oral corticosteroids, cancer, diabetes, occupational exposure to sunlight, and use of sunglasses. For mixed cataract, they were educational level, brown iris, diabetes, plasma glutathione peroxidase ($\log_{10}$) occupational exposure to artificial light, annual ambient solar radiation, and plasma transthyretin. For cataract surgery, they were educational level, smoking, diabetes, asthma, hypertension, plasma glutathione peroxidase ($\log_{10}$), and annual ambient solar radiation. For any cataract, they were educational level, plasma glutathione peroxidase ($\log_{10}$), brown iris, smoking, oral corticosteroids, cancer, cardiovascular disease, hypertension, diabetes, asthma, leisure exposure to sunlight, annual ambient solar radiation, occupational exposure to sunlight, occupational exposure to artificial light, use of sunglasses, and plasma transthyretin.

Tests for trend were performed by entering the biochemical variable in the logistic regression as a three-category variable instead of two independent dummy variables. All analyses were performed on computer (SAS ver. 9.1; SAS Institute, Inc., Cary, NC).

Results

Plasma carotenoids were highly intercorrelated (Table 1). The highest correlations were among lutein, zeaxanthin, and dehydro-lutein and among α- and β-carotene ($r > 0.70$). The other correlation coefficients ranged from 0.31 for α-carotene and lycopene to 0.62 for β-carotene and β-cryptoxanthin.

The associations of plasma carotenoids with the risk of ARM are presented in Table 2. Of the 640 subjects (1193 eyes) with complete data for ARM statistical analyses, 10 eyes (7 subjects) had late ARM, and 45 eyes (34 subjects) had early ARM. Because of the small number of subjects with late ARM, we pooled early and late ARM in all statistical analyses. Plasma lutein and zeaxanthin showed a strong inverse association with ARM. The association with plasma zeaxanthin was particularly strong. Compared with subjects who had low levels of zeax-

Table 2. Associations of ARM, Early or Late, with Plasma Carotenoids in the POLA Study

Plasma Carotenoid (μM)	Age- and Gender-Adjusted	Multivariate Adjusted*
Lutein		
<0.18	1.0	1.0
0.18–0.41	0.45 (0.22–0.94)	0.40 (0.18–0.89)
≥0.41	0.31 (0.09–1.05)	0.31 (0.09–1.07)
P for trend	0.03	0.04
Zeaxanthin		
<0.04	1.0	1.0
0.04–0.09	0.76 (0.37–1.57)	0.74 (0.35–1.59)
≥0.09	0.07 (0.01–0.61)	0.07 (0.01–0.58)
P for trend	0.005	0.005
Dehydro-lutein		
<0.03	1.0	1.0
0.03–0.07	0.51 (0.24–1.11)	0.52 (0.22–1.20)
≥0.07	0.38 (0.11–1.29)	0.39 (0.11–1.39)
P for trend	0.08	0.11
Total LZ†		
<0.25	1.0	1.0
0.25–0.56	0.48 (0.23–1.01)	0.45 (0.20–1.01)
≥0.56	0.21 (0.05–0.82)	0.21 (0.05–0.79)
P for trend	0.009	0.01
β-carotene		
<0.27	1.0	1.0
0.27–0.97	0.61 (0.27–1.39)	0.62 (0.28–1.35)
≥0.97	0.55 (0.18–1.71)	0.56 (0.18–1.75)
P for trend	0.29	0.29
α-carotene		
<0.04	1.0	1.0
0.04–0.20	0.94 (0.44–1.99)	0.93 (0.44–1.97)
≥0.20	0.33 (0.09–1.15)	0.30 (0.08–1.09)
P for trend	0.06	0.05
β-cryptoxanthin		
<0.13	1.0	1.0
0.13–0.47	1.20 (0.47–3.05)	1.18 (0.48–2.92)
≥0.47	1.08 (0.35–3.29)	1.16 (0.39–3.44)
P for trend	0.87	0.77
Lycopene		
<0.22		
0.22–0.71	0.90 (0.40–2.01)	0.92 (0.40–2.10)
≥0.71	0.34 (0.10–1.20)	0.38 (0.10–1.39)
P for trend	0.14	0.21

Estimations were performed using GEE logistic models, taking into account the data of both eyes and their correlation. Data are odds ratio (95% CI). $n = 55$ eyes with ARM.

* Multivariate adjustment for age, gender, smoking, lipid-standardized alpha-tocopherol, HDL-cholesterol, BMI.

† Total LZ = lutein + zeaxanthin + dehydro-lutein.

Table 3. Age- and Gender-Adjusted Associations of Plasma Lutein and Zeaxanthin with Cataract in the POLA Study

Plasma Carotenoid (μM)	Nuclear Only (86 Eyes)	Cortical Only (47 Eyes)	PSC Only (98 Eyes)	Mixed (93 Eyes)	Cataract Surgery (94 Eyes)	Any Cataract (418 Eyes)
Lutein						
< 0.17	1.0	1.0	1.0	1.0	1.0	1.0
0.17–0.41	0.79 (0.41–1.53)	0.80 (0.33–1.94)	1.17 (0.55–2.48)	0.87 (0.40–1.87)	0.83 (0.39–1.76)	0.85 (0.56–1.30)
≥0.41	0.60 (0.24–1.47)	0.75 (0.23–2.47)	1.26 (0.52–3.07)	0.75 (0.27–2.10)	1.07 (0.41–2.77)	0.82 (0.48–1.41)
P for trend	0.26	0.63	0.60	0.59	0.93	0.48
Zeaxanthin						
<0.04	1.0	1.0	1.0	1.0	1.0	1.0
0.04–0.09	0.47 (0.25–0.88)	0.60 (0.24–1.48)	1.22 (0.60–2.48)	1.00 (0.44–2.26)	0.69 (0.33–1.43)	0.70 (0.46–1.05)
≥0.09	0.25 (0.08–0.71)	1.09 (0.37–3.26)	0.84 (0.34–2.07)	0.66 (0.23–1.91)	0.75 (0.30–1.89)	0.57 (0.34–0.95)
P for trend	0.004	0.83	0.68	0.49	0.52	0.03
Dehydro-lutein						
< 0.03	1.0	1.0	1.0	1.0	1.0	1.0
0.03–0.07	0.81 (0.42–1.58)	0.84 (0.33–2.13)	1.05 (0.51–2.15)	1.53 (0.69–3.42)	0.98 (0.46–2.09)	0.90 (0.59–1.37)
≥ 0.07	0.34 (0.11–1.04)	0.89 (0.28–2.86)	0.97 (0.41–2.30)	0.74 (0.24–2.27)	0.86 (0.31–2.36)	0.69 (0.40–1.20)
P for trend	0.05	0.85	0.94	0.76	0.78	0.19
Total LZ*						
< 0.25	1.0	1.0	1.0	1.0	1.0	1.0
0.25–0.56	0.66 (0.34–1.29)	0.78 (0.32–1.90)	1.10 (0.53–2.28)	0.66 (0.32–1.37)	0.66 (0.32–1.38)	0.70 (0.46–1.07)
≥ 0.56	0.53 (0.21–1.36)	0.76 (0.23–2.52)	1.08 (0.45–2.62)	0.45 (0.16–1.30)	0.85 (0.33–2.18)	0.65 (0.38–1.11)
P for trend	0.17	0.65	0.87	0.13	0.67	0.11

Estimations were performed using GEE logistic models, taking into account the data of both eyes and their correlation. Data are odds ratio (95% CI).

* Total LZ = lutein + zeaxanthin + dehydro-lutein.

anthin (<0.04 μM), subjects with high levels of plasma zeaxanthin (>0.09 μM) had a 93% reduced risk of ARM. Globally, subjects with high total plasma lutein and zeaxanthin (>0.56 μM) had a 79% reduced risk of ARM compared with subjects with low total plasma lutein and zeaxanthin (<0.25 μM). Plasma dehydro-lutein, α- and β-carotene, β-cryptoxanthin, and lycopene did not show significant association with the risk of ARM. Further adjustment for smoking, lipid-standardized α-tocopherol, HDL cholesterol, and BMI did not materially affect the results (Table 2).

With respect to cataract, among the 1625 eyes (815 subjects) with complete data for cataract statistical analyses, 86 eyes (59 subjects) had nuclear only, 47 eyes (30 subjects) cortical only, 98 eyes (53 subjects) PSC only, and 93 eyes (66 subjects) mixed cataracts, and 94 eyes had cataract surgery (33 subjects with bilateral cataract surgery). The remaining 1207 eyes (574 subjects) without any type of cataract served as control subjects for all statistical analyses.

As shown in Table 3, after adjustment for age and gender, only plasma zeaxanthin showed a strong inverse association with nuclear cataract. Compared with subjects with low plasma zeaxanthin (<0.04 μM), those with high plasma zeaxanthin (>0.09 μM) had a 75% decreased risk of nuclear cataract. The other types of cataract did not show any significant association with plasma zeaxanthin. Globally, the risk of any cataract was reduced by 43% in subjects with high plasma zeaxanthin. Similarly, subjects with high plasma dehydro-lutein (>0.07 μM) had a significantly (66%) reduced risk of nuclear cataract, compared with subjects with low plasma dehydro-lutein (<0.03 μM; $P = 0.05$). By contrast, plasma lutein was not significantly associated with any type of cataract. Globally, total lutein, and zeaxanthin showed a trend toward an inverse association with nuclear, mixed, and any cataract that did not reach statistical significance.

These associations were not materially affected by further multivariate adjustment. In particular, the associations of plasma zeaxanthin with nuclear cataract (OR = 0.23; 95% CI: 0.08–0.68; *P* for trend = 0.003) and any cataract (OR = 0.53; 95% CI: 0.31–0.89; *P* for trend = 0.01) and of plasma dehydro-lutein with nuclear cataract (OR = 0.32; 95% CI: 0.10–1.02; *P* for trend = 0.04) remained statistically significant. The association of dehydro-lutein with any cataract became significant after multivariate adjustment (OR = 0.55; 95% CI: 0.31–0.97; *P* for trend = 0.04).

As shown in Table 4, plasma β-cryptoxanthin, α-and β-carotene, and lycopene did not show any significant associations with any type of cataract. However, the association of β-carotene with nuclear cataract became statistically significant after multivariate adjustment (OR = 0.38; 95% CI: 0.14–1.04; *P* for trend = 0.04).

Discussion

In the present study, high plasma zeaxanthin was associated with a markedly reduced risk of ARM and nuclear cataract. High plasma lutein and total lutein and zeaxanthin were also associated with a reduced risk of ARM, whereas they were not significantly associated with cataract. Although there were important correlations among carotenoids, we observed no important associations of ARM or cataract with the other studied carotenoids (α- and β-carotene, β-cryptoxanthin, lycopene), in line with the fact that only lutein and zeaxanthin are present in the retina and in the lens.

With respect to ARM, our results are consistent with those of a recent cross-sectional study performed in the United Kingdom.[12] The authors reported a significantly (50%) reduced risk of early or late ARM (OR = 0.5; 95% CI: 0.2–1.0; $P = 0.05$) in subjects with high plasma zeaxanthin (>0.05 μM), compared with subjects with low levels (<0.03 μM). The association with plasma lutein was weaker and did not reach statistical significance (OR = 0.6; 95% CI: 0.3–1.1; $P = 0.12$). In our study, the associations with plasma zeaxanthin and lutein were even stronger (OR = 0.07 and 0.31, respectively), and both were significant, perhaps because of to the higher values in the highest quintile of zeaxanthin (>0.09 μM) and lutein (>0.41 μM) in this Mediterranean population, probably associated with higher dietary intakes of these xanthophylls, which are

TABLE 4. Age- and Gender-Adjusted Associations of Other Plasma Carotenoids with Cataract in the POLA Study

Plasma Carotenoid (μM)	Nuclear Only (86 Eyes)	Cortical Only (47 Eyes)	PSC Only (98 Eyes)	Mixed (93 Eyes)	Cataract Surgery (94 Eyes)	Any Cataract (418 Eyes)
Beta-carotene						
< 0.27	1.0	1.0	1.0	1.0	1.0	1.0
0.27–0.97	0.63 (0.33–1.23)	1.67 (0.55–5.10)	1.01 (0.51–2.04)	0.77 (0.36–1.63)	0.83 (0.39–1.75)	0.89 (0.58–1.35)
≥ 0.97	0.42 (0.16–1.12)	1.10 (0.28–4.26)	0.51 (0.19–1.36)	0.50 (0.18–1.38)	0.81 (0.32–2.08)	0.69 (0.40–1.19)
P for trend	0.07	0.93	0.16	0.17	0.68	0.17
α-carotene						
< 0.04	1.0	1.0	1.0	1.0	1.0	1.0
0.04–0.20	1.30 (0.60–2.80)	0.99 (0.39–2.52)	0.88 (0.45–1.74)	0.78 (0.35–1.76)	0.69 (0.34–1.41)	0.88 (0.58–1.33)
≥ 0.20	0.76 (0.29–2.03)	0.97 (0.32–2.91)	0.72 (0.30–1.73)	0.54 (0.19–1.53)	0.49 (0.19–1.32)	0.71 (0.42–1.22)
P for trend	0.52	0.96	0.46	0.24	0.15	0.21
β-cryptoxanthin						
< 0.13	1.0	1.0	1.0	1.0	1.0	1.0
0.13–0.47	0.68 (0.35–1.31)	1.18 (0.46–3.04)	1.04 (0.53–2.04)	0.96 (0.47–1.98)	0.58 (0.28–1.18)	0.84 (0.55–1.26)
≥ 0.47	0.70 (0.29–1.68)	1.49 (0.48–4.68)	0.42 (0.15–1.18)	0.59 (0.22–1.60)	0.82 (0.34–1.98)	0.71 (0.42–1.20)
P for trend	0.44	0.49	0.09	0.30	0.70	0.20
Lycopene						
< 0.22	1.0	1.0	1.0	1.0	1.0	1.0
0.22–0.71	0.92 (0.46–1.82)	0.92 (0.36–2.33)	1.04 (0.51–2.12)	1.76 (0.79–3.93)	1.23 (0.57–2.64)	1.02 (0.67–1.56)
≥ 0.71	1.01 (0.41–2.51)	0.92 (0.27–3.13)	1.19 (0.49–2.88)	2.11 (0.71–6.27)	1.01 (0.36–2.85)	1.17 (0.68–2.01)
P for trend	0.99	0.89	0.70	0.16	0.92	0.58

Estimations were performed using GEE logistic models, taking into account the data of both eyes and their correlation. Data are odds ratios (95% CI).

mainly provided by leafy green vegetables such as spinach or broccoli.

Previous studies did not differentiate lutein from zeaxanthin. In the Eye Disease Case–Control Study, plasma lutein and zeaxanthin (LZ > 0.67 vs. < 0.25 μM) was associated with a reduced risk of neovascular ARM, with estimates close to the present study (OR = 0.3; 95% CI: 0.2–0.6).[10] Dietary LZ was also inversely associated with neovascular AMD (OR = 0.43; 95% CI: 0.2–0.7)).[9] Similarly, a recent case–control study from the Netherlands showed a significantly reduced risk of neovascular ARM in subjects with high dietary LZ (OR = 0.41; 95% CI: 0.20–0.91).[11] In the Third National Health and Nutrition Examination Survey (NHANES III), high plasma levels of LZ tended to be associated with a reduced risk of late AMD (OR = 0.6; 95% CI: 0.2–1.9), but this association was far from significant.[13] Similarly, high dietary intakes of LZ tended to be associated with reduced risk of late ARM (OR = 0.5; 95% CI: 0.1–1.6), but this association was significant only in subjects younger than 80 years (OR = 0.1; 95% CI: 0.0–0.9).[13] In a case–control study nested in the Beaver Dam Eye Study, the association between late ARM and plasma LZ was not significant (OR = 0.7; 95% CI: 0.4–1.4 for LZ > 0.17 vs. LZ < 0.17 μM).[14] In a 50% random sample of the Beaver Dam Eye Study, no significant associations were found between dietary LZ and prevalent early or late ARM[15] or incident early ARM.[16] Similarly, there were no significant associations of dietary LZ with incident early ARM in the Blue Mountains Eye Study.[17]

With respect to cataract, we found an association of nuclear cataract with plasma zeaxanthin but not with plasma lutein. In a cross-sectional study performed in the United Kingdom,[40] a nonsignificant inverse association of nuclear cataract with both zeaxanthin (OR = 0.7; 95% CI: 0.4–1.4) and lutein (OR = 0.8; 95% CI: 0.4–1.5) was found. In a Spanish case-control study, no significant associations were found between dietary lutein or dietary zeaxanthin and the risk of cataract.[24] However, the statistical analyses did not distinguish the different types of cataract. Another case–control study performed in Spain showed an increased risk of cataract in subjects with high plasma lutein (OR =1.8; 95% CI: 1.1–3.1) or zeaxanthin (OR = 2.2; 95% CI: 1.3–3.7).[25] However, age distribution was very different between cases and controls and potential confounding by known risk factors was not accounted for.

Previous studies have given results for pooled lutein and zeaxanthin, which may have obscured the association with cataract, if only zeaxanthin is associated with cataract. In our study, plasma total lutein and zeaxanthin were associated with an ORs of 0.53 for nuclear cataract and of 0.45 for mixed cataract, and with ORs close to 1 for cortical cataract and PSC. In a prospective analysis of the Beaver Dam Eye Study data, high dietary intakes of lutein and zeaxanthin were associated with a reduced risk of incident nuclear cataract (OR = 0.5; 95% CI: 0.3–0.8),[22] whereas in a subsample, the association with plasma lutein and zeaxanthin was in the same direction but did not reach statistical significance (OR = 0.7; 95% CI: 0.3–1.6).[41] In the Nutrition and Vision Project, high dietary intakes of lutein and zeaxanthin were associated with a reduced risk of nuclear cataract (OR = 0.52; 95% CI: 0.29–0.91),[23] whereas their associations with cortical cataract and PSC were not statistically significant.[42] Two large prospective studies also showed a reduced risk of cataract surgery in subjects with high dietary intakes of lutein and zeaxanthin.[20,21]

Although plasma lutein and zeaxanthin correlate highly, our results suggest a stronger association of plasma zeaxanthin than lutein, with the risk of ARM and cataract, consistent with the study from Gale et al.[12,40] The hypothesis of a more important role of zeaxanthin in retina and lens health is supported by several lines of evidence. First, the ratio of zeaxanthin to lutein is much higher in the central retina (1:1 in the macula, 2:1 in the fovea)[19,43] and in the lens (1:1)[19] than it is in the plasma (~1:5 in the present study), suggesting that the eye preferentially accumulates zeaxanthin. Moreover, although both lutein and zeaxanthin protect liposomal membranes from light induced oxidative stress, zeaxanthin appears to be a better photoprotector during prolonged UV exposure,[44] perhaps because there is a different orientation of lutein and zeaxanthin in biological membranes.[44] Zeaxanthin is also particularly effective in protecting lipid membranes against oxidation by peroxyl radicals.[45] More data are needed on the specific associations of lutein and zeaxanthin with ARM and cataract, both at the biological and epidemiologic levels.

Our study has several limitations. First, our sample underrepresents the older persons and overrepresents the middle and upper social classes, by comparison with the whole eligible population.[26] The subjects of this study may thus be healthier and have different lifestyles, in particular concerning diet and physical activity, than the general population. These differences are likely to have affected the distribution of plasma carotenoids or the prevalence of eye diseases. However, they are unlikely to have affected the association between eye diseases and plasma measurements. Moreover, although a selection bias cannot be dismissed, the prevalence rates of cataract and ARM and their associations with their known risk factors (i.e., smoking, diabetes, corticosteroids, and light exposure) are similar to those observed in other studies.[26,34,35]

Because samples were kept frozen at −80°C for approximately 7 years, it is possible that the observed carotenoid levels were underestimated in the present study due to long-term instability. For instance, for β-carotene, a 20% decrease in plasma level was observed over 7 years of storage at −80°C.[46] Because all samples were treated and stored in a similar manner, such a decrease is unlikely to have affected the associations between eye diseases and plasma measurements.

The relatively small number of subjects affected by ARM and cataract constitutes another limitation. In particular, due to the very low number of subjects with late ARM, we were unable to separate early and late ARM in statistical analyses. Our results therefore reflect mainly the associations of plasma carotenoids with early ARM. The small number of subjects in some cataract types (in particular cortical cataract) may also have generated insufficient statistical power to detect associations with plasma carotenoids. In particular, this may be one reason for the absence of association of plasma xanthophylls with mixed cataract and cataract surgery. Indeed, it is rather surprising that these two groups of cataract, which usually include a large number of nuclear opacities, were not significantly associated with zeaxanthin, whereas nuclear cataract was. Although their odds ratios were not statistically significant, they were in the same direction and were not significantly different from the one for nuclear cataract. These negative relationships probably contributed to the global statistically significant negative association with "any cataract."

In addition, cataract was graded directly at the slit lamp according to LOCS III, instead of photographic grading. This method may have caused misclassification of cataract status and therefore may have biased the observations toward the null hypothesis. Another limitation is due to the high number of comparisons made (up to 48 for cataract). We therefore cannot exclude that some of the observed associations were due to a chance finding.

In observational studies, the concern is always about confounding. We have therefore performed multivariate adjustments, to take into account all known risk factors for cataract and ARM. In addition to age, gender, and educational level, specific factors were used for the different types of cataract and ARM. The selected factors are those identified in previous analyses of the POLA Study for ARM[26,37,38] and cataract.[33–35,39] The associations of plasma zeaxanthin and lutein with eye diseases were not strongly affected by these adjustments.

Because this study is cross-sectional, we cannot assume that the low plasma zeaxanthin and lutein levels preceded the development of cataract or ARM. It is possible that participants with eye disease have modified their diets after the development of visual impairment. These results must therefore be confirmed in prospective studies. Whether increasing dietary intakes of lutein and zeaxanthin, through dietary modifications or nutritional supplements, have a preventive effect against ARM or cataract can be established only by interventional studies. The results of a recent small interventional study have suggested that lutein supplements may improve visual function in subjects with geographic atrophy.[47]

In conclusion, in this Mediterranean population, high plasma zeaxanthin levels were associated with reduced risk of ARM and nuclear cataract. High plasma lutein levels were also associated with reduced risk of ARM. These data are consistent with previous epidemiologic studies and suggest that lutein and zeaxanthin may be important for protecting against ARM and cataract, in particular in its nuclear localization. These data should be confirmed by other studies—in particular, prospective epidemiologic and interventional studies.

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APPENDIX

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