

A Central Dip in the Macular Pigment Spatial Profile Is Associated with Age and Smoking

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PURPOSE. To investigate the relationship between specific macular pigment (MP) spatial profiles and risk factors for age-related macular degeneration (AMD).

METHODS. The MP spatial profile of 484 healthy subjects was measured with customized heterochromatic flicker photometry (cHFP) and categorized into one of two profile types: typical exponential or atypical "central dip." Data on risk factors for AMD were obtained with a general health and lifestyle questionnaire. Dietary and serum concentrations of lutein (L) and zeaxanthin (Z) were also assessed.

RESULTS. The presence of the central dip MP spatial profile was significantly more common in older subjects (the mean \pm SD age of subjects with a central dip MP spatial profile was 46.9 ± 12 years, whereas the mean age of subjects with a typical MP spatial profile was 41.8 ± 12 years; $P = 0.004$) and in current cigarette smokers ($P = 0.031$). Also, there was a significant age-related decline in central MP optical density (MPOD; 0.25° retinal eccentricity), but in the men only ($r = -0.146$, $P = 0.049$).

CONCLUSIONS. A central dip in the MP spatial profile, seen in older subjects and in cigarette smokers, may represent an undesirable feature of macular pigmentation. Further research is needed in this area. (*Invest Ophthalmol Vis Sci.* 2010;51:6722-6728) DOI:10.1167/iovs.10-5344

The macula contains the highest density of cone photoreceptors in the retina and is responsible for detailed central color vision.¹ Age-related macular degeneration (AMD) is the leading cause of age-related blindness in the developed world.^{2,3} Increasing age, family history of AMD, and cigarette smoking⁴⁻⁶ are the three major risk factors for AMD; other putative risk factors include being of the female sex, obesity, light iris color, low dietary intake and low serum concentrations of lutein (L) and zeaxanthin (Z), and low macular pigment optical density (MPOD).⁷⁻⁹

At the macula, the carotenoids L, Z, and *meso*-Z (generated from retinal L) accumulate at high concentrations (to the exclusion of all other carotenoids) and are collectively referred to

as macular pigment (MP).¹⁰⁻¹² MP is a short-wavelength (blue) light filter¹ and a powerful antioxidant¹¹ and is therefore believed to protect against AMD.^{13,14} Consistent with the suggested protection that MP may afford against AMD, a recent study has shown that risk factors for AMD (including the three established risk factors: increasing age, family history of AMD, and cigarette smoking) are associated with a relative lack of MP⁷; however, the relationship between the spatial profile of MP and risk factors for this disease, if any, is not yet known.

To date, studies investigating the spatial profile of MP have reported its distribution as a first-order exponential decline with increasing retinal eccentricity.¹⁵⁻¹⁸ However, variations in its distribution have been reported.^{15,16,19} Recently, it has been shown that atypical MP spatial profiles are reproducible, when measured with customized heterochromatic flicker photometry (cHFP).¹⁹

The importance of such variations, if any, in the spatial profile of MP (e.g., the presence of a central dip) is not yet known, but may be related to the putative protective role of this pigment. For example, reduced MPOD at the center of the macula (i.e., the presence of a central dip) may be associated with increased risk of AMD (given the lower antioxidant activity and short-wavelength light-filtering capacity of the affected individual, when compared with an individual without such a central dip). Indeed, and consistent with this hypothesis, Trischmann et al.,²⁰ in a study of 400 subjects (253 with signs of early AMD, 147 without AMD), reported that eyes with AMD are more likely to display low central MPOD when compared with non-AMD eyes.

It appears that L, Z, and *meso*-Z play central roles in the macula; however, determinants of their concentration and factors that influence their spatial distribution remain unclear. The purpose of this study was to investigate the association, if any, between the MP spatial profile and established (and putative) risk factors for AMD.

METHODS

Subjects

Four hundred eighty-four subjects were recruited for this single-visit study. The subjects were recruited by a local poster campaign, by word of mouth in the college community, and by invitation. The study was approved by the Research Ethics Committee of Waterford Institute of Technology. The subjects were required to sign an informed-consent document before participating, and all experimental procedures adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria for participation were as follows: Caucasian race, age between 18 and 70 years, no evidence of ocular disease, visual acuity 6/12 or better in the study eye, and no current consumption of L and/or Z dietary supplements.

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Supported by the Medical Research Charities Group Grant, through Fighting Blindness, Ireland and the Health Research Board, Ireland.

Submitted for publication February 8, 2010; revised April 8, 2010; accepted June 1, 2010.

Disclosure: **M.L. Kirby**, None; **S. Beatty**, NutraSight Consultancy Limited (C); **E. Loane**, None; **M.C. Akkali**, None; **E.E. Connolly**, None; **J. Stack**, None; **J.M. Nolan**, NutraSight Consultancy Limited (C)

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Personal Details, Lifestyle, and Risk Factor Assessment

The following details were recorded for each subject by questionnaire: demographic data, best corrected visual acuity, refractive status, family history of eye disease, height (meters), weight (kilograms), body mass index (BMI, kilograms divided by meters squared), personal ophthalmic and medical history, medication use, smoking status (never, past, or current smoker), alcohol consumption, iris color, ethnicity, and ocular and dermatologic sun sensitivity.

Fundus and Iris Color Assessment

Fundus photography was performed with a nonmydriatic auto fundus camera (AFC-210; Nidek, Gamagori, Japan). Both eyes of each subject were photographed to screen for signs of any retinal disease. Iris photography was also performed to document and categorize subjects with respect to iris color. Subjects with brown or hazel iris color were classified as having dark irides, whereas subjects with blue, green, or gray iris color were classified as having light irides.

Measurement of MPOD

The spatial profile of MP was measured by using a customized version of heterochromatic flicker photometry (cHFP). We used a Macular Densitometer (Macular Metrics, Corp., Providence, RI), an HFP instrument that was slightly modified from a device described by Wooten and Hammond.²¹ A detailed description of the principle of HFP and its customization to accurately measure MP has been published by Kirby et al.¹⁹

To measure the spatial profile of MP, we performed measurements at the following degrees of eccentricity: 0.25°, 0.5°, 1°, 1.75°, and 7° (the reference point), obtained with the following sized target diameters: 30-minute, 1°, 2°, 3.5°, and 2°, respectively. Stimulus 5, our reference point, is a 2° diameter disc with its center located 7° from a red fixation point (i.e., the average of the inner arc, which defines the disc at 6.5° and the outer arc which defines the disc at 7.5°), as MPOD at this location is assumed to be optically undetectable and its distribution at this location is essentially flat.

The spatial profile for each subject was classified into one of two profile types based on individual MPOD results: The “exponential MP spatial profile” describes a steady decline in MPOD from the center (0.25°) to the periphery (7°), with each successive MPOD lower than the previous one (group 1; Fig. 1). The “central dip” MP spatial profile describes a dip in the central MP (0.25° retinal eccentricity), followed by an increase in MPOD at 0.5° retinal eccentricity, and finally a steady decline to the periphery. Subjects were classified as having a central

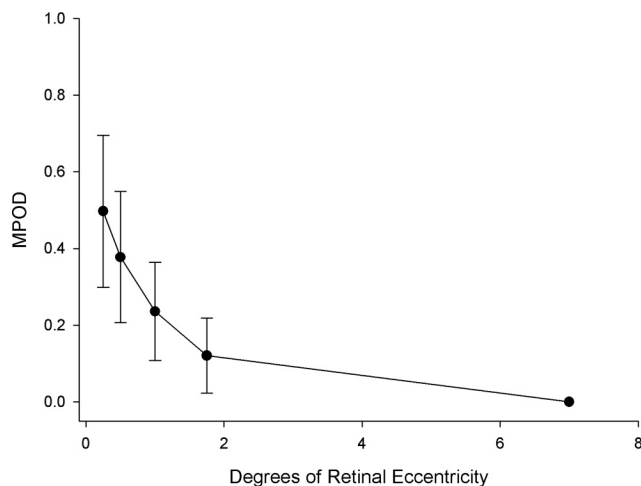


FIGURE 1. Average MP spatial profile for group 1, the typical exponential MP spatial profile. Data are expressed as the mean \pm SD.

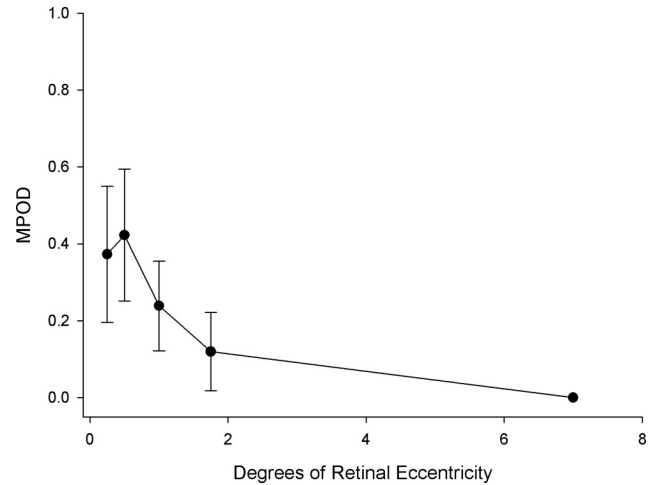


FIGURE 2. Average MP spatial profile for group 2, the central dip MP spatial profile. Data are expressed as the mean \pm SD.

dip MP spatial profile if the central MPOD (i.e., 0.25°) was lower than that at 0.5° (group 2; Fig. 2).

For each subject, the area of MPOD under the spatial profile curve was calculated by using the trapezoid rule, as follows: MPOD area = $\{[(\text{MPOD at } 0.25^\circ + \text{MPOD at } 0.5^\circ)/2] \times 0.25\} + \{[(\text{MPOD at } 0.5^\circ + \text{MPOD at } 1^\circ)/2] \times 0.5\} + \{[(\text{MPOD at } 1^\circ + \text{MPOD at } 1.75^\circ)/2] \times 0.75\} + \{[(\text{MPOD at } 1.75^\circ + \text{MPOD at } 7^\circ)/2] \times 5.25\}$, assuming an MPOD of 0 at 7° retinal eccentricity and also assuming a linear point-to-point fit between each successive point of measurement of MPOD, but a non-linear MP spatial profile overall. This MPOD area may give a better representation of actual MP quantity across the macula than does an individual optical density measurement at a single point of retinal eccentricity.

Food Frequency Questionnaire

Dietary intake was assessed with a self-administered, semiquantitative food frequency questionnaire developed by the Scottish Collaborative Group at the University of Aberdeen (Scotland, UK) and has previously been described by O'Connell et al.²²

High-Performance Liquid Chromatography

Serum concentrations of L and Z were analyzed by using high-performance liquid chromatography (HPLC). A description of our extraction procedure and detailed specifications of the HPLC device can be found in a paper recently published by Loane et al.²³

DSM Nutritional Products (Basel, Switzerland) provided the L and Z standards used to generate the response factors that were employed in calculating serum concentrations of L and Z. An internal standard, α -tocopherol acetate made up in ethanol (0.25 mg/L), was used to correct for the recovery of extractions for HPLC analysis and to assist in the quantification (α -tocopherol acetate recovery, 94% \pm 4%). All chromatograms were integrated manually by drawing a baseline and dropping perpendicular lines to quantify the peaks of interest (ChemStation software; Agilent Technologies, Palo Alto, CA).

Statistical Analysis

Pearson correlation coefficients were calculated to investigate the relationship between continuous variables (e.g., MPOD, at each degree of retinal eccentricity, and age). One-way analysis of variance and/or independent-samples *t*-tests were used to analyze the relationship between a continuous variable (e.g., MPOD) and a categorical variable (e.g., MP profile group, sex). Box plots and histograms were used to graphically illustrate these relationships. Our main method of analysis was multiple linear regression with MPOD as the dependent variable (individual models were generated for each degree of retinal eccen-

TABLE 1. Demographic and Anthropometric Data for All Subjects, Group 1 and Group 2

	All Subjects (<i>n</i> = 484)	Group 1 (<i>n</i> = 426)*	Group 2 (<i>n</i> = 58)†
Age, y‡	42 ± 13	41.8 ± 12.8	46.9 ± 12.1
Sex, <i>n</i> (%)			
Male	183 (37.8)	161 (37.8)	22 (37.9)
Female	301 (62.2)	265 (62.2)	36 (62.1)
Family history of AMD, <i>n</i> (%)			
Positive	157 (32.4)	140 (89.2)	17 (10.8)
Negative	322 (66.5)	281 (87.3)	41 (12.7)
Body mass index	26.4 ± 4.4	26.4 ± 4.5	25.9 ± 4.2
Smoking status, <i>n</i> (%)			
Never‡	272 (56.2)	248 (91.2)	24 (8.8)
Past	127 (26.2)	109 (85.8)	18 (14.2)
Current‡	85 (17.6)	69 (81.2)	16 (18.8)
Iris color, <i>n</i> (%) (<i>n</i> = 442)			
Light	320 (66)	288 (90)	32 (10)
Dark	164 (33.9)	138 (84.1)	26 (15.9)
Weekly alcohol intake, <i>n</i> (%)			
0 Units	96 (19.8)	83 (86.5)	13 (13.5)
1 Unit	54 (11.2)	49 (90.7)	5 (9.3)
2-5 Units	103 (21.3)	91 (83.3)	12 (11.7)
6-10 Units	133 (27.5)	116 (87.2)	17 (12.8)
>10 Units	96 (19.8)	86 (89.6)	10 (10.4)
MPOD			
0.25°§	0.48 ± 0.19	0.49 ± 0.19	0.37 ± 0.17
0.5°	0.38 ± 0.17	0.37 ± 0.17	0.42 ± 0.17
1.0°	0.23 ± 0.13	0.23 ± 0.12	0.23 ± 0.11
1.75°	0.12 ± 0.10	0.12 ± 0.09	0.11 ± 0.10
MPOD area	0.71 ± 0.42	0.71 ± 0.42	0.71 ± 0.42
Diet, mg/day			
Diet L	1.38 ± 1.21	1.36 ± 1.2	1.59 ± 1.3
Diet Z	0.20 ± 0.12	0.2 ± 0.12	0.2 ± 0.11
Serum, μmol/L			
Serum L	0.45 ± 0.23	0.44 ± 0.23	0.46 ± 0.23
Serum Z	0.21 ± 0.11	0.21 ± 0.12	0.2 ± 0.09

* Exponential MP spatial profile type.

† Central dip MP spatial profile type.

‡ Significant difference between groups at the 0.05 level.

§ Degree of retinal eccentricity.

|| Significant difference between groups at the 0.01 level.

tricity measured) and risk factors for AMD as potential explanatory variables. Binary logistic regression was used to analyze the relationship between MPOD spatial profile group and risk factors for AMD (all analyses: SPSS, ver. 15; SPSS, Chicago, IL).

RESULTS

Demographics

The anthropometric and lifestyle data for all subjects are presented in Table 1.

Macular Pigment Optical Density

Mean ± SD MPOD (at each measured degree of retinal eccentricity) and MPOD area for all subjects are presented in Table 1. Four hundred twenty-six (88%) of the 484 subjects had an exponential MP spatial profile (group 1; Fig. 1), and 58 (12%) had a central dip MP spatial profile (group 2; Fig. 2).

MPOD with Respect to Established Risk Factors for AMD

Age. There was no relationship between age and MPOD at any of the eccentricities measured (0.25°, 0.5°, 1.0°, or 1.75°) or between age and MPOD area ($P > 0.05$, for all). However, when the data were analyzed separately in the male and female subjects, there was a statistically significant inverse relation-

ship between increasing age and MPOD at 0.25° retinal eccentricity, for men only ($r = -0.146$; $P = 0.049$; Fig. 3).

The mean ± SD age of the subjects in group 1 (exponential MP spatial profile) was 41.8 ± 13 years, whereas the mean ± SD age of the subjects in group 2 (central dip MP spatial

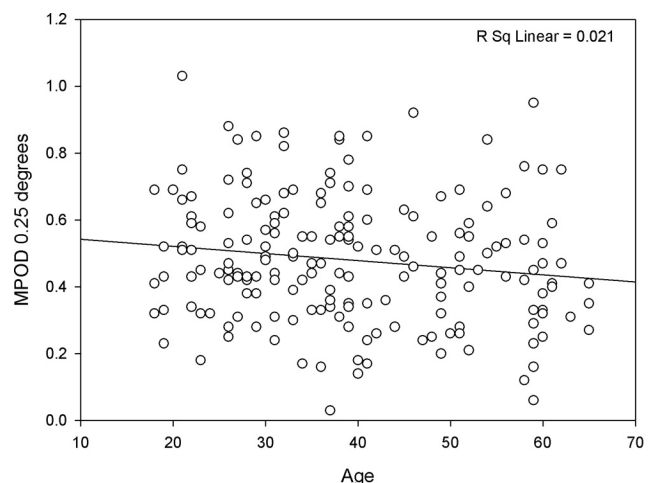


FIGURE 3. MPOD at 0.25° retinal eccentricity versus age.

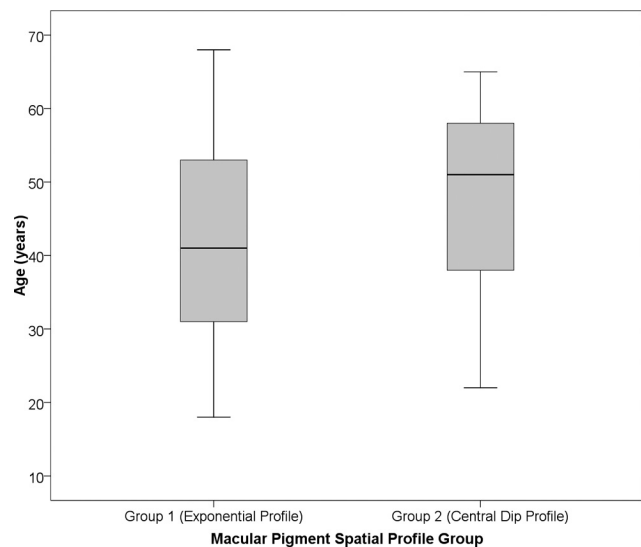


FIGURE 4. Age with respect to MP spatial profile group.

profile) was 46.9 ± 12 years (Table 1). There was a statistically significant difference between these two groups with respect to age ($P = 0.004$; Fig. 4).

Family History of AMD. The subjects with a confirmed family history of AMD ($n = 157$) had a mean \pm SD MPOD at all eccentricities measured (0.25° , 0.5° , 1.0° , and 1.75°) that was statistically comparable to that in the subjects with no known family history of disease ($n = 322$; $P > 0.05$, for all eccentricities). The presence or absence of a confirmed family history of AMD was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.549$; Table 1).

Cigarette Smoking. There was no statistically significant difference in MPOD, at any of the eccentricities measured, nor in MPOD area, attributable to smoking status (i.e., never, past, current; $P > 0.05$ for all). However, there was a statistically significant relationship between MP spatial profile group type and cigarette smoking (Fig. 5, Table 2 [$P = 0.031$]; Table 3 [$P = 0.021$]). The percentage of subjects with a central dip MP spatial profile was 8.8% for the never smokers, rising to 14.2% for the past smokers, and the 18.8% for current smokers (Table 2). The logistic regression output in Table 4 shows that, with

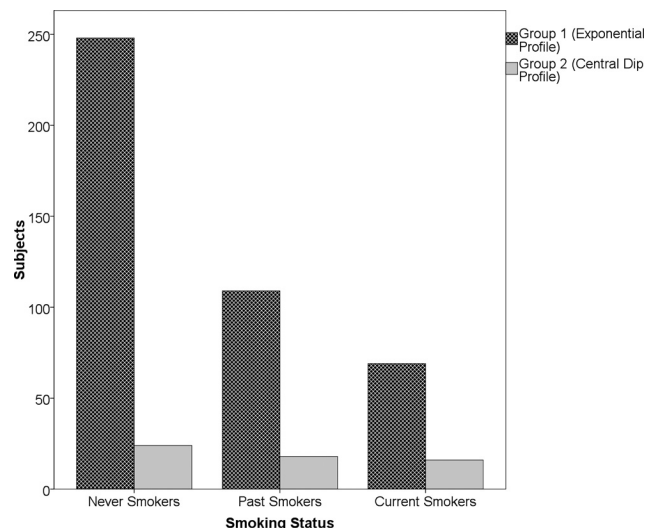


FIGURE 5. MP spatial profile group with respect to smoking status.

TABLE 2. Cross-tabulation of Smoking Status versus MP Profile Group Type

	MP Spatial Profile Type		
	Group 1*	Group 2†	Total
Never smoker	248 (91.2)	24 (8.8)‡	271 (100)
Past smoker	109 (85.8)	18 (14.2)	127 (100)
Current smoker	69 (81.2)	16 (18.8)	85 (100)
Total	426 (88)	58 (12)	484 (100)

Data are expressed as n (%).

* Typical exponential MP spatial profile.

† Central dip MP spatial profile.

‡ Significantly different from current smokers at the 0.05 level.

adjustment for age, the never smokers were significantly less likely to have a central dip MP spatial profile than were the current smokers ($P = 0.005$) and also that the percentage of central dip MP spatial profiles was lower in the past smokers than in the current smokers, but not significantly so ($P = 0.104$).

MPOD with Respect to Putative Risk Factors for AMD

Sex. There was no statistically significant difference in MPOD (at any of the eccentricities measured), nor in MPOD area, between the male and female subjects ($P > 0.05$, for all). Sex was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.984$; Table 1).

Obesity. There was a weak, but statistically significant, inverse relationship between BMI and MPOD at 0.25° , 0.5° , and 1.0° retinal eccentricity ($r = -0.093$ to -0.131 ; $P < 0.05$, for all). There was also a weak, but statistically significant, inverse relationship between BMI and MPOD area ($P = 0.047$, $r = -0.091$). BMI was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.390$; Table 1).

Dietary L and Z. Data on dietary intake of L and Z are presented in Table 1. There was no statistically significant relationship between dietary intake of L or Z and MPOD (at any of the eccentricities measured) or MPOD area ($P > 0.05$, for all). Dietary intake of L and Z was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.182$ and 0.983 , respectively; Table 1).

Serum L and Z. Data on serum concentrations of L and Z are presented in Table 1. There was a positive and statistically significant relationship of serum concentrations of L and Z to MPOD (at each degree of eccentricity measured) and MPOD area ($P < 0.05$, for all). Serum concentrations of L and Z were unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.629$ and 0.312 , respectively; Table 1).

Iris Color. Subjects with dark-colored irises had a significantly higher MPOD at 0.5° and 1.0° than did subjects with light-colored irises ($P = 0.007$ and $P = 0.045$), respectively. Iris color was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.069$; Table 1).

Alcohol Consumption. There were no statistically significant differences between MPOD (at any of the eccentricities measured) and any of the five categories of alcohol consumption (see Table 1; $P > 0.05$, for all). Alcohol consumption was unrelated to MP spatial profile group status (i.e., presence or absence of a central dip; $P = 0.922$; Table 1).

Relation of MPOD with Risk Factors for AMD, as Assessed by Multiple Linear Regression

Multiple linear regression analysis, with indicator variables used as categorical variables, was performed to analyze the

TABLE 3. Binary Logistic Regression Analysis for Relationship of MPOD Spatial Profile Type to Known and Putative Risk Factors for AMD

	B	SE	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)
Age	0.035	0.012	8.541	1	0.003	1.036	1.012-1.061
Cigarettes			7.731	2	0.021		
Never smoker	-0.996	0.358	7.729	1	0.005	0.369	0.183-0.745
Past smoker	-0.642	0.394	2.65	1	0.104	0.526	0.243-1.14
Constant	-2.885	0.579	24.822	1	0.000	0.056	

The reference group for the cigarettes variable is the current smokers group. 95% CI, 95% confidence interval.

relationship between MPOD at each degree of eccentricity measured and the following known and putative risk factors for AMD: age, cigarette smoking, family history of AMD, sex, BMI, dietary L, dietary Z, serum L, serum Z, iris color, and alcohol consumption.

Variables for all these factors were included initially in a multiple linear regression model, with MPOD as the dependent variable, run separately for each retinal eccentricity measured. Statistically nonsignificant variables were then removed, one by one, using the 5% level of significance as the criterion for removal. The final regression model, for each degree of retinal eccentricity measured and for MPOD area, is presented in Table 4; these models explained 15%, 14%, 10%, and 0.04% of the variance in MPOD at the eccentricities 0.25°, 0.5°, 1.0°, and 1.75°, respectively; Fig 6).

Relation of MPOD Spatial Profile Group with Risk Factors for AMD, as Assessed by Binary Logistic Regression

Binary logistic regression analysis, with indicator variables as the categorical variables, was performed to analyze the relationship between MPOD spatial profile group and the following known and putative risk factors for AMD: age, cigarette smoking, family history of AMD, sex, BMI, dietary L, dietary Z, serum L, serum Z, iris color, and alcohol intake.

Variables for all these factors were included initially in a binary logistic regression model, with MPOD spatial profile group as the dependent variable. Statistically nonsignificant

variables were then removed, one by one, with the 5% level of significance as the criterion for removal. The final regression model, for MPOD spatial profile group, is presented in Table 3; this model showed age and cigarette smoking to be independent significant predictors of MP spatial profile group type.

DISCUSSION

Previous studies have shown that healthy subjects (i.e., subjects without AMD) have relatively less MP in the presence of all established,⁷ and several putative,^{7,24} risk factors for AMD. The purpose of this study was to investigate the association, if any, between the spatial profile of MPOD and these risk factors (i.e., age, family history of AMD, cigarette smoking, sex, obesity, dietary carotenoid intake, and iris color). We collected risk factor data and MP spatial profile data of 484 healthy subjects. The MP spatial profile of each subject was categorized into one of two profile types: the typical exponential profile (group 1) or the central dip profile (group 2).

Analyzing our study population as a whole ($n = 484$), we found the three established risk factors for AMD—increasing age, cigarette smoking, and family history of the disease—were unrelated to MPOD at any measured degree of retinal eccentricity. When we split our study population by sex, we found a weak, but statistically significant inverse relationship between age and central MPOD (0.25° retinal eccentricity), but in males only. The relationship between increasing age and

TABLE 4. Multiple Linear Regression Analysis with Respect to MPOD at Each Eccentricity Measured and MPOD Area and Known and Putative Risk Factors for AMD

Model	Unstandardized Coefficients		Standardized Coefficients		Sig.
	B	SE	B	t	
Dependent variable: MPOD at 0.25° retinal eccentricity					
Constant	0.373	0.019		19.347	0.000
Serum L, $\mu\text{mol/L}$	0.287	0.038	0.331	7.606	0.000
MPOD profile type	-0.131	0.027	-0.214	-4.921	0.000
Dependent variable: MPOD at 0.5° retinal eccentricity					
Constant	0.251	0.017		14.444	0.000
Serum L, $\mu\text{mol/L}$	0.27	0.033	0.36	8.222	0.000
Iris color	0.037	0.016	0.103	2.348	0.019
Dependent variable: MPOD at 1.0° retinal eccentricity					
Constant	0.154	0.013		11.736	0.000
Serum L, $\mu\text{mol/L}$	0.168	0.025	0.303	6.75	0.000
Iris color	0.025	0.012	0.092	2.057	0.040
Dependent variable: MPOD at 1.75° retinal eccentricity					
Constant	0.083	0.01		8.335	0.000
Serum L, $\mu\text{mol/L}$	0.088	0.02	0.207	4.483	0.000
Dependent variable: MPOD area					
Constant	0.493	0.042	11.649	0.000	
Serum L, $\mu\text{mol/L}$	0.508	0.084	0.275	6.045	0.000

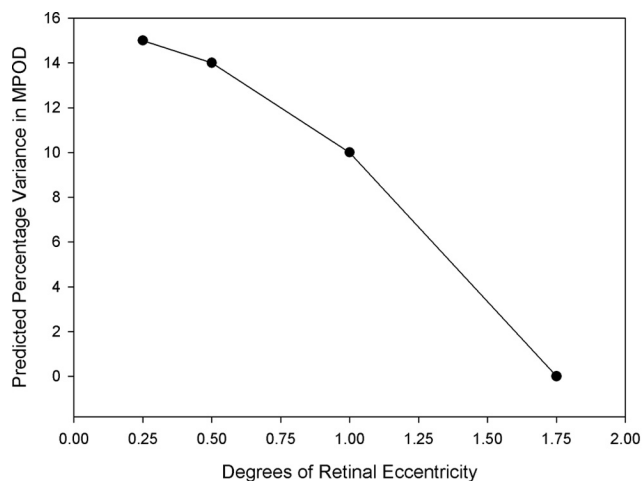


FIGURE 6. Predicted variance in MPOD at each degree of retinal eccentricity measured. The data in this graph are also presented in Table 4.

MPOD has been widely reported in the literature.^{7,25,26} The results, however, are somewhat inconsistent.

The absence of this relationship in female subjects is consistent with the findings of the Carotenoids in Age-Related Eye Disease Study (CAREDS), a cross-sectional study of 1698 female subjects, in which MPOD was found to be unrelated to age or smoking (at any measured degree of retinal eccentricity).²⁷ Sex differences in the metabolism, transport, and accumulation of carotenoids have been documented.^{24,28,29} These studies report that female subjects have lower average MPOD, but higher adipose tissue concentrations and higher circulating serum L concentrations than do male subjects. It is possible, therefore, that the L contained in the adipose tissue and circulating in the serum of female subjects may act as a buffer against any decline in MPOD with increasing age.

We report a positive and statistically significant relationship between serum concentrations of L and MPOD (at all degrees of retinal eccentricity measured). This finding is unsurprising, given the exclusive dietary origin of MP and has been reported previously in the literature.^{30,31} Also, we found that light iris color (i.e., blue, green, or gray) was associated with having significantly lower levels of MPOD (at 0.5° and 1.0° retinal eccentricity), when compared with that present with dark iris color (i.e., brown or hazel). This finding has also been supported in the literature and may represent a greater transmission of short-wavelength light in eyes with lighter colored irides than in eyes with darker colored irides (given that there was no significant difference between subjects with light and dark irides with respect to age, sex, smoking status, BMI, or alcohol intake), leading to increased free radical production in such individuals and a consequential depletion of their MP.⁹

A key and novel finding of our study was the association between established risk factors for AMD (i.e., age and cigarette smoking) and MPOD spatial profile group type. We found that age was a significant predictor of MP spatial profile group type, with the younger subjects having the typical exponential MP spatial profile (group 1), whereas the older subjects were more likely to have a central dip in their MP spatial profile (group 2), even after adjustment for smoking habits. It should be noted that the subjects in group 1 were significantly younger (5 ± 12 years) than those in group 2. Previous investigators who reported atypical MP spatial profiles, measured using fundus autofluorescence, reported no such association with age.^{15,16} It has been suggested that the spatial profile of MP may be affected by unique features of the foveal anatomic

architecture.¹ For example, Kirby et al.,¹⁹ using HFP, recently reported an association between a wider foveal depression and the presence of a secondary peak (equivalent to the central dip group defined herein) in the MP spatial profile. However, foveal width was not measured in our study.

Cigarette smoking also emerged from our logistic regression model as a significant predictor of MP spatial profile group type (group 1 or 2). The presence of the typical exponential MP spatial profile was significantly more common in the subjects who never smoked cigarettes when compared with the profile in the past and current cigarette smokers. Conversely, the presence of the central dip MP spatial profile was significantly more common in the current cigarette smokers than in the past or never smokers, even after adjustment for age. Several studies have found cigarette smoking to be a negative predictor of MPOD.^{7,32} Although such a finding was not reproduced in this study, we report a novel association between the central dip MP profile group and current cigarette smoking.

To explain our finding, we suggest that the known increased levels of oxidative stress associated with cigarette smoking may have contributed to the association.^{32,33} Furthermore, the observation that the central dip MP spatial profile was more common in the current smokers than in the past smokers (albeit to a nonsignificant level) and in comparison to that in the never smokers (to a statistically significant level), suggests that smoking status influences the MP spatial profile centrally. Indeed, a dose-response relationship between cigarette smoking and MPOD levels has been reported in the literature.⁷

Of particular relevance to this study are the recently published findings by Connolly et al.,³⁴ who reported on serum and MPOD (measured at 0.25°, 0.5°, 1.0°, and 1.75° retinal eccentricity) in response to supplementation with *meso-Z* (7.3 mg; the dominant carotenoid in formulation), L (3.7 mg), and Z (0.8 mg). In their study, 4 of the 10 subjects studied displayed an atypical or central dip (i.e., lower MPOD at 0.25° degrees than at 0.5° degrees of retinal eccentricity) in their MPOD spatial profiles at baseline. However, after just 8 weeks of supplementation, all four subjects displayed the more typical exponential MPOD spatial profile, after augmenting their (0.25°) MPOD centrally. Of note, *meso-Z* (the dominant carotenoid in that study formulation) is the predominant Z-isomer at the foveal center.²⁰ The presence of *meso-Z* at the macula is attributed to generation from retinal L (as it is not normally found in a typical Western diet).²⁰ Thus, Connolly et al. speculated that subjects who initially display a central dip MPOD spatial profile are unable to convert L to *meso-Z* at this location. In relation to our finding, it is tempting to hypothesize that increased oxidative stress, a factor common to both older age and cigarette smoking, may prevent the conversion of L to *meso-Z*, in such subjects; however, further study is clearly warranted in this area.

Given the cumulative and chronic nature of AMD, it is reasonable to suggest that any putative protective effect of MP is necessary from a relatively young age and throughout an individual's lifetime. This study was designed to further our understanding of the determinants of MP and its spatial distribution. In conclusion, older subjects and cigarette smokers were more likely to display a central dip in their MP spatial profile. Furthermore, and in light of the established link of age and cigarette smoking with AMD, and consistent with the recent postmortem study by Trieschmann et al.,²⁰ who reported that AMD subjects were more likely to display low central MPOD than were non-AMD subjects, we speculate that the central dip MPOD spatial profile represents an undesirable distribution of MP.

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