

Macular Pigment and Risk for Age-Related Macular Degeneration in Subjects from a Northern European Population

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PURPOSE. Age and advanced disease in the fellow eye are the two most important risk factors for age-related macular degeneration (AMD). In this study, the authors investigated the relationship between these variables and the optical density of macular pigment (MP) in a group of subjects from a northern European population.

METHODS. The optical density of MP was measured psychophysically in 46 subjects ranging in age from 21 to 81 years with healthy maculae and in 9 healthy eyes known to be at high-risk of AMD because of advanced disease in the fellow eye. Each eye in the latter group was matched with a control eye on the basis of variables believed to be associated with the optical density of MP (iris color, gender, smoking habits, age, and lens density).

RESULTS. There was an age-related decline in the optical density of macular pigment among volunteers with no ocular disease (right eye: $r^2 = 0.29$, $P = 0.0006$; left eye: $r^2 = 0.29$, $P < 0.0001$). Healthy eyes predisposed to AMD had significantly less MP than healthy eyes at no such risk (Wilcoxon's signed rank test: $P = 0.015$).

CONCLUSIONS. The two most important risk factors for AMD are associated with a relative absence of MP. These findings are consistent with the hypothesis that supplemental lutein and zeaxanthin may delay, avert, or modify the course of this disease. (*Invest Ophthalmol Vis Sci.* 2001;42:439-446)

Age-related macular degeneration (AMD) is the leading cause of legal blindness in the Western world,¹ and its prevalence is likely to increase as a consequence of increasing longevity.² Although the pathogenesis of AMD remains poorly understood, mechanisms believed to be causative include genetic factors,³ cumulative light damage,^{4,5} free radical injury,⁶ and hemodynamic processes.⁷ The putative role of all these processes can be attributed, at least in part, to oxidative retinal injury.⁸

Human macular pigment (MP) consists of the two hydroxycarotenoids, lutein (L) and zeaxanthin (Z), with concentrations that peak at the center of the fovea.⁹ MP is an effective filter of

damaging blue light, which causes photo-oxidative retinal injury, because of its absorbance spectrum and its prereceptor location.¹⁰ Further, L and Z are powerful antioxidants with the ability to quench the triplet state of photosensitizers and singlet oxygen,^{11,12} to react with free radicals,¹³ and to retard the peroxidation of membrane phospholipids.¹⁴ Consequently, it has been hypothesized that MP protects against AMD. Because the macular pigment is entirely of dietary origin,¹⁵ a protective effect would have important health care implications.

There are many variables that have been investigated as potential risk factors for AMD. These include: exposure to sunlight,^{4,5,16-20} tobacco use,^{16,17,21,22} light iris color,^{16-18,23-27} race,^{28,29} genetic predisposition,³⁰⁻³² female gender,^{4,17,28,33} cardiorespiratory disease,^{16,34,35} diet,^{36,37} and hypermetropia.^{16,35} However, considerable controversy persists regarding all these putative risk factors. AMD in the fellow eye and increasing age, however, are two risk factors on which there is consensus.^{21,33,34,38-41} It was this observation that prompted us to correlate MP optical density measurements with age and to compare MP density measurements in the 'healthy' fellow eye of patients with unilateral neovascular AMD with matched eyes not at high risk of development of AMD.

MATERIALS AND METHODS

Subjects

Healthy Subjects. Forty-six white volunteers were recruited. Subjects were in good general health, and a comprehensive ophthalmic examination failed to identify any ocular disease. The following data were recorded for each subject: age, gender, smoking habits, iris color (classified as blue-gray, hazel-green, or brown-black, as judged by an independent observer using standardized iris photographs), and lens density (Lens Opacification Classification System ([LOCS] III)).⁴²

High-Risk Eyes. Nine white patients with advanced neovascular AMD in one eye, but no macular abnormalities in the fellow (high-risk) eye, were recruited. A diagnosis of neovascular AMD was made according to the criteria published by the International ARM Epidemiologic Study Group and required the demonstration of choroidal neovascularization in the presence of soft drusen and/or pigmentary changes.⁴³ Each high-risk eye was matched with that of a control subject based on iris color, age (± 10 years), smoking habits, gender, and lens density. Optical density of MP was then compared in a case-control manner. The matching process was masked to MP measurements.

The project was endorsed by the Central Manchester Local Research Ethics Committee (reference number, CEN/98/100). Informed consent was obtained from all subjects, and the tenets of the Declaration of Helsinki were observed.

Measurement of Macular Pigment

Apparatus. The apparatus used to derive the optical density of MP, using heterochromatic flicker photometry (HFP), is described elsewhere⁴⁴ and is schematically represented in Figure 1. The apparatus consists of a three-channel free-viewing (non-Maxwellian view)

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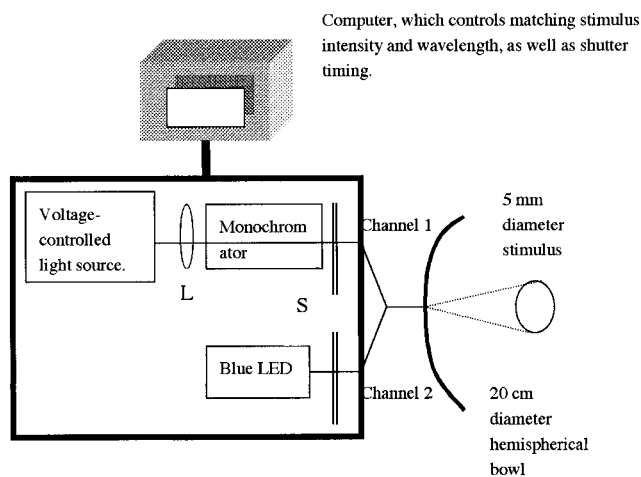


FIGURE 1. Simplified representation of the fiberoptic apparatus used to generate a heterochromatic flickering stimulus. L, aspheric condensing lens used to focus the lamp filament onto the entrance slit of the monochromator; S, shutter.

optical system. Channel 1 provides the matching stimulus with a wavelength that can be varied in increments of 1 nm (bandwidth: 6 nm). Channel 2 provides the blue reference light, an LED, the output spectrum of which peaks at 476 nm (calibrated with a spectrophotometer). The blue LED was set to give a stimulus luminance of 20 candelas (cd/m^2), thus providing retinal illumination of approximately 2.6 log troland (td).

Channels 1 and 2 are superimposed using a Y-shaped glass fiberoptic bundle to provide the test stimulus at the center of a matte white hemispheric bowl. The background was illuminated with a 10-W halogen bulb behind a color temperature filter (LEE filters 201; Lee, Andover, UK; full color temperature correction blue, tungsten to daylight, 3200–5700°K) to give a background illumination of 1.9 log td.

Channels 1 and 2 each contain a shutter driven by a pen motor (AL20/10; Lectromed, Letchworth Garden City, UK), thus allowing the test stimulus to alternate between the two channels. A computer program is used to control the wavelength and intensity of the matching stimulus (channel 1).

Procedure. To test the validity of and establish the optimum settings for our technique of MP measurement, we performed preliminary investigations in 12 healthy subjects. Sessions began with a 5-minute period of dark adaptation, during which time the procedure was explained. The viewing distance was 30 cm, thus resulting in a stimulus size of 0.95° , and the fellow eye was occluded throughout the procedure.

With the subject fixating centrally and after increasing the luminance of channel 1 (the matching stimulus) to a level of obvious flicker at a frequency of 25 Hz, the subject was instructed to reduce the luminance of the matching stimulus until flicker could no longer be appreciated (the first point of no flicker). Then, the subject continued to reduce the luminance until the flicker reappeared, which he or she then increased until flicker was once again eliminated (the second point of no flicker). The midpoint of this range of no flicker was taken as the matching luminance. When a subject could not eliminate the flicker entirely, a range of minimum flicker was recorded. This procedure was then repeated while the subject fixated a target located at a nasal eccentricity of 6° .

The absorption spectrum for MP was generated by taking optical density measurements, with central and peripheral fixation, between 450 and 560 nm in 12 subjects, in increments of 10 nm between 450 and 480 nm and in increments of 20 nm between 480 and 560 nm. The differing wavelengths were presented in a pseudorandom fashion, and three to six readings were taken for each wavelength.

When we were satisfied that our method of measuring MP optical

density was valid and reproducible, subsequent measurements involved recording the matching luminance using test stimuli of 476 and 560 nm only. In each case, five readings were taken on two separate occasions.

Derivation of MP Optical Density. The principle behind MP optical density measurements derived from HFP is as follows. A blue reference light close to the spectral optical density peak of MP (476 nm) alternates with a light that is not absorbed by the pigment (say 560 nm), and flicker is eliminated when the perceived luminance of the two lights is equalized. MP reduces the relative sensitivity of the central retina at various wavelengths by a factor equivalent to the fraction of incident light that it absorbs. Therefore, as MP is optically undetectable at an eccentricity of 6.5° , the difference between the matching luminances obtained from central and peripheral viewing can be used as a measure of MP optical density.

If the luminance of the reference L_{476} is constant and L_λ (the matching luminance) is variable, the absorption spectrum of MP is calculated by:

$$\Delta OD_\lambda = \log_{10}(L_{p\lambda}/L_{c\lambda})$$

where $L_{c\lambda}$ is the matching luminance setting for central fixation, $L_{p\lambda}$ is the matching luminance setting for peripheral fixation, and ΔOD_λ is the optical density difference of MP at wavelength λ compared with that of the reference 476 nm.

The absorption spectrum is normalized at 560 nm, because absorption by MP is zero at and above 560 nm. The optical density of MP is therefore calculated by

$$OD_{MP} = \log_{10}(L_{p560}/L_{c560})$$

Dietary Carotenoid Intake

Nutritional information was obtained by interview, using a food frequency questionnaire (FFQ)⁴⁵ which was modified to take account of published data regarding the carotenoid content of foodstuffs^{46,47} so that dietary intake of L and Z could be evaluated. No measures were taken to control for seasonal variation in the availability of foods, because it has been shown that MP optical density remains stable for long periods in subjects who consume a relatively constant diet,⁴⁸ reflecting the low biologic turnover of the carotenoids in the retina.⁴⁹ Because the intake of most nutrients correlates positively with total energy intake,⁵⁰ the nutrient values were appropriately adjusted and are expressed in milligrams per 1000 calories (kilocalories). When nutrient supplements were used, only the dietary component of the nutrient was energy adjusted and then added to the supplemented amount. Energy and nutrient values were retrieved from UK food tables.⁵¹ Dietary intake of L and Z was calculated as nutrient content of food \times portion weight \times frequency of consumption.

Statistical Analysis

Regression analysis and analysis of variance (ANOVA) were used to test age associations. Statistical comparisons between high-risk eyes and their matched pairs were made using the nonparametric Wilcoxon signed rank test, or the paired Student's *t*-test when the data came from a normal distribution. The reproducibility and test-retest variability of MP measurements are expressed as the coefficient of repeatability ($2\sqrt{2}$ variance within subjects).⁵² This coefficient represents the value below which the difference between two successive readings, or two sessions, will lie with 0.95 probability.

RESULTS

Results are expressed as mean \pm SD.

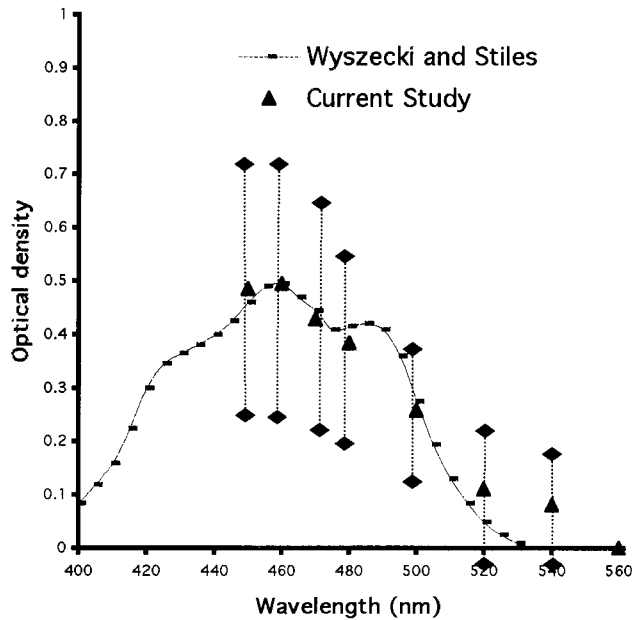


FIGURE 2. The mean absorption spectrum (\pm SD) of 12 volunteers in relation to the composite standard absorption spectrum of Wyszecki and Stiles.⁵³

Macular Pigment

Absorption Spectrum of Macular Pigment. The average absorption spectrum, and the individual absorption spectra, of MP generated by HFP closely matched the composite curve of Wyszecki and Stiles,⁵³ thus confirming the validity of our technique (Fig. 2). Reproducibility and intersession variability are given by the coefficients of repeatability of 0.08 and 0.09, respectively.

Healthy Subjects. The ages of the 46 volunteers without evidence of ocular disease ranged from 21 to 81 years (mean, 51 ± 18). The male-to-female ratio was 21:25, and 13 volunteers were current smokers. Iris color was classified as blue-gray in 21 subjects, hazel-green in 14 subjects, and brown-black in the remaining 11. Among the healthy volunteers, no association between age and gender (ANOVA: $P = 0.58$), tobacco use ($P = 0.8$), or iris color ($P = 0.07$) was noted.

The mean optical density of MP was 0.289 ± 0.156 (range, 0.024–0.646), for the right eye. The corresponding values for the left eye were 0.299 ± 0.159 (range, 0.031–0.596). There was good interocular agreement of MP optical density (simple regression: $r = 0.866$; $P < 0.0001$), with a maximum right-left difference of 0.135, and the measurements were statistically similar for fellow eyes (Wilcoxon signed rank test: $P = 0.68$; Fig. 3). A statistically significant age-related decline in the optical density of MP was observed (right eye: $r^2 = 0.24$, $P = 0.0006$; left eye: $r^2 = 0.29$, $P < 0.0001$; Fig. 4).

High-Risk Eyes. Nine healthy eyes predisposed to AMD (high-risk eyes) exhibited a mean MP optical density of 0.147 ± 0.144 (range, 0–0.346). The corresponding values for healthy eyes with no macular disease in either eye (standard-risk eyes), but matched for age (± 0 years), gender, iris color, smoking habits, and lens density were 0.311 ± 0.206 (range, 0.027–0.724). High-risk and standard-risk eyes were statistically similar in age (paired Student's t -test: $P = 0.18$), nuclear opalescence (0.18), nuclear color ($P = 0.45$), cortical opacification ($P = 0.86$), and posterior subcapsular cataract ($P = 0.71$; Table 1). Further, reproducibility of MP measurements was similar for high-risk and standard-risk eyes, expressed by the coefficients of repeatability of 0.1 and 0.12, respectively. Of note, parafoveal matching luminances

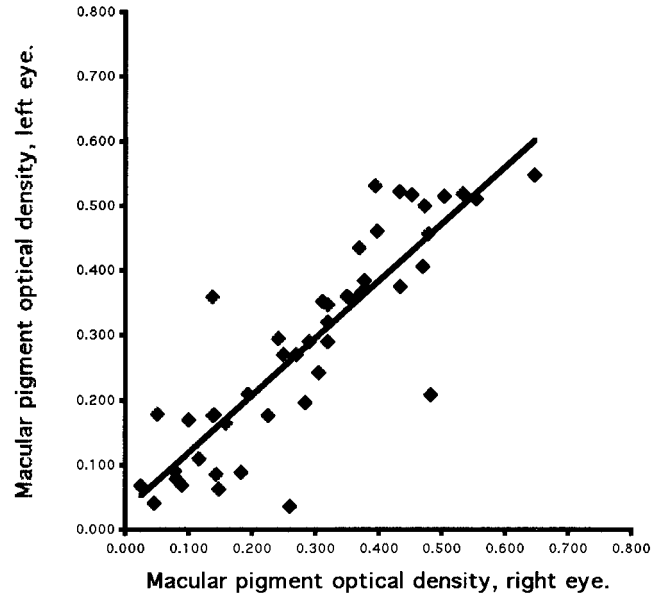


FIGURE 3. MP optical density in the right and left eyes of 46 healthy volunteers. There was good interocular agreement of MP optical density (simple regression: $r = 0.866$; $P < 0.0001$), with a maximum right-left difference of 0.135.

were similar for standard-risk and AMD-predisposed eyes (high-risk eyes: $L_{p\lambda} = 1.56 \pm 0.16$; standard-risk eyes: $L_{p\lambda} = 1.65 \pm 0.19$; Wilcoxon signed rank test: $P = 0.26$), indicating that peripheral medium- and long-wavelength cone spectral sensitivity

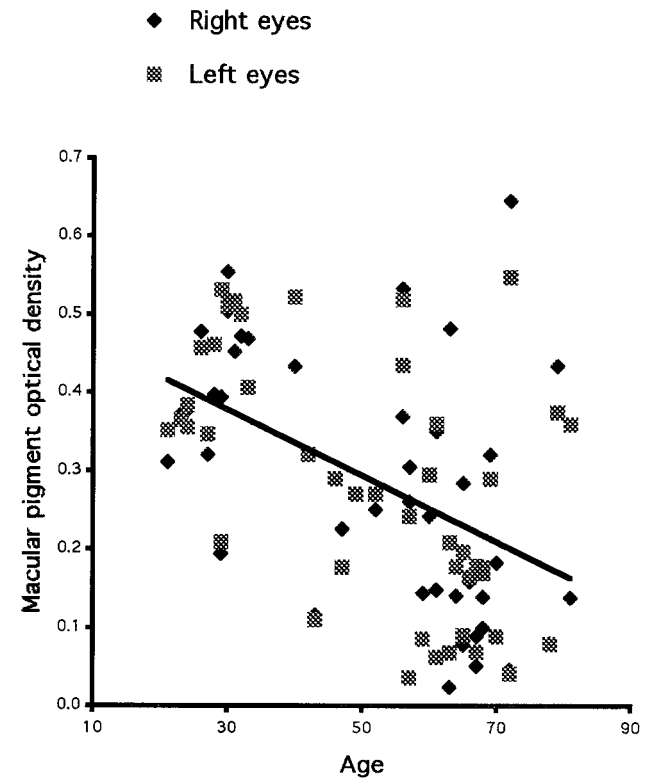


FIGURE 4. Relationship between MP optical density and age in 46 healthy control subjects. A statistically significant age-related decline in the optical density of MP was observed (right eye: $r^2 = 0.24$, $P = 0.0006$; left eye: $r^2 = 0.29$, $P < 0.0001$).

TABLE 1. Details of Factors Believed to be Related to MP Optical Density

Sex, Iris Color, and Tobacco Use	High-Risk Eye (n = 9)						Standard-Risk Eye (n = 9)					
	Age	No	Nc	C	P	MPOD	Age	No	Nc	C	P	MPOD
Female, hazel-green, smoker	61	2	2	1	1	0.03	61	1.9	2	0	0	0.72
Male, brown, smoker	73	2.5	2.8	1.0	1.0	0.0	72	2.5	2.2	4.0	1.0	0.03
Female, brown, smoker	66	2.1	2.0	1.0	0.9	0.18	58	1.5	0.8	0.5	0.5	0.37
Female, brown, nonsmoker	76	2.0	2.0	0.2	0.2	0.32	73	2.3	2.7	0.8	1.0	0.4
Male, blue, smoker	72	2.5	2.8	1.5	0.8	0.0	68	2.5	2.5	1.0	1.0	0.17
Male, blue-grey, nonsmoker	67	2.7	2.9	0.5	0.5	0.19	65	2.4	2.7	0.7	0.7	0.26
Male, blue-grey, nonsmoker	71	2.2	2.0	1.0	1.0	0.35	78	3.0	3.2	1.0	2.5	0.47
Male, hazel-green, nonsmoker	81	3.3	3.0	2.8	3.0	0.27	71	2.2	1.8	0.2	0.2	0.21
Male, blue, nonsmoker	68	2.2	2.5	0.2	0.2	0.0	66	2.5	2.2	0.2	0.3	0.18

No, nuclear opalescence; Nc, nuclear color; C, cortical cataract; P, posterior subcapsular cataract; MPOD, macular pigment optical density.

was similar for the matched pairs. Eight of the nine high-risk eyes had less MP than their matched control eyes, and the difference was statistically significant (Wilcoxon signed rank test: $P = 0.015$; Table 1).

Dietary Carotenoids

For the 46 healthy individuals, the mean daily absolute and energy-adjusted values for dietary intake of L and Z are given in Table 1. There was no demonstrable relationship between absolute or energy-adjusted intake of L, Z, or a combination of these carotenoids and MP optical density (Table 2; Figs. 5, 6).

A comparison of dietary consumption of carotenoids in subjects with healthy eyes predisposed to AMD and the matched control subjects showed no statistically significant difference in absolute or energy-adjusted intake of L, Z, carotenoid equivalent or L and Z combined (Table 3).

DISCUSSION

In this study, we used a free-viewing HFP to measure the optical density of MP in 46 healthy subjects and demonstrated an age-related decline in the optical density of the pigment in a group from a northern European population. Furthermore, we observed significantly less MP in nine healthy eyes known to be at high risk for AMD compared with nine matched eyes at no such risk.

The mean optical density of MP in this study was 0.289 ± 0.156 , higher than measurements taken in 217 subjects from Arizona (0.22 ± 0.13) in a recent study,⁵⁴ but comparable with most previous reports.^{55,56} There are two possible explanations for the discrepancy between our results and those of the Arizona study. First, Hammond and Caruso-Avery used a ref-

erence for MP measurement at only 4° retinal eccentricity, where MP is still optically detectable, and slightly lower values are therefore unsurprising. And second, all subjects reported in the current study have been living in the northwest of England since childhood, where ambient light levels are much lower than in Arizona, and it is possible that MP is depleted in response to the oxidant load arising from greater cumulative light exposure. The interindividual variability of MP measurement among our subjects, represented by a range of 0.0124 to 0.646 ± 0.156 , is entirely consistent with previous reports.^{54,57}

The current results showing an age-related decline in MP optical density are contrary to some of the early studies^{57,58} but consistent with the most recent.⁵⁴ It is important to note, however, that the early reports took no account of recently identified variables that are believed to be related to the optical density of MP, such as iris color,⁵⁹ tobacco use,⁶⁰ gender,⁵⁵ and lens density.⁶¹ In 2000, Hammond and Caruso-Avery⁵⁴ reported a statistically significant inverse relationship between the optical density of MP and age ($r = -0.14$; $P < 0.02$) among subjects living in Arizona. However, it was unclear whether the ages of the study group were related to the variables that are associated with MP density. This is of particular importance, because those associations were confirmed in that study.⁵⁴ In

TABLE 2. Daily Dietary Intake of L and Z

Variable	Absolute Dietary Intake (mg/day)	Energy-Adjusted Dietary Intake (mg/1000 kcal per day)
L and Z	3.7 ± 3.5	0.015 ± 0.013
L	2.9 ± 2.7	0.012 ± 0.01
Z	$0.82 \pm .23$	0.003 ± 0.001

Data are expressed as mean \pm SD. Intakes were assessed by a modified Food Frequency Questionnaire in 46 healthy subjects. There was no demonstrable relationship between absolute or energy-adjusted intake of L, Z, or a combination of these carotenoids, and MP optical density (Simple regression analysis. Absolute dietary intake. L: $r^2 = 0.017$, $P = 0.38$; Z: $r^2 = 0.007$, $P = 0.95$; L + Z: $r^2 = 0.016$, $P = 0.4$; energy-adjusted dietary intake: L: $r^2 = 0.027$, $P = 0.27$; Z: $r^2 = 0.002$, $P = 0.92$; L + Z: $r^2 = 0.025$, $P = 0.3$).

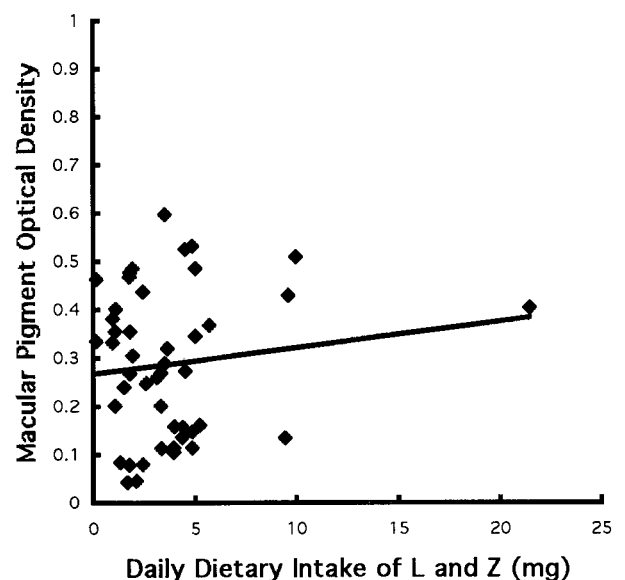


FIGURE 5. Relationship between absolute daily dietary intake of L and Z (in milligrams) and the optical density of MP in 46 healthy volunteers.

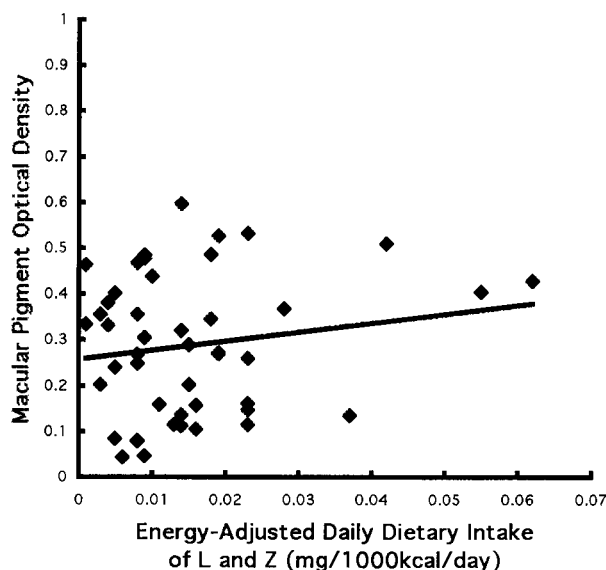


FIGURE 6. Relationship between energy-adjusted daily dietary intake of L and Z (in milligrams/1000 kcal per day) and the optical density of MP in 46 healthy volunteers.

the present study, there was no relationship between any of these variables and age. It is of interest that age and years spent in Arizona were positively correlated in Hammond and Caruso-Avery, allowing for the possibility that the age-related decline in MP optical density was attributable to cumulative exposure to very high ambient levels of light. That we have reproduced this finding in subjects from a northern European population indicates that other factors play a role.

The age-related decline in MP optical density must be attributable to either inadequate uptake or excessive depletion of the retinal carotenoids. The decline of MP optical density with increasing age may simply reflect the age-related loss of photoreceptors and their axons in which L and Z are found,⁶² especially in view of the demonstration by Elsner et al.⁶³ of the close spatial relationship between cone photopigment and MP distribution. However, because cones are relatively spared in age-related loss⁶⁴ and because our technique of HFP excluded rod contributions, this is unlikely. Alternatively, depletion of MP may result from utilization of L and Z in response to the age-related increase in oxidant load.⁶⁵⁻⁶⁸

Given that micronutrient deficiencies are seen in 18% to 40% of the elderly population,⁶⁹⁻⁷¹ the age-related decline in MP may be nutritional in origin. However, because dietary carotenoid intake is difficult to measure, reflected in the wide variability of reported values for L and Z consumption (0.8-4 mg/day),^{72,73} this is difficult to investigate. Of note, a recent study has confirmed a significant and positive correlation be-

tween dietary intake of carotenoids and serum levels in the elderly, with the exception of L and Z, indicating that the macular carotenoids may be inadequately absorbed in this age group.⁷⁴ Further, because carotenoids act synergistically with α -tocopherol and ascorbate, deficiency of either of these vitamins results in excessive depletion of its carotenoid coantioxidant.^{75,76} Beyond dietary and absorptive factors, it is also possible that age-related changes in carotenoid transport in blood and accumulation of L and Z in the retina may be important.

Of nine eyes at high-risk of AMD and nine eyes at no such risk, when paired eyes were matched on the basis of variables associated with MP optical density, less MP was seen in the predisposed eye in eight cases. The bilaterality of AMD has recently been investigated in the Blue Mountains Eye Study, in which it was reported that both eyes were affected in 80% of patients with early or late AMD.⁷⁷ Of 30 fellow eyes in subjects with unilateral neovascular AMD, early AMD was seen in 20 (66%), atrophic AMD in 7 (23%), and a healthy macula in only 3 (10%). The second eye of patients with unilateral neovascular AMD is at high risk of development of the condition because of the significant age-related increase in the bilaterality of neovascular AMD, even after adjusting for variables such as age, smoking, and family history.^{39,77} The incidence of choroidal neovascularization in the contralateral eye in cases of unilateral neovascular AMD has been estimated to lie between 28% and 35% at 4 years,^{39,40,78,79} with a 12% risk per annum.⁴¹ Of the nine high-risk eyes reported here, three exhibited soft drusen with pigmentary changes within 18 months of testing, and a further three showed these changes with choroidal neovascularization.

The relative absence of MP in eyes predisposed to AMD should be interpreted in the context of the excellent interocular agreement of MP measurements demonstrated in this study and in previous studies.^{80,81} In other words, low MP optical density in the fellow eye of a patient with neovascular AMD indicates that MP was probably absent in the diseased eye, although the latter cannot be measured because of fibrovascular scarring and loss of central vision. Further, because we matched eyes in terms of putative risk factors for AMD, which are reportedly associated with MP optical density, the observed absence of MP in the predisposed eyes appears to be an independent association with high risk for AMD. However, whether this deficiency of MP resulted in neovascular AMD in the diseased eye and will do the same in the healthy eye or is the result of subclinical disease warrants discussion.

Because MP is located within some part of the photoreceptor cell⁸² or its membrane,⁸³ and because cone and rod systems appear to be functionally impaired in early AMD,^{84,85} it is possible that photoreceptor loss in preclinical AMD may result in depletion of the pigment. However, this mechanism is unlikely to have played a role in our study, because sensitivity was similar for AMD-predisposed eyes and age-matched control eyes at the parafovea, where AMD typically begins. Further,

TABLE 3. Daily Dietary Intake of L and Z

Variable	Absolute Intake			Energy-Adjusted Intake		
	AMD Subjects	Matched Controls	<i>P</i>	AMD Subjects	Matched Controls	<i>P</i>
L and Z	5.2 ± 6.7	4.9 ± 6.4	0.8	0.021 ± 0.026	0.016 ± 0.015	0.48
L	3.9 ± 5.1	3.7 ± 4.9	0.77	0.016 ± 0.02	0.012 ± 0.012	0.47
Z	1.25 ± 0.34	1.1 ± 0.3	0.42	0.005 ± 0.001	0.004 ± 0.001	0.58

Energy-adjusted intake is in milligrams/1000 kilocalories per day. *P* is the result of a paired Student's *t*-test. Data are for nine subjects with advanced AMD in one eye and nine control subjects matched for age, iris color, gender, race, lens density, and smoking habits. There was no statistically significant difference between the two groups in absolute or energy-adjusted intake of L, Z, carotenoid equivalent, or L and Z combined.

Curcio et al.⁶² have shown a differential loss of rods and cones in AMD, with sparing of foveal cones and relative sparing of parafoveal cones in early disease. In brief, therefore, because the healthy predisposed eyes exhibited no clinical signs of disease and rods did not contribute to HFP measurements because we used a frequency of 25 Hz, we do not believe that photoreceptor dropout accounts for the absence of MP we observed in these eyes. This conclusion is consistent with the findings of Bone et al.,⁸⁶ who found that L and Z concentrations, as measured by high-performance liquid chromatography (HPLC), were significantly reduced in the central and peripheral retina of eyes with AMD, suggesting that the loss of retinal carotenoids is not the result of the disease process.

It is interesting, however, that some predisposed eyes had greater quantities of MP than some of the standard-risk eyes. In other words, our finding does not support the view that there is a critical value below which AMD is likely to develop. Rather, the results suggest depletion of preexisting MP and are therefore consistent with the view that the retinal carotenoids are used in response to an age-related process, possibly oxidative stress.⁶⁶ Clearly, the main limitation of the present study rests on the small sample size, which reflects the rarity of healthy fellow eyes in patients with unilateral neovascular AMD.

The evidence in support of the view that MP protects against AMD has been reviewed elsewhere.⁸⁷ The Eye Disease Case-Control Study (EDCC) reported that a high dietary intake and high serum levels of L and Z were associated with a reduced chance of development of AMD.^{36,88} Parallels between several putative risk factors for AMD and an absence of MP have been observed by Hammond et al.,⁵⁹ including light iris color, cigarette smoking,⁶⁰ female gender,⁵⁵ loss of visual sensitivity,⁸⁹ and increasing lens density.⁶¹ Furthermore, reduced concentrations of L and Z have been demonstrated in the macula and whole retina of human donor eyes with early AMD compared with control subjects.⁹⁰ Weiter et al.⁹¹ noted that the area of central sparing seen in cases of annular macular degeneration, including cases of atrophic AMD, correlated strongly with the lateral extent of MP, which may be due to the absorptive properties of the pigment, in that the lipofuscin fluorophore A2E is known to mediate blue-light-induced apoptosis of RPE cells.^{92,93} Indeed, it has even been suggested that the focal reduction in RPE lipofuscin concentration at the fovea is attributable to the protection afforded to the photoreceptor outer segments, the phagocytosed elements of which contribute to lipofuscinogenesis⁹⁴ by the MP.^{95,96} Although these findings are consistent with the plausible rationale that MP protects the central retina from blue light damage and oxidative stress, they should be interpreted in the context of our current and incomplete understanding of the disease and with full appreciation of the limitations of the observational nature of the studies involved.

The hypothesis that MP reduces the risk of development of AMD is particularly enticing because MP is entirely of dietary origin, thus suggesting that the most common cause of blind registration in the western World could be delayed, or even averted, with appropriate dietary modification. Hammond et al.⁹⁷ have shown that dietary supplements of spinach and corn, representing approximately four times as much L and two to three times as much Z as a typical diet, result in a significant increase in the optical density of MP and the serum concentration of L in most subjects. Of note, after discontinuation of the modified diet, serum levels of the carotenoids returned to normal but MP optical density remained augmented, reflecting the low turnover of these compounds in the retina. However, the subjects involved varied in age from 30 to 65 years and are therefore not representative of the population at risk for AMD.

It remains uncertain whether the age-related decline in MP optical density or the relative absence of MP in predisposed eyes is the result of inadequate dietary intake of L and Z or some other mechanism, and this is an area that requires further investigation. To our knowledge, there are no World Health Organization (WHO) guidelines for optimal nutritional intake of specific carotenoids, and the recommendation of preparations containing these micronutrients cannot be justified on the basis of current evidence. Nevertheless, because MP is entirely of dietary origin, it seems prudent to encourage our patients to eat a balanced diet rich in fruits and vegetables, especially those that are yellow, orange, or dark green.⁷²

In conclusion, we have shown that the two most important risk factors for AMD, age and advanced disease in the fellow eye, are associated with reduced optical density of MP. Ultimately, longitudinal studies involving serial measurements of MP and serum levels of L and Z in a large cohort of subjects are needed to establish whether supplemental L and Z augments MP in those subjects at risk of development of AMD and whether such MP augmentation can delay, avert, or modify the course of the disease.

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