Macular Pigment and Visual Function in Patients With Glaucoma: The San Diego Macular Pigment Study

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PURPOSE. Although recent studies have shown that macular pigment (MP) is significantly lower in glaucoma patients, this relationship merits further investigation.

METHODS. This cross-sectional study included 85 glaucoma patients and 22 controls. All subjects had standard automated perimeter (SAP) and retinal nerve fiber layer (RNFL) thickness measurements. Intake of lutein (L) and zeaxanthin (Z) was estimated using a novel dietary screener. The Heidelberg Spectralis dual-wavelength autofluorescence (AF) technology was employed to study the relationship between MP and glaucoma. The association between MP volume and glaucoma was investigated using linear regression models accounting for potential confounding factors.

RESULTS. Glaucoma patients had significantly worse SAP mean deviation (MD) and lower RNFL thickness in the study eye compared to control subjects (P < 0.001 for both). MP (volume) was comparable between groups (P = 0.436). In the univariable model, diagnosis of glaucoma was not associated with MP volume (R² = 1.22%; P = 0.257). Dietary intake of L and Z was positively and significantly related to MP in the univariable (P = 0.022) and multivariable (P = 0.020) models.

CONCLUSIONS. These results challenge previous studies that reported that glaucoma is associated with low MP. Dietary habits were found to be the main predictor of MP in this sample. Further research is merited to better understand the relationship between glaucoma, MP, and visual performance in these patients.

Keywords: glaucoma, carotenoids, macular pigment, visual function

Glaucoma

Glaucoma is a group of optic neuropathies that have in common a progressive degeneration of retinal ganglion cells (RGCs) and their axons, resulting in a characteristic appearance of the optic disc and a concomitant pattern of visual field loss. Assessment and estimation of the extent of the disease have been traditionally made using standard automated perimeter (SAP) and retinal nerve fiber layer (RNFL) measurements through optical coherence tomography (OCT).

In addition, previous studies have recognized that glaucomatous damage also affects macular structures. Therefore, evaluation of macular components may be a valuable method of assessment of the disease. In fact, approximately 50% of RGCs are located within 4.5 mm (16°) of the foveal center, a region that represents only 7.3% of the total retina area. Further, the macula is vital for central vision, and therefore, loss of macular RGCs is likely to be of particular importance for vision-related quality of life.

The macular carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ) selectively accumulate in the central retina (macula lutea), where they are collectively referred to as macular pigment (MP). The anatomic (central and prereceptorial location), biochemical (antioxidant and anti-inflammatory), and optical (short-wavelength [blue] light-filtering) properties of the macular carotenoids make these ideal candidates to enhance visual function, a hypothesis that has been confirmed in subjects free of retinal disease, and in patients with early (nonvisually consequential) age-related macular degeneration (AMD). AREDS2, a study that examined the role of supplementation of co-antioxidants in combination with L and Z, reported a favorable effect for patients with intermediate AMD by reducing the progression to advanced forms of the disease. Further, Akuffo et al. demonstrated that supplementation with L, Z, and MZ resulted in significant increases in MP and improvements in contrast sensitivity and other measures of visual function in patients with nonadvanced AMD (1-8 on the AREDS 11-step severity scale).

To date, four studies have shown that MP is significantly lower in patients with open-angle glaucoma compared to control subjects. Of note, in those studies, MP was measured using either the one-wavelength fundus reflectance method or customized heterochromatic flicker photometry (CHFP). However, the reflectance method used is limited because it uses only one wavelength, and therefore does not account for lens absorption. In addition, accurate measures of...
reflectance assume that retinal pigment epithelium melanin and other light absorbers have the same distribution across the retina. The cHFP technique, commonly referred to as the gold standard for measuring MP, is a psychophysical technique and therefore influenced by the operator’s implementation of the test and subjects’ performance (i.e., their ability to take instructions to eliminate flicker using different-sized targets). Also, this technique is time-consuming, and measuring a spatial profile of MP can take up to 30 minutes per eye, with subject fatigue making this test difficult to complete for some subjects, especially so in patients with visual field loss (e.g., glaucoma patients).

In our study we have taken advantage of recent application of the Heidelberg Spectralis (HRA+OCT Multicolor) for measuring MP objectively using dual-wavelength autofluorescence (AF) (see below for description of methods). Here, we report on MP profiles in patients with glaucoma and healthy control subjects, and investigate the relationship between MP and visual function in these groups.

METHODS

This was a cross-sectional study that included participants from a prospective longitudinal study designed to evaluate optic nerve structure and visual function in glaucoma, the Diagnostic Innovations in Glaucoma Study (DIGS). The study was conducted at the Hamilton Glaucoma Center and Shiley Eye Institute of the Department of Ophthalmology, University of California San Diego (UCSD). Participants were longitudinally evaluated according to a preestablished protocol that included regular follow-up visits in which patients underwent clinical examination and several other imaging and functional tests. Methodologic details have been described previously. Written informed consent was obtained from all participants, and institutional review board and human subjects committee approved all methods. All methods adhered to the tenets of the Declaration of Helsinki for research involving human subjects, and the study was conducted in accordance with the regulations of Health Insurance Portability and Accountability Act.

All participants underwent comprehensive ophthalmologic examination including review of medical history, visual acuity measured using the Early Treatment Diabetic Retinopathy Study chart (letter acuity was expressed as the logarithm of minimum angle of resolution), contrast sensitivity (CS) assessment using the Pelli-Robson contrast sensitivity chart (Precision Vision, La Salle, IL, USA), slit-lamp biomicroscopy, intraocular pressure measurement, gonioscopy, stereoscopic optic disc photography, and SAP using Swedish Interactive Threshold Algorithm Standard with 24-2 strategy of the Humphrey Field Analyzer IIi, model 750 (Carl Zeiss Meditec, Inc., Dublin, CA, USA). Visual acuity was measured using the Early Treatment Diabetic Retinopathy Study chart, and letter acuity was expressed as the logarithm of minimum angle of resolution. Only subjects with open angles on gonioscopy were included. Subjects with open angles on gonioscopy were included. Subjects were excluded if they presented any other ocular or systemic disease that could affect optic nerve or visual field.

Two participant groups were included in the study as follows: case participants (patients with confirmed presence of glaucoma, see below) and control participants (subjects free of glaucoma). Inclusion criteria were as follows: Glaucoma was defined by the presence of repeatable abnormal SAP tests (pattern standard deviation with $P < 0.05$ and/or a Glaucoma Hemifield Test outside normal limits) and corresponding optic nerve damage in at least one eye. Control subjects had no evidence of optic nerve damage and had normal SAP tests in both eyes. Controls were recruited from the general population through advertisements and from staff and employees of UCSD. All visual fields were evaluated by the UCSD Visual Field Assessment Center (VisFACT). Only reliable SAP tests were included (less than 33% fixation losses or false-negative errors, and less than 15% false-positive errors). They were also reviewed for artifacts, fatigue, or learning effects, inappropriate fixation, evidence that the visual field results were caused by a disease other than glaucoma (e.g., homonymous hemianopia) and inattention; tests were excluded if such artifacts were present.

All subjects had measurements of weight and height obtained at the time of testing. These were used to calculate body mass index (BMI) for each subject, as the quotient of mass (in kilograms) divided by the square of height (in meters).

Spectral-Domain Optical Coherence Tomography (SD-OCT)

Spectralis SD-OCT (software version 5.4.7.0; Heidelberg Engineering, Dossenheim, Germany) was used to measure peripapillary RNFL thickness in the present study. The device uses a dual-beam SD-OCT and a confocal laser-scanning ophthalmoscope (cSLO) that employs a super-luminescent diode light with a center wavelength of 870 nm and an infrared scan to provide simultaneous images of ocular microstructures. A real-time eye tracking system is incorporated that couples the cSLO and SD-OCT scanners to adjust for eye movements and to ensure that the same location of the retina is scanned over time. For RNFL thickness a total of 1536 A-scan points were acquired from a 3.45-mm circle centered on the optic disc. All images were reviewed by UCSD Imaging Data Evaluation and Analysis Center to ensure the scan was centered, that the signal strength was more than 15 decibels (dB), and that there were no artifacts. Scans that were inverted or clipped or those that showed excoistent retinal pathologic abnormalities were excluded. The accuracy of the segmentation of each retinal layer also was checked for errors. The SD-OCT RNFL thickness parameter used in the study was the global RNFL thickness corresponding to the average of all measures in the peripapillary circle.

Dietary and Supplement Carotenoid Assessment

Dietary intake of L and Z was estimated using a crude carotenoid screener known as the “L/Z screener” developed by Elizabeth Johnson (Tufts University, Boston, MA, USA). This screener gives a dietary score (from 0 to 75), which can be categorized as follows: estimate low intake, category 1: 0 to 15 (≤2 mg/day); estimate medium intake, category 2: 16 to 30 (5–13 mg/day); estimate high intake, category 3: 31 to 75 (>13 mg/day). This tool has been described in detail elsewhere. Due to sample size, subjects with low and medium intake were combined in subsequent analysis.

In addition, participants were asked if they were consuming any commercially available food supplements. If participants answered yes, the researchers queried the supplement brand in order to investigate if the supplement contained L, Z, and/or MZ. Supplementation use, defined as recent consumption of a food supplement containing L, Z, and/or MZ, was coded as no or yes for analyses.

Measurement of Macular Pigment

MP was measured using the Spectralis HRA+OCT Multicolor (Heidelberg Engineering, Heidelberg, Germany) based on a previously described protocol. In brief, the protocol used 20 × 15 volume scans, 19 scans each 259 μm apart at high

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Table 1. Demographic and Clinical Characteristics of Subjects Included in the Study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Glaucoma, n = 85</th>
<th>Controls, n = 22</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>72.5 ± 10.1</td>
<td>70.0 ± 10.3</td>
<td>0.414†</td>
</tr>
<tr>
<td>Sex, n (%) female</td>
<td>45 (50.6)</td>
<td>17 (77.3)</td>
<td>0.030‡</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td>0.945‡</td>
</tr>
<tr>
<td>White</td>
<td>57 (67.1)</td>
<td>16 (72.7)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>18 (21.2)</td>
<td>5 (22.7)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>7 (8.2)</td>
<td>1 (4.6)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (2.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.6 ± 5.8</td>
<td>29.1 ± 5.6</td>
<td>0.053‡</td>
</tr>
<tr>
<td>MP volume</td>
<td>8716.6 ± 3902.8</td>
<td>7660.6 ± 3750.2</td>
<td>0.436†</td>
</tr>
<tr>
<td>Central MP, 0.23º</td>
<td>0.52 ± 0.19</td>
<td>0.49 ± 0.18</td>
<td>0.543§</td>
</tr>
<tr>
<td>Diet, estimated L and Z intake, n (%) yes</td>
<td>30.1 ± 13.3</td>
<td>36.3 ± 14.4</td>
<td>0.056§</td>
</tr>
<tr>
<td>Carotenoid supplement</td>
<td>26 (30.6)</td>
<td>6 (27.3)</td>
<td>0.762‡</td>
</tr>
<tr>
<td>Visual acuity, logMAR</td>
<td>0.04 ± 0.14</td>
<td>0.05 ± 0.12</td>
<td>0.410†</td>
</tr>
<tr>
<td>Contrast sensitivity</td>
<td>1.40 ± 0.30</td>
<td>1.53 ± 0.13</td>
<td>0.066†</td>
</tr>
<tr>
<td>SAP 24-2 MD, dB</td>
<td>−6.4 ± 7.4</td>
<td>0.1 ± 1.0</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>RNFL thickness, µm</td>
<td>71.6 ± 16.8</td>
<td>86.4 ± 14.7</td>
<td>&lt;0.001§</td>
</tr>
</tbody>
</table>

LogMAR, logarithm of the minimum angle of resolution. Values are presented as mean ± standard deviation, unless otherwise noted. † Wilcoxon rank-sum test. § Fisher’s exact test. ‡ Student’s t-test.

The association between MP volume and diagnosis of glaucoma was investigated using linear regression models, where MP volume was used as dependent variable and glaucoma diagnosis as independent variable. We initially ran univariable models evaluating the association of each variable with the outcome. Subsequently, multivariable models were used, adjusting for potential confounding factors such as age, BMI, and dietary intake of L and Z. All statistical analyses were performed using commercially available software Stata, version 14 (StataCorp LP, College Station, TX, USA). The α level (type I error) was set at 0.05.

Results

The study included 85 glaucoma patients and 22 control subjects. Table 1 presents demographic and clinical characteristics of participants for each group, and whether the groups were statistically similar or different. In brief, groups were statistically comparable in age (P = 0.414), but there was a lower percentage of females in the glaucoma group compared with the control group (50.6% vs. 77.3%, respectively; P = 0.030). There was no significant difference in race, BMI, visual acuity, or contrast sensitivity of studied eye between groups. As expected, glaucoma subjects had significantly worse SAP mean deviation (MD) results in the studied eye when compared to control subjects (−6.4 ± 7.4 dB vs. 0.1 ± 1.0 dB, respectively; P < 0.001). Also, global RNFL thickness in the study eye of glaucoma patients was significantly lower than in controls (71.6 ± 16.8 µm vs. 86.4 ± 14.7 µm, respectively; P < 0.001).

MP (volume) was statistically comparable between glaucoma patients and controls (8716.6 ± 3901.8 vs. 7660.0 ± 3750.2, respectively; P = 0.436), even after controlling for dietary intake of L and Z and carotenoid supplement use.

Table 2 presents univariable and multivariable linear regression models for explaining MP volume. Again, in the univariable model, diagnosis of glaucoma was not associated with MP volume (R² = 1.22%; P = 0.257). Age, sex, race, visual acuity, contrast sensitivity, SAP MD, and OCT RNFL were also not statistically significantly associated with MP volume. Subjects with higher BMI had an association close to significance with lower MP volume (R² = 3.59%; P = 0.052). High dietary intake of L and Z had a statistically significant association with MP volume in the univariable (R² = 4.92%; P = 0.022) and multivariable (P = 0.020) models.

Discussion

Over the last two decades there has been growing interest in the role of the macular carotenoids for visual performance (in diseased and nondiseased eyes) and for prevention of AMD.3–5,12,24,25 These nutrients are now also being studied for their potential role to enhance cognitive function, with promising initial findings.26 This study, however, was designed to compare MP measurements in glaucoma patients to controls, and to investigate the association between MP and visual function in these groups.

Our study demonstrated no association between MP and glaucoma diagnosis. Also, we did not find any association between MP and variables that are usually used to assess and estimate the extent of glaucoma, such as SAP and SD-OCT. Although we did identify that MP was related to dietary L and Z intake (i.e., via diet and/or food supplements), controlling for dietary intake of L and Z did not alter our main finding (i.e., that MP was not related to glaucoma status). Of note, our finding that dietary intake of L and Z is related to MP levels is consistent with the exclusive dietary origin of these nutrients and a recent meta-analysis that confirmed a dose-response relation.
relationship between dietary/supplement intake of L, Z and MZ, and MP levels.27

Interestingly, these results demonstrated that mean diet scores, obtained using the L/Z screener, were much higher in the San Diego glaucoma population compared with an Irish glaucoma population. Nolan et al.22 in a recent study performed in Waterford (Ireland) reported a diet score of 24 (3–13 mg/day) in the sample (of similar age) studied, compared with a score of 36 (>13 mg/day) in the current sample. This finding suggests that the participants from the San Diego population consume, on average, more L and Z (estimated combined). Of note, the differences seen in dietary intake of these carotenoids was also reflected in the MP data, with the average MP volume score in the San Diego population of 7660 compared to the Irish population of 6426.

Four earlier studies have suggested that MP may have an important role in glaucoma. These studies reported that glaucoma patients tend to have lower MP values compared to control subjects (see Table 3).13–15 However, results from the current study are not in agreement with these earlier reports, because MP levels in glaucoma patients were not significantly different from those in the control group. First, we reports, because MP levels in glaucoma patients were not significantly different from those in the control group. First, we significantly different from those in the control group. First, we significantly different from those in the control group. First, we significantly different from those in the control group. First, we significantly different from those in the control group. First, we significantly different from those in the control group. First, we significantly different from those in the control group. First, we significantly different from those in the control group. First, we significantly different from those in the control group.

Our study also demonstrated no significant relationship between MP and the gold standard tests used to evaluate glaucoma, that is, SAP MD and SD-OCT RNFL. Ji et al.15 and Igras et al.13 also did not find significant associations between SAP MD and MP. Although Siah et al.14 did not report on the relationship between SAP MD and MP, in a more recent study from their group based on the same population, they reported that MP was significantly correlated with SAP MD results.16

When referring to RNFL profile, Ji et al.15 and Siah et al.14 did not find any association with MP, and these findings are consistent with our work. Of note, Ji et al.15 and Igras et al.13 did not assess dietary intake of L and Z in their studies, and therefore interpretation of their findings must take this limitation into consideration.29

Previous reports have documented CS losses in glaucoma patients, even when presenting a relatively good visual acuity.32 Loughman et al.33 have demonstrated that an increase in MP resulted in significant improvements in CS. The anatomic (central and prereceptorial location), biochemical (antioxidant and anti-inflammatory), and optical (short-wavelength [blue] light-filtering) properties of the macular carotenoids make these ideal candidates to enhance visual function, and therefore MP measurements in glaucoma patients might be valuable due to the disease characteristics and symptoms that typically result with a loss of visual function. In addition, future studies should be performed in order to evaluate the role of specific carotenoid supplementations on visual function in glaucoma patients.

Our study had limitations. Due to the cross-sectional design, we were not able to clearly determine the temporal relationship between MP measurement and carotenoid supplement intake. It is also important to acknowledge that our case/control design has limitations given that the ratio of cases to controls is 1:3.8. It is known that the statistical confidence in a case–control design increases with an increase in ratio of

### Table 2. Results of the Univariable and Multivariable Linear Regression Models for Explaining Macular Pigment Volume*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariable Model Coefficient (95% CI)</th>
<th>P</th>
<th>Multivariable Model Coefficient (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glaucoma</td>
<td>1056.0 (–780.8 to 2892.9)</td>
<td>0.257</td>
<td>958.6 (–908.86 to 2818.78)</td>
<td>0.312</td>
</tr>
<tr>
<td>Diet, high L and Z intake</td>
<td>1712.9 (257.5 to 3181.1)</td>
<td>0.022</td>
<td>1775.59 (290.16 to 3261.03)</td>
<td>0.020</td>
</tr>
<tr>
<td>Age, per decade older</td>
<td>523.0 (–415.3 to 106.1)</td>
<td>0.388</td>
<td>393.89 (–533.74 to 1121.53)</td>
<td>0.285</td>
</tr>
<tr>
<td>Sex, female</td>
<td>413.7 (–1089.7 to 1916.1)</td>
<td>0.587</td>
<td>–96.59 (–224.07 to 30.88)</td>
<td>0.136</td>
</tr>
<tr>
<td>Race, American</td>
<td>60.0 (–758.2 to 1878.3)</td>
<td>0.948</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, per 1 kg/m² higher</td>
<td>–126.0 (–252.9 to 0.9)</td>
<td>0.052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual acuity, per 0.1 logMAR higher</td>
<td>3122.0 (–8482.7 to 2238.7)</td>
<td>0.251</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity</td>
<td>–1148.1 (–3746.0 to 1449.8)</td>
<td>0.383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD SAP 24-2, per 1 dB lower</td>
<td>–11.5 (–17.5 to 94.5)</td>
<td>0.830</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCT RNFL thickness, per 1 μm thinner</td>
<td>–9.2 (–53.1 to 34.8)</td>
<td>0.681</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CI, confidence interval.

* Multivariable model was adjusted for age, BMI, and dietary carotenoid intake.

### Table 3. Clinical Characteristics of Studies Demonstrating Relationship Between Macular Pigment Optical Density (MPOD) and Glaucoma*

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age, y</th>
<th>Population</th>
<th>BMI</th>
<th>Diagnosis</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study†</td>
<td>72.8 ± 11</td>
<td>60.7% Caucasian</td>
<td>27 ± 6.1</td>
<td>POAG</td>
<td>0.52 ± 0.19</td>
</tr>
<tr>
<td>Igras et al.‡‡</td>
<td>69.0 ± 11</td>
<td>N/R</td>
<td>N/R</td>
<td>90% POAG</td>
<td>0.25</td>
</tr>
<tr>
<td>Siah et al.‡‡</td>
<td>67</td>
<td>N/R</td>
<td>25.5</td>
<td>51.1% POAG</td>
<td>0.23</td>
</tr>
<tr>
<td>Ji et al.‡‡</td>
<td>42.3 ± 16.9</td>
<td>Chinese</td>
<td>22.2 ± 2.5</td>
<td>POAG</td>
<td>0.116</td>
</tr>
</tbody>
</table>

POAG, primary open-angle glaucoma; N/R, not reported.

† MPOD was measured using physical (objective) dual-wavelength autofluorescence.
‡‡ MPOD was measured using customized heterochromatic flicker photometry.
§§ MPOD was measured using one-wavelength fundus reflectance.
control to case participants up to 4:1, beyond which there is no more benefit; therefore our design falls significantly short of the optimal scenario. In addition, we were not able to investigate the longitudinal relationship between SAP MD and SD-OCT RNFL measurements with MP. Future longitudinal studies should be able to clarify this issue. Although SAP is the gold standard for glaucoma functional assessment, due to its simplicity it is likely that it does not fully capture certain dynamic aspects of vision that may be important in performing daily activities, such as driving, reading, or navigating the environment. Previous studies have demonstrated that patients with glaucoma had a higher incidence of motor vehicle collisions and performed worse on wayfinding tasks. Therefore future studies should be performed in order to evaluate the relationship between MP and driving and/or navigating the environment. We did not report cataract status of our population, and MP measurements obtained via dual-wavelength AF are known to be influenced by cataract status. In addition, we did not report smoking or cognitive status from the population studied and these could have impacted MP measurements. However, it is unlikely that this could have influenced the main conclusions of the study.

In conclusion, patients diagnosed with glaucoma in this study had comparable MP levels to control subjects. Our results disagree with previous studies reporting on MP in patients with glaucoma. However, it is our view that there is no explicit reason as to why patients with glaucoma would be lacking in MP levels compared to controls. Although not tested in our study, we do believe (based on a biologically plausible rationale and many published clinical trials) that enrichment of MP may be important to enhance visual function in patients with glaucoma. Further research is merited to better understand the relationship between glaucoma and macular carotenoids, and the impact of enriching these nutrients in these patients.

Acknowledgments

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