Spatial Profile of Macular Pigment and Its Relationship to Foveal Architecture

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Purpose. Macular pigment (MP) is composed of two dietary carotenoids, lutein and zeaxanthin, and a carotenoid generated by the retina, meso-zeaxanthin. There is large intersubject variability in peak optical density, spatial profile, and lateral extent of macular pigment, and it has been suggested that foveal architecture may play a role in this variability. This study is an initial investigation of the relationship between the spatial profile of macular pigment and foveal architecture.

Methods. Sixty normal subjects were enrolled (one was eventually excluded). The spatial profile of macular pigment optical density (MPOD) was measured by customized heterochromatic flicker photometry (cHFP). High-resolution macular thickness maps were obtained by optical coherence tomography. Four parameters were analyzed: (1) minimum foveal thickness (MFT) at the intersection of six radial scans; (2) central foveal thickness (CFT) averaged over the central 1 mm of the fovea; (3) foveal width identified as the region lacking a nerve fiber layer; and (4) foveal width measured from crest to crest. Lifestyle and vision information were obtained by questionnaire.

Results. The mean ± SD MPOD at 0.25° eccentricity was 0.49 ± 0.23 and at 0.5° eccentricity, 0.41 ± 0.21. A first-order decreasing exponential function accounted for most of the variance of the MP profile averaged across subjects ($r^2 = 0.99$). MPOD measured at 0.25° was unrelated to both measures of foveal thickness for the entire study group ($r = 0.03$, $P = 0.81$, and $r = -0.08$, $P = 0.57$, respectively). Similarly, MPOD measured at 0.5° was unrelated to foveal thickness in the entire study group ($r = 0.12$, $P = 0.36$ and $r = -0.05$, $P = 0.71$, respectively). However, when analyzed separately in the nonwhite subjects, the relationship between MPOD at 0.25° and MFT was positive and significant ($r = 0.59$, $P = 0.01$), but remained unrelated to CFT ($r = 0.20$, $P = 0.41$). Similarly, in the nonwhite subjects, the relationship between MPOD at 0.5° and MFT was positive and significant ($r = 0.68$, $P < 0.01$), but again was unrelated to CFT ($r = 0.23$, $P = 0.32$). There was no significant relationship between MPOD and either measure of foveal thickness in the white subjects. In the entire study group, there was a positive and significant relationship between foveal width and MPOD averaged across the fovea ($r = 0.41$, $P < 0.01$) and between foveal width and MP integrated across the fovea ($r = 0.41$, $P < 0.01$).

Conclusions. Foveal MP was positively and significantly related to foveal width in the entire study group. This relationship may be determined by the greater length of the cone axons (Henle fibers) in wider foveas. MPOD was unrelated to foveal thickness in the white subjects. However, in the nonwhite subjects there was a positive association between MFT and MPOD at the 0.25° and 0.5° eccentricities, suggesting that other personal characteristics modulate the MPOD–retinal thickness relationship. (*Invest Ophthalmol Vis Sci.* 2008;49:2134–2142) DOI:10.1167/iovs.07-0933

The fovea is a specialized part of the retina, as it provides the sharpest visual acuity and best color discrimination. A pigment, composed of the xanthophyll carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin, accumulates within the fovea where it is known as macular pigment (MP). Lutein and zeaxanthin are of dietary origin, whereas meso-zeaxanthin is formed in the retina by conversion from lutein.[1] MP is yellowish in color and is deposited preferentially in the photoreceptor axon and inner plexiform layers of the retina.[2] Because of its yellow color and position anterior to the photoreceptor outer segments, MP acts as a short-wavelength light filter for the foveal photoreceptors.[2,3]

The optical density and spatial distribution of MP have been shown to vary dramatically among individuals.[4–8] In 1997, Hammond et al.[9] described the MP profile in the human fovea as having a central peak that, in their averaged data, decreases approximately exponentially to optically undetectable levels at 6° to 8° eccentricity. In their study, they investigated MP optical density (MPOD) by using heterochromatic flicker photometry (HFP) and found that in approximately 40% of individuals, the locus near 1° eccentricity exhibited positive deviations from the exponential function higher than 0.05 optical density units, which they referred to as subpeaks or shoulders.[9] The shape of the spatial distribution of MPOD is thought to be accounted for, in part, by the spatial distribution of the cone photoreceptors (via binding sites for lutein and zeaxanthin), which also decreases rapidly from the center of the fovea outward.[10]

Snodderly et al.[2] measured the distribution of MP in individual retinal layers of monkeys by two-wavelength densitometry. Their results showed that, within the layer structure of the retina, the greatest concentrations of MP are found in the photoreceptor axons at the fovea, with relatively high concentrations found in the inner plexiform layer outside the foveola.[2,3] Although some of the monkey retinas exhibited a single central peak with quasimonotonic decline, others showed a distinct trimodal distribution, with a secondary peak at 200 to 300 μm from the fovea. Snodderly et al.[2] hypothesized that the variability in MP distribution that they observed was due to the differences between individuals in the size of the foveal de-

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pression, thereby suggesting that foveal architecture may influence the spatial profile of MP across the central retina.

Recent studies in which autofluorescence imaging was used to measure spatial patterns of MPOD in humans have extended the analysis of the MPOD spatial profile. In these studies, Delori et al.[11] and Berendschot and van Norren[12] found that some human subjects had trimodal MPOD profiles, whereas others did not. Snodderly et al.[2] and Delori et al.[11] hypothesized that these differences could have an anatomic basis. Determining the relationship between foveal anatomic architecture and the MPOD spatial profile in normal human subjects was the goal of our study.

Methods

Subjects

Sixty healthy subjects volunteered to participate in the study, which was approved by the Human Assurance Committee of the Medical College of Georgia (MCG). Informed written consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

Subjects were recruited to this single-visit study via posters and word of mouth at MCG. The inclusion criteria were age of 18 to 60 years and refractive error between −5 and +5 D. The exclusion criteria were any ocular disease and refractive error outside −5 to +5 D. It is important to note that, of the total 59 subjects included in the study (1 of the original 60 was excluded for reasons described later), the average refractive correction for those with a refractive error (n = 31, all myopes) was −3.26 D, which corresponds to a 6.5% overestimation of MPOD at the most central point.

Demographic and Lifestyle Details

The following details were recorded for each volunteer by means of a demographic and lifestyle questionnaire: Snellen visual acuity, lens prescription, general health status, tobacco use, body mass index (BMI; defined as kilograms body weight/per square meter of height), ethnic background, skin color, and iris color.

Measurement of the Spatial Profile of Macular Pigment

The densitometer described by Wooten et al.[13] was slightly modified to obtain more extensive spatial profiles of MPOD by HFP. For these measurements the subject views a stimulus that alternates between a wavelength band absorbed by MP and a band that is not. The subject adjusts the radiance of the wavelength band absorbed by MP to minimize the percept of flicker. The range of alternation rates at which flicker is not perceived is called the null zone. Primarily because of interindividual differences in temporal (i.e., flicker) sensitivity, it is optimal to customize the HFP task for each subject by selecting the alternation rate to achieve a narrow null zone and a precise setting. We have termed this customized (c)HFP. Selecting the best flicker rate for each subject enables one to accommodate the variation in flicker sensitivity due to such factors as age and disease.[14,15] If differences among subjects in flicker sensitivity are not accounted for (i.e., a fixed flicker frequency is used), then a subject with low flicker sensitivity (i.e., low critical fusion frequency [CFF]) will most likely experience a large null flicker zone. Although the subject may be able to complete the task by eliminating flicker from the test target, the settings are likely to be variable, and subjects may exhibit systematic bias toward one end of the null range, resulting in either over- or underestimation of MPOD. Alternatively, a subject with a high CFF may not be able to eliminate flicker from the test target, which would make the task difficult to complete. As reported by Snodderly et al.,[16] the problem of flicker sensitivity can be addressed by introducing, as a preliminary test, a CFF task involving a single-wavelength band outside the absorption band of MP. Based on an individual's CFF, the optimal HFP flicker frequency that facilitates good subject performance and reduces measurement error can be estimated. In addition, an algorithm can be developed to estimate optimal HFP flicker frequencies for each retinal locus, including the reference locus. To this end, we developed an algorithm, based on data from five normal subjects, to guide flicker frequency settings for the different eccentricities during MPOD testing.

The second methodological consideration involves a test stimulus configuration in which the radiances of the two alternating components are inverse-yoked. In other words, when a subject adjusts the blue component to be more intense, the luminance of the green component is commensurately decreased and vice versa. This procedure keeps the brightness of the test stimulus relatively constant. We regard this approach as an improvement, because some subjects find changes in brightness distracting when they perform the task. This aspect of cHFP is not customized by the experimenter, but is automatically customized for each subject, because their settings reflect their own ocular absorption and retinal sensitivity.

The procedure used in the present study was modeled after that described in CAREDS (Carotenoids and Age-Related Eye Disease Study; Snodderly et al.[16]), including the viewing of a training video, determination of the subject's CFF, and development of an algorithm to predict flicker frequencies appropriate for the different loci tested under HFP conditions.

The edge of a test stimulus has been shown by HFP to determine the no-flicker threshold out to at least 2° eccentricity.[9,17,18] For each stimulus condition or location, subjects usually made four judgments of no flicker. The range of null flicker was considered too wide if the subject provided radiances that differed from one another by more than ~15% (~0.07 MPOD).

Test stimuli were presented in natural view and near the center of a 6°, 2.75-cd/mm^2, 470-nm circular background. Light for the alternating measuring and reference fields and the background was produced by 20-nm half-bandpass LEDs with peak energy at 458, 500, 570, and 470 nm, respectively (Nichia Corp., Mountville, PA). The radiance of the LEDs was controlled by constant current, high-frequency electronic pulses. The measuring and reference fields were superimposed and presented out of phase with an alternation rate optimized (as noted) for each subject and for each condition. Once the rate of alternation was optimized, subjects adjusted the radiance of the 458- and 500-nm measuring fields (which was counterbalanced with the 3.0-cd/mm^2, 570-nm reference field [minimal MP absorbance] to maintain constant luminance) until a no-flicker point was achieved.

MPOD was measured at eccentricities of 0.25°, 0.5°, 1°, 1.75°, 3°, and 5° along the horizontal meridian of the temporal retina (as MP measurements for all subjects were performed in the right eye) relative to a reference location at 7° eccentricity. We also report the integrated MP, determined by calculating the area under an exponential fit to the subjects' spatial distribution data.

Optical Coherence Tomography Analysis

High-resolution macular thickness maps were obtained by optical coherence tomography (OCT; model 3000; Carl Zeiss Meditec, Inc., Dublin, CA). The Stratus OCT generates cross-sectional images (tomograms) of the retina with ≤10-μm resolution. The retinal thickness is calculated as the distance between the vitreoretinal interface and the retinal pigment epithelium (RPE; Fig. 1). Topographic maps are produced by obtaining six consecutive cross-sectional scans at equally spaced angular orientations (30°) in a radial spoke pattern centered on the fovea.

Scan analysis was performed with the built-in Stratus OCT software version 4.1 (Carl Zeiss Meditec, Inc.). We also used Stratus OCT review software version 4.0 which allowed for detailed investigation and interpretation of all OCT scans performed. Retinal map analysis was performed in the right eye of all subjects (n = 60). In addition, 10 subjects were examined for interocular symmetry, and 10 subjects were examined for test–retest reliability (see the Results section).

OCT scans were performed on undilated eyes, as it has already been shown that mean macular thickness and macular volume (as

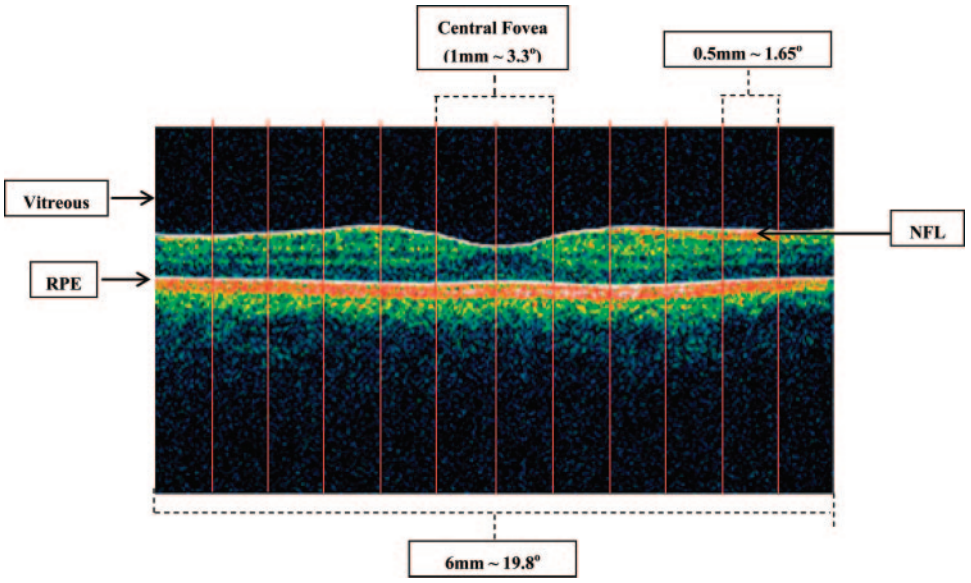

FIGURE 1. OCT image of the central retina of a horizontal 180° scan showing parameters of foveal architecture. Parameters of foveal architecture reported include: MFT (average retinal thickness at point of intersection of six radial scans); CFT (average retinal thickness within the central 1 mm (~3.3°) diameter zone); foveal width (measure 1: identified as width of retina where nerve fiber layer was absent; measure 2: identified as distance from peak foveal crest to crest). NFL, nerve fiber layer; RPE, retinal pigment epithelium.

measured with OCT) are not significantly affected by pupillary dilation.[19] To confirm this finding, we measured central foveal thickness (CFT) in 10 subjects before and after pupil dilation and found good agreement between measurements ($r^2 = 0.96$, $P < 0.01$). The mean difference (±SD) before and after pupil dilation, for CFT measurements, was 2.14 ± 4.22 μm. In these 10 subjects, pupil dilation was achieved with phenylephrine 2.5% and tropicamide 1%.

By means of the retinal map analysis option, the OCT software provides a mean ± SD for the minimum foveal thickness (MFT). Ideally, if the subject's fixation is stable and the retinal boundary detection is correct, then the MFT for each of the six scans should be the same, and the SD should be 0. Therefore, reproducibility of scans was assessed by calculating the MFT SD/MFT ratio. For this study, scans with MFT SD/MFT ratios greater than 0.1 were deemed inaccurate (possibly indicating poor subject fixation). After this criterion, one subject was removed from study analysis, as his MFT SD/MFT ratio was 0.16. Therefore, reliable retinal map data for 59 of the 60 subjects recruited were available for analysis.

We felt that it was important to assess all our OCT scans for stable subject fixation, as a recent report by Liew et al.[20] presented an OCT scan that appeared to be misaligned. They presented OCT thickness charts from two subjects, one with a thin fovea (135 μm centrally) and low MPOD (0.03 optical density units) and one with a thick fovea (222 μm centrally) and high MPOD (0.68 optical density units). However, it is clear from Figure 3B in Liew et al.[20] (i.e., a subject with a thick fovea and high MPOD) that the OCT scan was misaligned, as evidenced by the presence of the nerve fiber layer and inner retinal layers at the center of this image. Such a misalignment results in an inflated thickness measurement, thus introducing errors into any comparisons. However, we are confident that all OCT thickness data reported in our study are accurate, due to the precautionary measures taken, as mentioned earlier.

We report on the following characteristics of foveal architecture (Fig. 1): MFT (average retinal thickness at point of intersection of six radial scans), CFT (average retinal thickness within the central 1-mm [~3.3°] diameter zone), and foveal width. Foveal width (micrometers) was identified with two techniques and measured with the calipers provided by the OCT software: measure 1, identified as region of fovea where nerve fiber layer was absent; and measure 2, identified as distance from peak foveal crest to crest. We used the horizontal meridian for both foveal width assessments. All measurements were performed by a study investigator who was masked to subject identification.

Statistical Analysis

A commercial statistical software package (SPSS, ver. 11; SPSS, Chicago, IL) was used for analysis. In addition, graphic software (Origin; ver. 5; Microcal, Northampton, MA) was used to plot the spatial profile of MPOD and also to determine the best-fitting curve to describe this profile.

All variables investigated exhibited a typical normal distribution. Means ± SDs are presented in the text. Pearson correlation coefficients were calculated, to investigate bivariate relationships. The significance between group differences was determined by one-way ANOVA, independent samples *t*-test, or paired-sample *t*-test, depending on the analysis in question.

We performed standard post hoc testing (Bonferroni and Tukey HSD adjustment) that automatically adjusts the family-wise error introduced by multiple comparison testing. These adjustments are "built into" most statistical packages and are expressly meant for unplanned comparisons. Intergrader agreement of foveal width measurements was assessed by using the intraclass correlation. We used the 5% level of significance throughout our analysis.

For two subjects, MPOD at 0.25° eccentricity was greater than 3 SDs above the mean, and their data were therefore considered outliers. All analyses were performed with and without these outliers included.

RESULTS

Macular Pigment Optical Density

Mean MPODs measured at each eccentricity are summarized in Table 1. Mean MPOD at 0.25° was positively and significantly related to mean MPOD at all other eccentricities ($r = 0.59$–0.94; $P < 0.01$, for all). A first-order decreasing exponential function fit the averaged subjects' profile data well ($r^2 = 0.99$; Fig. 2). For 6 of the 59 subjects analyzed (10%; five females and one male), peak MPOD was at 0.5° of eccentricity, whereas four of the subjects displayed peak MPOD at 1° of eccentricity (7%, three females and one male). We found no obvious association between the secondary trimodal peak and ethnicity or foveal architecture. It is possible that the presence of other subpeaks was missed because of the limited number of reference points used.

The white subjects had significantly lower mean MPODs ($P < 0.01$, at all eccentricities; Fig. 3) than each of the nonwhite subject groups, both individually (Indian: $n = 5$; Asian: $n = 6$; Hispanic/Spanish: $n = 3$; Black; $n = 4$) and when

TABLE 1. The Demographic, Lifestyle and Anthropometric Data with Respect to MPODs in the 59 Subjects

Characteristic	*n*	%	Degrees of Eccentricity						
			0.25°	0.5°	1°	1.75°	3°	5°	0.25°, 0.5°, 1°, 1.75°
Entire study group	59	100	0.49 ± 0.23	0.41 ± 0.21	0.27 ± 0.17	0.10 ± 0.11	0.10 ± 0.07	0.02 ± 0.04	0.23 ± 0.13
Age (y)									
18–32	33	56	0.54 ± 0.24	0.44 ± 0.23	0.30 ± 0.18	0.11 ± 0.10	0.08 ± 0.08	0.02 ± 0.04	0.25 ± 0.13
33–46	11	19	0.50 ± 0.23	0.40 ± 0.21	0.29 ± 0.19	0.13 ± 0.15	0.09 ± 0.09	0.03 ± 0.04	0.24 ± 0.14
47–60	15	25	0.40 ± 0.19	0.34 ± 0.15	0.20 ± 0.12	0.06 ± 0.06	0.03 ± 0.03	0.01 ± 0.04	0.17 ± 0.08
Sex									
Male	24	41	0.48 ± 0.18	0.37 ± 0.13	0.24 ± 0.11	0.08 ± 0.06	0.06 ± 0.05	0.01 ± 0.03	0.20 ± 0.07
Female	35	59	0.51 ± 0.26	0.44 ± 0.25	0.29 ± 0.20	0.12 ± 0.13	0.08 ± 0.09	0.03 ± 0.05	0.24 ± 0.15
Ethnic background									
White	41	69	0.43 ± 0.17*	0.34 ± 0.13*	0.22 ± 0.13*	0.07 ± 0.07*	0.05 ± 0.06*	0.01 ± 0.03*	0.19 ± 0.08*
Nonwhite‡	18	31	0.64 ± 0.29*	0.55 ± 0.28*	0.39 ± 0.20*	0.18 ± 0.13*	0.12 ± 0.08*	0.05 ± 0.05*	0.32 ± 0.16*
Iris color									
Blue-grey	16	27	0.41 ± 0.16	0.32 ± 0.13	0.22 ± 0.14	0.07 ± 0.09	0.04 ± 0.07	0.01 ± 0.02	0.18 ± 0.08
Green-hazel	16	27	0.46 ± 0.18	0.38 ± 0.14	0.22 ± 0.12	0.07 ± 0.06	0.06 ± 0.03	0.03 ± 0.03	0.21 ± 0.08
Brown, black	27	46	0.56 ± 0.28	0.48 ± 0.26	0.33 ± 0.20	0.13 ± 0.13	0.09 ± 0.09	0.03 ± 0.05	0.27 ± 0.16
Smoking status									
Current/past	11	19	0.39 ± 0.18	0.33 ± 0.15	0.21 ± 0.15	0.08 ± 0.08	0.05 ± 0.08	0.01 ± 0.03	0.18 ± 0.10
Never	48	81	0.52 ± 0.24	0.43 ± 0.22	0.29 ± 0.17	0.11 ± 0.11	0.08 ± 0.07	0.02 ± 0.04	0.24 ± 0.13
Body mass index									
BMI ≤25	34	58	0.54 ± 0.25	0.46 ± 0.24†	0.32 ± 0.18†	0.11 ± 0.10	0.09 ± 0.07	0.03 ± 0.04	0.26 ± 0.14
BMI >25	25	42	0.43 ± 0.18	0.34 ± 0.15†	0.21 ± 0.14†	0.08 ± 0.11	0.05 ± 0.07	0.01 ± 0.04	0.19 ± 0.10

* $P < 0.01$.
† $P < 0.05$.
‡ Nonwhite Indian: $n = 5$; Asian: $n = 6$; Hispanic/Spanish: $n = 3$; Black: $n = 4$.

combined ($n = 18$) as a single nonwhite group (Table 1). Also, removing the two MP outliers from analysis and controlling for age did not alter the findings ($P < 0.05$ for all).

The mean age of the sample was 35.6 ± 11.4 years (range, 19–57 years). For all degrees of eccentricity, there was a trend toward an age-related decline in MPOD ($r = -0.165$ to -0.284), with a statistically significant age-related decline in MPOD found at eccentricities of 0.25°, 1°, and 3° ($r = -0.252$, $P = 0.049$; $r = -0.278$, $P = 0.033$; $r = -0.284$, $P = 0.030$, respectively). These significant relationships remained, even after controlling for foveal width and foveal thickness ($P < 0.05$ for all).

However, as two subjects in our sample had peak MPODs (0.25°) that were more than 3 SD above the mean, we reanalyzed our data after exclusion of these subjects (statistically, the data of these subjects were deemed outliers). The strength of these relationships was weakened ($r = -0.132$ to -0.265), but a statistically significant age-related decline in MPOD remained at 1° and 3° eccentricity ($r = -0.259$, $P = 0.049$ and $r = -0.265$, $P = 0.046$, respectively). However, after adjustment for ethnic background, there was no demonstrable relationship between increasing age and MPOD at any degree of eccentricity ($P > 0.05$ for all).

Subjects with a desirable BMI (≤25) had higher mean MPODs than did subjects with an undesirable BMI (>25), and the differences were significant at 0.5° and 1° retinal eccentricity ($P < 0.05$ for both; Table 1).

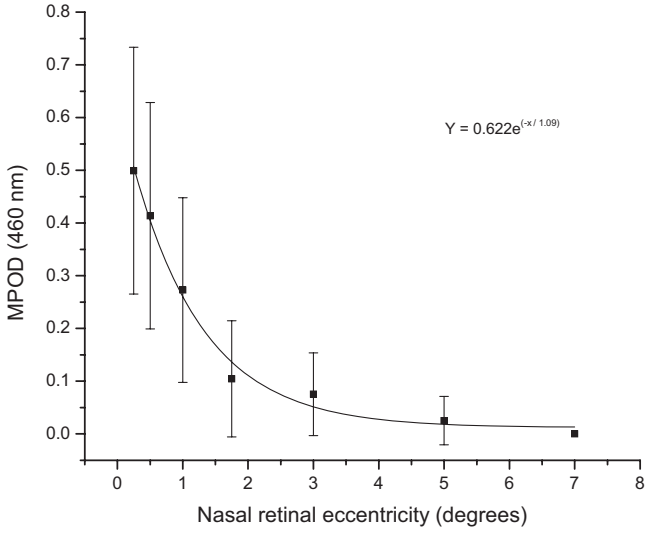

FIGURE 2. Mean spatial profile of MPOD.

Foveal Thickness

Mean MFT (average retinal thickness at the point of intersection of six radial scans), CFT (average retinal thickness within the central 1-mm [~3.3°] diameter zone), foveal width (identified as the region of the fovea where the nerve fiber layer was absent [measure 1]; and from peak foveal crest-to-crest [measure 2]) are summarized in Table 2. The mean MFT and CFT for the entire study group were 162 ± 19 and 204 ± 16 μm, respectively. White subjects had significantly greater CFTs ($P < 0.05$) and narrower foveal width measurements than did nonwhite subjects ($P < 0.05$).

The between-session variability of OCT measurements was assessed in 10 subjects, to assess reproducibility of foveal thickness measurements. Good agreement was found between readings recorded on the two separate occasions with mean differences (test 1 − test 2) of 0.1 ± 4.9 μm for MFT and −0.7 ± 3.3 μm for CFT. Also, we investigated interocular symmetry for both MFT and CFT measurements in the same 10 subjects and found a high degree of agreement for both of these measurements. The mean difference between eyes (right eye minus left eye) was −0.86 ± 7.81 μm for MFT and 2.14 ± 4.22 μm for CFT.

Relationships between MPOD and Foveal Architecture

For these analyses, relationships between OCT parameters and MPODs were studied at comparable retinal locations. First, we

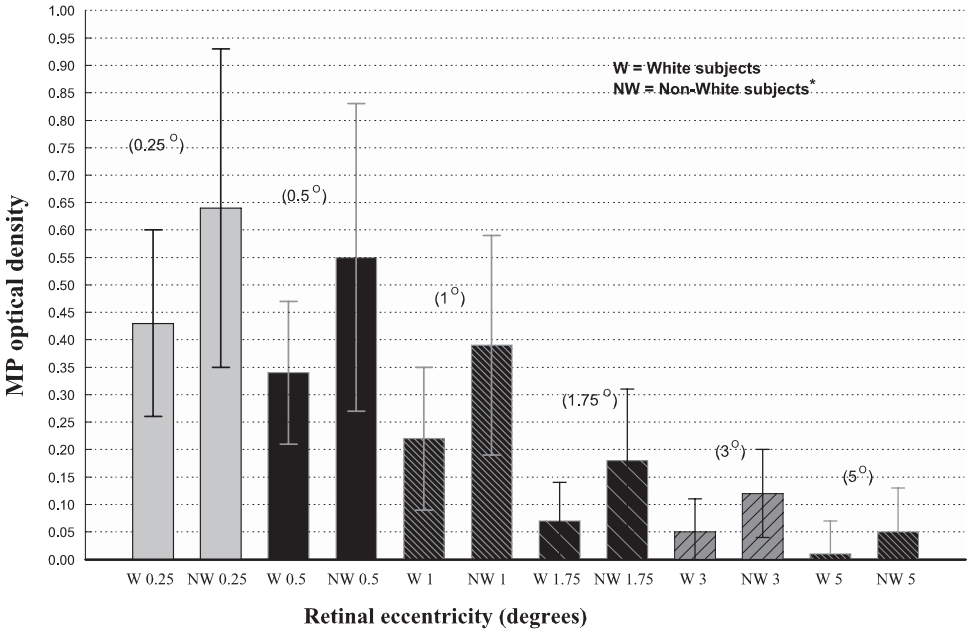

FIGURE 3. MPOD for the white and nonwhite subjects, at various degrees of retinal eccentricity. *Indian: $n = 5$; Asian: $n = 6$; Hispanic/Spanish: $n = 3$; Black: $n = 4$.

present analyses based on the entire study group, and then analyses based on sex and ethnic-based subgroups. For each analysis, the correlations were recalculated without the MP outliers and after adjustment for age. The significance of the correlations was unaltered by these control analyses.

Association of MFT and CFT with MPOD in the Entire Study Group

MPOD at 0.25° was unrelated to MFT and CFT (Table 3). Similarly, MPOD at 0.5° eccentricity was unrelated to MFT and CFT (Table 3). Moreover, CFT was unrelated to the averaged MPOD for the corresponding retinal locations (eccentricities 0.25°, 0.5°, 1°, and 1.75°; $r = -0.153$, $P = 0.247$).

Association of MFT and CFT with MPOD Analyzed Separately in the Males and Females

MPOD at 0.25° was unrelated to MFT and CFT, when the data were analyzed separately for male and female subjects (Table 3). Similarly, MPOD at 0.5° eccentricity was unrelated to MFT and CFT for both male and female subjects (Table 3). Furthermore, for both sexes, CFT was unrelated to the averaged MPOD for the corresponding retinal locations (eccentricities of 0.25°, 0.5°, 1°, and 1.75°; males: $r = 0.17$, $P = 0.42$; females: $r = -0.24$, $P = 0.16$).

Relation of MFT and MPOD, Analyzed Separately in the Whites and Nonwhites

When the data were analyzed for the white subjects only, MPOD at 0.25° was unrelated to MFT (Table 3; Figure 4). However, in the remaining 18 nonwhite subjects (Indian: $n = 5$; Asian: $n = 6$; Hispanic/Spanish: $n = 3$; Black; $n = 4$), there was a strong positive and significant relationship between MPOD at 0.25° and MFT (Table 3; Figure 4), which remained even after the MP outlier data were removed ($r = 0.52$, $P < 0.05$). Similarly, MPOD at 0.5° was unrelated to MFT (Table 3) in the white subjects, whereas there was a strong positive and significant relationship between MPOD at 0.5° and MFT for the nonwhite subjects (Table 3).

CFT and MPOD, Analyzed Separately in the Whites and Nonwhites

When the data were analyzed separately for the white and nonwhite subjects, MPOD at 0.25° and 0.5° was unrelated to CFT (Table 3). Also, CFT was unrelated to the average MPOD

TABLE 2. Foveal Thickness and Foveal Width Measurements with Respect to Sex and Ethnicity

Characteristic	*n*	%	CFT (μm) [1 mm]	MFT (μm)	Foveal Width (μm) [measure 1]*	Foveal Width (μm) [measure 2]†
Entire study group	59	100	204 ± 16	162 ± 19	1244 ± 211	1371 ± 215
Sex						
Male	24	41	207 ± 15	160 ± 19	1183 ± 210	1402 ± 221
Female	35	59	202 ± 16	164 ± 18	1287 ± 203	1327 ± 202
Ethnic background						
White	41	69	208 ± 15‡	165 ± 19	1204 ± 201‡	1304 ± 214‡
Nonwhite§	18	31	196 ± 15‡	156 ± 16	1336 ± 208‡	1443 ± 205‡

MFT, average retinal thickness at point of intersection of six radial scans; CFT, average retinal thickness around the central 1 mm (≈3.3°) diameter zone; foveal width was measured in two ways (see below).

* Measured as area of fovea where nerve fiber layer was absent.

† Measured as foveal crest-to-crest using calipers provided by Stratus OCT software.

‡ $P < 0.05$.

§ Nonwhite Indian: $n = 5$; Asian: $n = 6$; Hispanic/Spanish: $n = 3$; Black: $n = 4$.

TABLE 3. Parson Correlation Matrix Showing the Relationship between Foveal Thickness Values and MPOD at 0.25° and 0.5° of Retinal Eccentricity

	CFT (μm) [across 1 mm]	MFT (μm)
Entire study group (n = 59)		
MPOD (0.25°)	$r = -0.08$	$r = 0.03$
MPOD (0.5°)	$r = -0.05$	$r = 0.12$
Male subjects (n = 24)		
MPOD (0.25°)	$r = 0.22$	$r = 0.14$
MPOD (0.5°)	$r = 0.28$	$r = 0.20$
Female subjects (n = 35)		
MPOD (0.25°)	$r = -0.18$	$r = -0.02$
MPOD (0.5°)	$r = -0.13$	$r = -0.07$
White subjects (n = 41)		
MPOD (0.25°)	$r = -0.01$	$r = -0.10$
MPOD (0.5°)	$r = 0.03$	$r = -0.01$
Nonwhite subjects† (n = 18)		
MPOD (0.25°)	$r = 0.18$	$r = 0.59$*
MPOD (0.5°)	$r = 0.23$	$r = 0.67$*

MFT and CFT are as defined in Table 2.

* Correlation is significant at the 0.01 level.

† Nonwhite subjects: Indian: n = 5; Asian: n = 6; Hispanic/Spanish: n = 3; Black: n = 4.

in the corresponding retinal locations, when the data of the white and nonwhite subjects were analyzed separately ($r = -0.10$, $P = 0.54$ and $r = 0.12$, $P = 0.63$, respectively).

Foveal Width, MPOD, and Integrated MP in the Entire Study Group

The between-session variability of OCT measurements was assessed in 10 subjects to determine the reproducibility of foveal width measurements (measure 1: identified as the region of the fovea where the nerve fiber layer was absent; measure 2: identified as the distance from peak foveal crest to crest). Good agreement was found between readings recorded on the two separate occasions with mean differences (test 1 − test 2) of -7.33 ± 46.9 μm (0.67%) for foveal width measure 1 ($P = 0.599$, paired t-test) and 3.08 ± 56.01 μm (0.45%) for foveal width measure 2 ($P = 0.852$, paired t-test). Also, in the same 10 subjects, we assessed the intraclass correlation coefficient (ICC) for foveal width measurements 1 and 2, to assess intergrader agreement (among the four graders) and found an ICC of 0.950 for method 1 and 0.980 for method 2. This result provides strong evidence of agreement among the four graders on all measurements of interest. The between-session agreement and intergrader agreement we report for both methods of foveal width assessment used in this study provides confidence in these nonstandardized measurements of foveal width.

The relationship between foveal width and averaged MPOD across the fovea (eccentricities of 0.25°, 0.5°, 1°, 1.75°, and 3°) was positive and significant, with both methods of determining foveal width (Table 4; Fig. 5). Similarly, the relationship between foveal width and integrated MP across the fovea was positive and significant with both methods (Table 4). Of note, removing the MP outliers did not alter the significance of these relationships ($P < 0.05$, for all).

Foveal Width, MPOD, and Integrated MP Analyzed Separately in the Males and Females

The relationship between foveal width and averaged MPOD across the fovea remained positive and significant with both methods of determining foveal width, when the data were analyzed separately in the male and female subjects (Table 4). Similarly, the relationship between foveal width and integrated MP across the fovea remained positive and significant with both methods of measurement, when the data were analyzed separately for the male and female subjects, with the exception of the measure 2 foveal width–MP association in male subjects, which was positive but not statistically significant (Table 4).

Foveal Width, MPOD, and Integrated MP Analyzed Separately in the Whites and Nonwhites

The relationship between foveal width and averaged MPOD across the fovea remained positive and significant with both

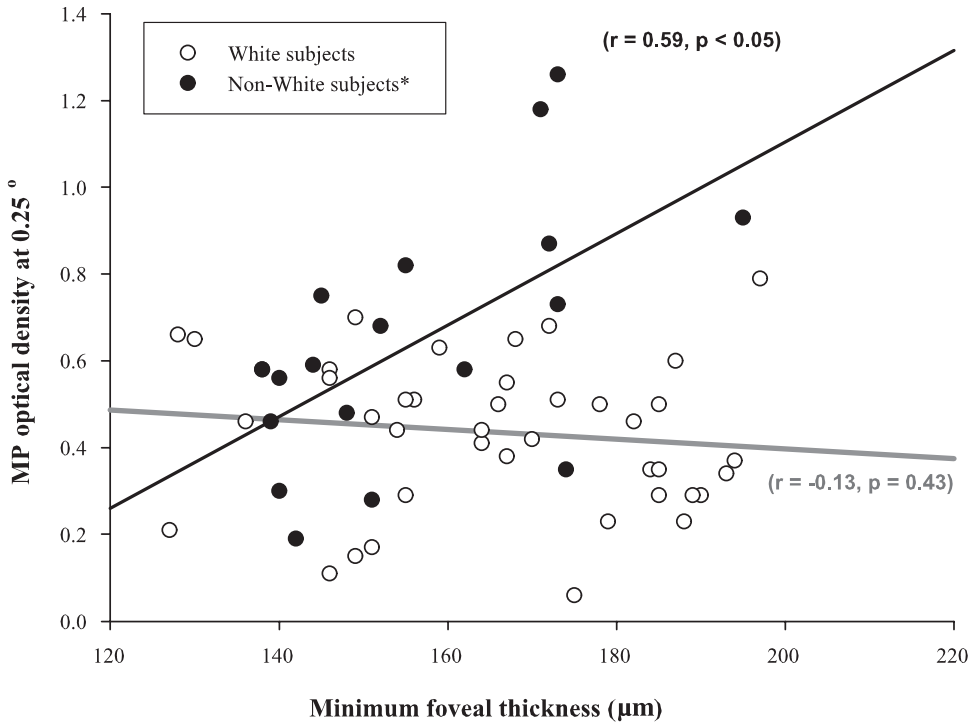

FIGURE 4. The relationship between MPOD at 0.25° foveal eccentricity and MFT (for the white and nonwhite subjects). *Indian: n = 5; Asian: n = 6; Hispanic/Spanish: n = 3; Black; n = 4. MFT is the average retinal thickness at the point of intersection of six radial scans.

Table 4. Parson Correlation Matrix Showing the Relationship between Foveal Width Measures and Averaged MPOD across the Fovea and Integrated MP across the Fovea

	Foveal Width (μm) [measure 1]*	Foveal Width (μm) [measure 2]†
Entire study group (n = 59)		
Average MPOD across fovea	r = 0.41‡	r = 0.32§
Integrated MP across fovea	r = 0.41‡	r = 0.32§
Male subjects (n = 24)		
Average MPOD across fovea	r = 0.55‡	r = 0.376§
Integrated MP across fovea	r = 0.50‡	r = 0.22
Female subjects (n = 35)		
Average MPOD across fovea	r = 0.36§	r = 0.33§
Integrated MP across fovea	r = 0.37§	r = 0.34§
White subjects (n = 41)		
Average MPOD across fovea	r = 0.37§	r = 0.28
Integrated MP across fovea	r = 0.36§	r = 0.27
Nonwhite subjects‖ (n = 18)		
Average MPOD across fovea	r = 0.30§	r = 0.26
Integrated MP across fovea	r = 0.30§	r = 0.25

Averaged MPOD across the fovea is the average MPOD, as measured at the following degrees of retinal eccentricity (0.25°, 0.5°, 1°, 1.75° and 3°); integrated MP across the fovea is the area under the spatial distribution curve; foveal width was measured in two ways (shown below).

* Measured as area of fovea where the nerve fiber layer was absent.

† Measured as foveal crest to crest, with the calipers provided by Stratus OCT software (Carl Zeiss Meditec, Inc.).

‡ Correlation is significant at the 0.01 level.

§ Correlation is significant at the 0.05 level.

‖ Nonwhite subjects: Indian: n = 5; Asian: n = 6; Hispanic/Spanish: n = 3; Black: n = 4.

methods of determining foveal width, when the data were analyzed separately for the white and nonwhite subjects, with the exception of the measure 2 (identified from peak foveal crest to crest) foveal width–MP association in nonwhite subjects, which was positive but not statistically significant in both subgroups (Table 4). Similarly, the relationship between foveal width and integrated MP across the fovea remained positive with both methods of measurement, when the data were analyzed separately for the white and nonwhite subjects (Table 4). However, and similar to the results with method 1, the relationship between foveal width measure 2 and integrated MP across the fovea did not reach statistical significance in either the white or the nonwhite subjects (Table 4).

Discussion

Several studies have reported on MFT and CFT for the normal population.[19–26] The mean (±SD) MFT (162 ± 19 μm) and CFT (204 ± 16 μm) thicknesses in our study are comparable to data in those previous reports. We found that MPOD at 0.25° and 0.5° eccentricity was unrelated to MFT and CFT. Consistent with this, CFT was unrelated to the averaged MP for the corresponding retinal location (eccentricities of 0.25°, 0.5°, 1°, and 1.75°).

Few studies have been undertaken to investigate the relationship between central retinal architecture and the spatial profile of MP.[20,21,27,28] However, consistent with our findings, Kanis et al. recently measured MPOD by using fundus reflectometry and foveal thickness by using OCT in 37 subjects (36 of whom were white) and found that there was no significant linear association between MFT and MPOD (r = 0.05, P = 0.78) or between CFT and MPOD (r = −0.04, P = 0.82).[28]

Aleman et al.[21] measured inner retinal thickness in the central 1° by using OCT in a group of 49 patients (75 eyes) with retinitis pigmentosa (RP) or Usher syndrome (US) and 19 normal subjects (27 eyes) without ocular disease. They found that MPOD (at 0.5°, measured with HFP) was positively and significantly related to retinal thickness in the patient group (r = 0.57, $P < 0.001$) and to a lesser extent in the normal subjects (r = 0.39, P = 0.12). Their latter, non–statistically significant, observation is consistent with findings in our study. Aleman et al.[21] hypothesized that the low MP levels in the patients with retinal disease were due to the loss of inner retinal tissue known to occur in outer retinal degenerations[29] and concluded that, in a normal retina (without disease), the relationship between retinal thickness and MP is more complex. This notion is consistent with a study by Duncan et al.,[27] who found a strong positive relationship between central retinal thickness and MP at 0.5° (r = 0.66, P = 0.003) in a group of 13 patients with a diagnosis of choroideremia.

Liew et al.[20] measured MPOD by using autofluorescence and HFP, and CFT and MFT were measured with OCT. They reported that MP at 0.5° was positively and significantly related to MFT and CFT, when measured with HFP (r = 0.33, $P < 0.01$ and r = 0.29, $P < 0.01$, respectively). Similarly, they found that peak MP, measured with autofluorescence, was positively and significantly related to MFT and CFT (r = 0.33, $P < 0.01$ and r = 0.29, $P < 0.01$, respectively) and that the average MP for the central 1° area was positively and significantly related to MFT and CFT (r = 0.40, $P < 0.01$ and r = 0.33, $P < 0.01$, respectively). They suggested that the amount of MP at the fovea may be related to retinal thickness at the central foveal depression. Of note, the principal conclusion of Liew et al. contrasts with that of the present study and that recently reported by Kanis et al.,[28] and the discrepancy may be due to methodological differences. As outlined in our methods section, it appears that some of the OCT scans in Liew et al.[20] may have been misaligned, which would have resulted in inaccurate foveal thicknesses. In addition, their study was confined to female twin subjects.

In this study, we found that white subjects had significantly thicker central foveas and larger MFTs than did nonwhite subjects. This finding is consistent with a study performed by Huynh et al.,[30] who found that MFT and CFT were significantly thicker in white than in East Asian children. Of interest, we found no demonstrable relationship in our data between

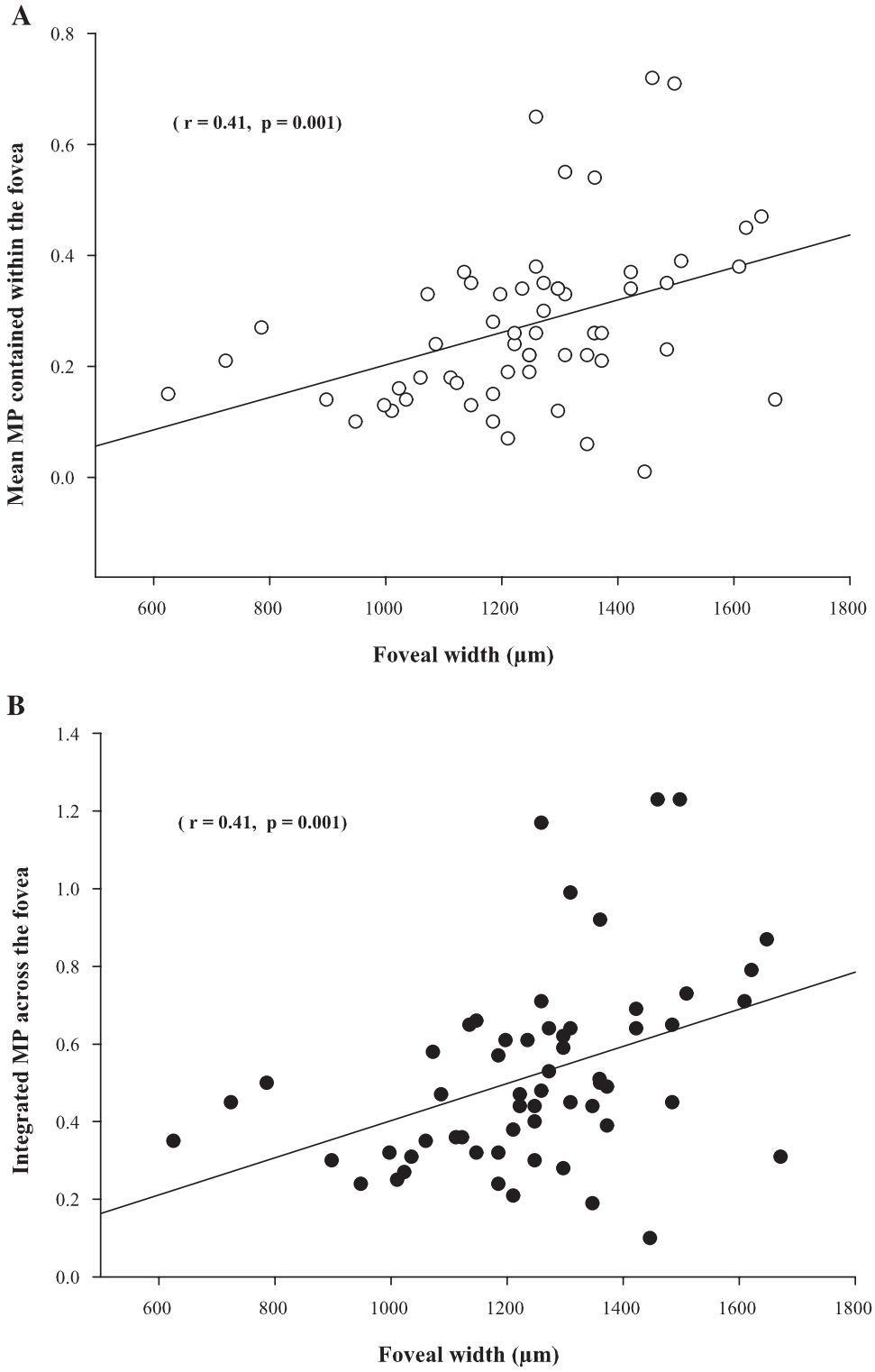

FIGURE 5. (**A**) The relationship between mean MPOD within the fovea and foveal width. Foveal width denotes the region where the nerve fiber layer was absent (measure 1). Mean MP contained within the fovea is mean MPOD for 0.25°, 0.5°, 1°, 1.75°, and 3° of retinal eccentricity. (**B**) The relationship between integrated MP within the fovea and foveal width ($r = 0.41$, $P < 0.05$). Foveal width: the region where the nerve fiber layer was absent (measure 1). Integrated MP within the fovea: integrated MP for retinal eccentricities of 0.25°, 0.5°, 1°, 1.75°, and 3°.

MPOD at 0.25° of eccentricity and MFT in our white subjects; whereas, in the nonwhite subjects, we found a strong positive and significant relationship between these variables. As outlined earlier, this finding is in agreement with that of Kanis et al.,[28] who reported no association between MFT or CFT and MPOD in their study group, which was almost entirely composed of white subjects (37 white subjects and 1 subject of Surinam-Portuguese ancestry).

Finally, we report a significant and positive relationship between foveal width and average MPOD across the fovea that was unaffected by sex or ethnic background. Also, we report on foveal width and integrated MP across the fovea, as integrated MP is thought to correspond more closely to the chemical quantity of the macular carotenoids. Similarly, we found a positive and significant relationship between foveal width and integrated MP across the fovea, which is unsurprising, given that in a wider fovea, the cone axons (Henle fibers) where MP is densest,[2] are longer and may store more MP.

The importance of recognizing a relationship between foveal width and MPOD rests on the fact that in total, only 12%

to 22% of the overall variation in MP can be accounted for by explanatory variables such as dietary intake of lutein and zeaxanthin, serum concentrations of L and Z, cigarette smoking, waist circumference, body mass index, and female sex. Of note, this is the first time that a specific feature of foveal architecture—namely, foveal width—has been shown to account for an additional small percentage of the variance in MP levels.[31,32] It is important to note, however, that a large percentage of the variation in MP remains yet unexplained (roughly 75%). Hopefully, future genetic studies with respect to MP levels and studies investigating the transport (e.g., association of L and/or Z with lipoproteins) and uptake of L and Z into the retina (association of L and/or Z with retinal binding proteins) (Balashov-Katz N, et al. *IOVS* 1999;43:ARVO Abstract 1151) will further assist in our understanding of the large, and as yet unexplained, variation in MP levels.

In conclusion, foveal MP is positively and significantly related to foveal width, regardless of sex or ethnic background, whereas it is unrelated to retinal thickness in our entire study group, with the exception of the positive and significant relationship found between MFT and MPOD for nonwhite subjects. These results indicate that the spatial profile of MPOD is related to an individual's foveal architecture and that this relationship is more complex than previously thought. Further studies in which novel techniques are used for retinal imaging, such as the next generation of OCT, and validated imaging-based methods for measuring MP should facilitate further exploration of our findings.

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