ABSTRACT

**Purpose.** Macular pigment (MP) acts as a prereceptoral filter which selectively absorbs short wavelengths. It has the potential to alter color vision but the literature is conflicting on whether it does and, if so, to what extent, possibly reflecting differences between color mechanisms and color tests. This study was designed to identify and investigate relationships, if any, between MP optical density (MPOD) and color sensitivity using a battery of techniques to quantify the color vision of color-normal observers.

**Methods.** Color vision was assessed with the Farnsworth-Munsell 100-Hue test (FM100), Moreland match on the HMC anomaloscope, and a customized short wavelength automated perimetry (SWAP) technique at the foveola and at 1, 2, 3, 4, and 5° eccentricity. MPOD spatial profile was measured using customized heterochromatic flicker photometry.

**Results.** Total error scores and % partial error scores on the FM100 were uncorrelated to MPOD. Moreland matches showed a significant long wavelength shift with MPOD at between 1 and 3° (at 1.75°, \( r = 0.489, p < 0.001 \)). Sensitivities on customized SWAP (cSWAP) using foveal targets were significantly inversely correlated with MPOD at both 1.75° (\( r = -0.461, p < 0.001 \)) and 3° (\( r = -0.393, p < 0.001 \)). Partial correlation analysis suggests that none of these findings can be attributed to age effects within the range 18 to 40 years.

**Conclusions.** Our findings suggest that dietary supplementation to increase MPOD is unlikely to adversely affect hue discrimination. The association of MPOD with cSWAP may be a temporally limited effect to which the visual system normally adapts. We suggest that cSWAP may provide a clinical tool for assessing short-wavelength foveal sensitivity. (Optom Vis Sci 2011;88:1-●●●)

Key Words: hue discrimination, anomaloscope, SWAP, macular pigment

Macular pigment (MP), consisting of the carotenoids lutein, zeaxanthin, and meso-zeaxanthin, is concentrated at the macula and is not detectable optically beyond about 7° from the foveal center. Of these carotenoids, the zeaxanthins predominate at the fovea whereas lutein dominates beyond the fovea. The extent of macular pigmentation has recently been found to be related to the width of the foveal cup, as assessed by optical coherence tomography. Because these pigments are located in the fibers of Henle at the foveola and in the inner nuclear layer beyond the fovea, they act as a prereceptoral filter and are believed to contribute a variety of potentially beneficial properties for vision, including reduction of the effects of chromatic aberration (though not supported by Engles et al.), improvement of spatial vision and contrast enhancement, increased photopic increment sensitivity, reduced glare sensitivity in some studies but not others, and increased critical flicker frequency. Hue discrimination and color vision in general are most acute at the fovea corresponding to increased cone density, specialized anatomic relationships and minimal spatial summation in this region (although with appropriate stimulus size scaling, surprisingly good color vision is possible beyond the fovea). It is plausible that color discrimination at a small angular subtense would be influenced by the optical density (OD) of MP at the fovea. Indeed, it has long been speculated that interobserver differences in color matching by color-normal observers are at least partly because of differences in macular pigmentation. Also, it is known that even subjects with ophthalmoscopically normal fundi exhibit substantial variations in MPOD, contributing to a range of prerece-
The color vision tests used in this study were (a) hue discrimination using the FM100 test, (b) hue matching using the Moreland match on an anomaloscope, and (c) short wavelength automated perimetry (SWAP) increment thresholds using a customized procedure (cSWAP) to provide optimal foveal and parafoveal stimuli. This study has clinical implications for the visual effects of dietary supplementation of patients with age-related macular degeneration and at-risk patients.

**METHODS**

Identical instrumentation and test protocols were used in the Macular Pigment Research Group laboratories in Dublin and Waterford, Ireland.

**Subjects**

One hundred two healthy subjects aged 18 to 40 years and resident in either Dublin or Waterford, Ireland, were recruited to participate in this dual-center study, which was approved by Research Ethics Committees of Waterford Institute of Technology and of Dublin Institute of Technology. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

Potential subjects underwent a full eye examination. The exclusion criteria comprised: any ocular pathology (including abnormal macula appearance or cataract); corrected visual acuity <6/9 in the better eye; refractive error outside −6 to +6 diopters; and defactive color vision. One eye only of each subject was tested, that with better corrected acuity. Full color vision data were available for 84 subjects.

**Color Threshold/Sensitivity Techniques**

**The FM100 test (X-Rite UK, Poynton)**

This test was administered under color-corrected fluorescent lighting supplied by a pair of 15W 46 cm lamps (The Daylight Co., London, UK) providing minimum luminance of 94 cd·m⁻² reflected from each color sample as measured with a spot telephotometer. Maximum background luminance reflected from the supplied black sample trays was 12 cd·m⁻². Color temperature is rated at 6400° K. Subjects were allowed to review the arrangement in each tray if they so requested.

Individual error scores and total error scores (TES), summed across the visible spectrum and purple hues, were determined using the software supplied by the manufacturer. Partial error scores (PES) were used to assess hue discrimination specifically among blue and cyan hues using samples 50 to 68 and 36 to 54, respectively, and were divided by TES to obtain percentage values (%PES).

**Anomaloscope**

This test was administered using the Moreland match on an HMC MR anomaloscope (type 7700: Oculus, Wetzlar, Germany). This provides a 2° field within which 436 and 490 nm sources are matched to a mixture of 480 and 589 nm, the latter mixture providing a brightness match. Control of stimuli and calculation of blue/green mixture were achieved with the anomaloscope under computer control using the manufacturer’s software. Neutral preadaptation was not used as this
was found to produce transient adaptation effects on stimulus saturation. Stimuli were presented under continuous viewing mode. After practice, subjects toggled the mixture to obtain four matches, two each with the mixture preset to blue bias and green bias. The mean of six blue/green matches was calculated for each subject to obtain the midpoint.

**Customized Short-Wavelength Automated Perimetry**

Foveal and parafoveal increment sensitivities were measured using an adaptation of the standard SWAP routine on a Humphrey Field Analyzer 2i (Carl Zeiss Medetec, Jena, Germany). Yellow (530 nm) background luminance was 100 cd.m\(^{-2}\). Size V targets of 440 nm and 200 ms duration subtending 1.7° at the eye were presented at 0, 1, 2, 3, 4, and 5° eccentricity from a fixation target. The number of targets at each eccentricity beyond the foveal center varied from 4 to 20. On each presentation, a single target was presented. Increment thresholds were obtained using the SWAP adaptive staircase full thresholding technique. Subjects were given 3 min to adapt to the background before testing began. Sensitivity for each eccentricity was the mean of values for all targets in the group at that eccentricity.

**Macular Pigment Optical Density**

MPOD was measured by customized HFP (cHFP) using a densitometer (Macular Metrics Corp., Providence, RI), which alternates 460 and 550 nm stimuli, the former being maximally absorbed by MP whereas the latter is not absorbed by MP. A spatial profile of MPOD was obtained by performing five measurements at each eccentricity (0.25, 0.5, 1, 1.75, and 3°), and at 7°, to provide a reference point at which MP is optically undetectable. Further details have been published elsewhere.\(^2^9\) This instrument and technique have been shown to be valid and have high reproducibility.\(^3^0\)

**Statistical Methods**

Data were analyzed using PASW Statistics 17 (SPSS, Chicago, IL). Correlation coefficients and first-order partial correlation coefficients were calculated using the Pearson product-moment method because scatter-plots showed no evidence of non-linearity. Statistical analysis was based on two-tailed tests and interpreted with reference to 0.05 significance levels and Bonferroni correction.

**RESULTS**

Fig. 1 shows the MPOD spatial profile. These data compare well with previously published data using the same cHFP method.\(^3\) Mean (±SD) MPOD for the 0.25° stimulus was 0.45 (±0.18), range 0.16 to 0.93.

Mean (±SD) hue discrimination TES for our subjects was 55 (±23), comparable with Kinnear and Sahaie’s data for the 30 to 39 age group.\(^3^1\) TES was found not to correlate significantly (p > 0.001 after Bonferroni correction). Possible associations between MPOD and (1) short wavelength hue discrimination in the region of peak absorption by MP and (2) discrimination at the short wavelength end of the expected axis of a type III acquired color vision defect were investigated by calculating %PES for color samples 50 to 68 and 36 to 54, respectively, i.e., %PES = MPOD / 0.239 + 33.92. An example of this analysis is provided in Fig. 2, which is a scattergram of %PES for FM100 samples 36 to 54 against MPOD at 1.75° eccentricity. Despite an apparent trend of increased %PES with higher MPOD, both (1) and (2) were found to be non-significantly significant.
TABLE 1.
Correlations between color vision variables and MPOD

<table>
<thead>
<tr>
<th>MPOD</th>
<th>%PES</th>
<th>Moreland midpoint</th>
<th>cSWAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B/G 36–54</td>
<td>B 50–68</td>
<td>Fovea</td>
</tr>
<tr>
<td>0.25°</td>
<td>r₀</td>
<td>-0.188</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>r₁</td>
<td>-0.183</td>
<td>0.121</td>
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<tr>
<td></td>
<td>p₀</td>
<td>0.084</td>
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<td>df₀</td>
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<td>83</td>
</tr>
<tr>
<td>0.5°</td>
<td>r₀</td>
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<td>0.094</td>
</tr>
<tr>
<td></td>
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<td>0.099</td>
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<tr>
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<td>p₀</td>
<td>0.195</td>
<td>0.393</td>
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<td>df₀</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>1°</td>
<td>r₀</td>
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<tr>
<td></td>
<td>r₁</td>
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<td></td>
<td>p₀</td>
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<td>83</td>
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<td>1.75°</td>
<td>r₀</td>
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<td>0.113</td>
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<tr>
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<td></td>
<td>p₀</td>
<td>0.040ᵇ</td>
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<td>df₀</td>
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<td>83</td>
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<tr>
<td>3°</td>
<td>r₀</td>
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<tr>
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<td>r₁</td>
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<td>p₀</td>
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<td>0.034ᵇ</td>
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<tr>
<td></td>
<td>df₀</td>
<td>83</td>
<td>83</td>
</tr>
</tbody>
</table>

ᵃSignificant with correction for a 5 by 9 correlation matrix.
ᵇp ≤ 0.05 without Bonferroni correction.

Correlated (p > 0.001 with Bonferroni correction) to MPOD at all eccentricities.

The anomaloscope Moreland match midpoints were found to be negatively correlated to MPOD at all eccentricities (Table 1 and Fig. 3), indicating a shift toward green mixtures to match cyan. The coefficient was maximal for MPOD at 1.75°, corresponding to the anomaloscope stimulus diameter of 2°. MPOD at 1.75° accounted for 23.9% of variability (r²) in Moreland match data. The anomaloscope Moreland match midpoints were found to be negatively correlated to MPOD at all eccentricities (Table 1 and Fig. 3), indicating a shift toward green mixtures to match cyan. The coefficient was maximal for MPOD at 1.75°, corresponding to the anomaloscope stimulus diameter of 2°. MPOD at 1.75° accounted for 23.9% of variability (r²) in Moreland match data.

DISCUSSION

Our hue discrimination data do not support the findings of Moreland and Dain, who found a significant increase in both TES and PES in the blue-green region with their MP1 carotenoid filter of 1.0 maximum absorbance. We found no statistically significant association between MPOD at any retinal eccentricity and TES or PES after application of Bonferroni correction. This discrepancy may be a reflection of the nature of Moreland and Dain’s filter, which was considerably denser than typical MPOD values; it exceeded the MPOD of all our subjects at and between 1.75° and 3°. The maximal proportion of variability in cSWAP attributable to MPOD (r²) is 21.2% (for foveolar cSWAP and MPOD at 1.75°).

It is possible that an artificial filter creates short-term changes in color vision and that an autoregulatory process adjusts retinal and/or cortical color mechanisms on a long-term basis in response to their naturally occurring MPOD. This hypothesis is supported by data showing a consistent shift in achromatic locus over a 3 months period for cataract patients postsurgery, by color con-
stancy effects for blue and green targets despite crystalline lens brunescence,33 and by evidence of plasticity of adult neural color mechanisms.34 Rodriguez-Carmona et al.26 found no correlation between YB thresholds and MPOD using a technique in which threshold color differences were measured for detection of movement of a stimulus within a checkered array.

We did not assess the association, if any, of MPOD across subjects with color appearance other than by using the HMC anomaloscope Moreland match. Using this technique, we found that midpoint data were surprising in that subjects with high MPOD required less blue to match cyan; this finding was consistent for MPOD at all eccentricities. No directly comparable data exist in the literature, although Stringham and Hammond17 found that YB cancellation thresholds were constant across the retina despite significant MPOD variability across the retinal region tested. It is of interest that in one study of Moreland match midpoint data, no difference was reported between postcataract patients with short wavelength-absorbing intraocular lenses and those with clear intraocular lenses.35

The cSWAP data show relatively constant sensitivity across the retina beyond the foveola (Fig. 5) despite substantial differences in MPOD across the retina (Fig. 1). This finding is consistent with that of Stringham et al.36 who used Maxwellian-view multichannel optics except that they found slightly lower sensitivity at the foveola compared with parafovea using 16 subjects of similar age to those in this study. This suggests that parafoveal (but not foveolar) cSWAP may provide a valid clinical test of SWS cone function. The fact that we found statistically significant inverse correlations between short-wave sensitivity for the foveal stimulus and MPOD at two eccentricities does not in fact contradict Stringham et al.’s conclusions; our correlations relate to differences between subjects rather than to averaged measures across the retina which would not take into account the effects of intersubject variance in both SWS cone sensitivity and MPOD at any single retinal locus.

We hypothesize that the fact that SWS cone sensitivity exhibited significant inverse associations with MPOD, whereas hue discrimination thresholds showed no significant associations with
MPD, may be related to temporal differences between the two measures. It is possible that, by using short stimulus presentations, the cSWAP technique (200 ms) produces transient effects quite different to those found with much longer presentations such as those of the FM100 test.

Confounding variables which might influence the relationship between MPD and color vision include iris and choroidal pigmentation, age, stimulus size, and pupil diameter. The effect of iris pigment density has been studied by Woo and Lee, who found that Asians have poorer PES in the blue quadrant, and by Hammond and Caruso-Avery, who reported that subjects with darker irides had higher MPD. Because all subjects in this study were white, the density range of both iris pigment and choroidal pigment was limited, and yet MPD was found to correlate significantly with color sensitivity across a variety of measures. We suggest that our findings are independent of iris pigmentation, although such pigmentation is a factor in a less racially homogenous group of subjects.

The effect of age on hue discrimination, in the blue-green spectral region in particular, is well known and is partly because of wavelength-selective loss of light transmission by the aging crystalline lens. An age effect on MPD has also been reported, some studies having shown a statistically significant age-related decline in MPD. It is therefore possible that age is a confounding factor influencing our findings on MPD and hue discrimination in the blue-green spectral region. A similar age effect is possible in relation to SWS cone function as measured by cSWAP. Although our subjects were restricted to the age range 18 to 40 years, and our exclusion criteria included any evidence of cataract, potentially confounding contributions attributable to age cannot be dismissed. However, inspection of Table 1 shows that first-order partial correlation coefficients with age as the control variable are very similar to 0-order coefficients. In no case did a significance level change from significant to non-significant by controlling for age. We therefore suggest that our observed associations between MPD and both Moreland midpoint and cSWAP are independent of age within the age range of this study (18 to 40 years, mean age ±SD = 29 ± 6 years). However, the age factor may be important in older subjects.

Stimulus size and location are known to affect both color vision and measures of MPD. In this study, MPD was measured using targets subtending between 30 min and 3.5° at eccentricities between 0 and 3°. Color thresholds were measured using centrally fixated targets subtending ~1.5° (FM100), 2° (anomaloscope), and 1.7° at between 0 and 5° eccentricity (cSWAP). A clear pattern is evident from our data: MPD correlated consistently across size and eccentricity parameters with cSWAP and Moreland midpoint. MPD values were reported in this study at a range of eccentricities to assess the consistency of correlations, and because retinal images extend beyond their geometric optical limits as a result of aberrations, diffraction, and scatter. Furthermore eye movements produce translational shift of retinal images in a natural viewing environment.

The practical implications of this study are two-fold. First, dietary supplementation to increase MPD is not likely to adversely affect hue discrimination. However, a longitudinal study of the effects of supplementation on color vision is needed to support this. Second, we have shown that appropriate customization of a standard clinical automated perimetry test (cSWAP) provides a potential clinical test for foveal SWS-cone sensitivity, although this awaits confirmation by a concordance study using Maxwellian view instrument.

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