Measurement of Macular Pigment Optical Density in a Healthy Chinese Population Sample

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PURPOSE. Macular pigment may protect against age-related macular degeneration (AMD) by its capacity to absorb blue light and scavenge free radicals. Current information on human macular pigment density has been largely from studies on Caucasian populations. The purpose of this study was to assess macular pigment density and its determinant factors in a Chinese population sample.

METHODS. Macular pigment optical density (MPOD) was measured in a healthy Chinese population using heterochromatic flicker photometry (HFP). Participants received a standard ophthalmic examination, and only subjects who were confirmed not to have any eye diseases except mild age-related cataract were included in the study. Demographic and lifestyle data and general health status were recorded by questionnaire.

RESULTS. A total of 281 unrelated healthy Chinese individuals, including 96 males and 185 females, with ages ranging from 17 to 85 years, participated in the study. The mean and standard deviation of MPOD levels were 0.56 ± 0.19, 0.49 ± 0.18, 0.36 ± 0.15, and 0.19 ± 0.12, respectively, at 0.25°, 0.5°, 1.0°, and 1.75° eccentricity points. A significant age-related decline in MPOD was observed at 0.25° (P = 0.014). Females tended to have relatively lower levels of MPOD than males at 0.25° (P = 0.21), 0.5° (P = 0.025), and 1.0° (P = 0.16). No statistically significant association of MPOD was observed with body mass index or smoking status.

CONCLUSIONS. Macular pigment density measured by HFP tended to decline with aging in this healthy Chinese population sample. Females may have lower levels of MPOD than males. (Invest Ophthalmol Vis Sci. 2012;53:2106–2111) DOI:10.1167/iovs.11-8518

The macula is the center of the posterior retina and can be discerned clinically as the area of yellowish pigmentation. Macular pigment, composed of three carotenoids—lutein, zeaxanthin, and meso-zeaxanthin—is believed to improve visual performance by reducing chromatic aberration and glare sensitivity.2–4 Through its ability to absorb high-energy short-wave blue light and its antioxidative property, macular pigment is also hypothesized to protect the macula from light-induced and oxidative damage, which may lead to age-related loss of function and macular diseases.5,6 Although obtaining direct evidence for this hypothesis is difficult, a variety of indirect evidence supports this hypothesis. For example, epidemiologic studies suggest that a high intake of lutein and zeaxanthin and high blood levels of these carotenoids are related to a reduced risk of age-related macular degeneration (AMD).7–9 Lutein and zeaxanthin are entirely of dietary origin and cannot be synthetized by the human body. Foods such as green leafy vegetables, corn, squash, broccoli, peas, and egg yolks are a rich source of lutein and zeaxanthin.10 In healthy subjects, it has been shown that blood or retinal levels of carotenoids can be modified by dietary intake or supplementation.11–13 However, whether increasing blood or retinal levels of carotenoids can offer additional protection from degenerative changes of the retina remains to be proven.

It is well-known that the prevalence of AMD varies among different populations. Prevalence of early and late AMD in a Chinese population, for example, was reported to be lower than that in Caucasians.14,15 In addition to the differences in genetic susceptibility,16–18 traditional Chinese foods with higher concentrations of carotenoids19 may translate to higher macular pigment and contribute to the relatively lower prevalence of AMD in the Chinese population. Current information regarding human macular pigment optical density (MPOD), however, is mainly from studies on Caucasian populations. To the best of the authors’ knowledge, only two studies reported MPOD in small samples of Hong Kong Chinese.20,21 The present study aimed to measure MPOD in a relatively larger sample of a healthy Chinese population in urban Beijing and to evaluate its determinant factors.

SUBJECTS AND METHODS

Study Participants and Clinical Evaluation

The study was performed under approval of the Ethics Committee of Beijing Tongren Hospital. Informed consent was obtained from participants after explanation of the purpose and procedure and possible consequences of the study. The research procedures followed...
the tenets of the Declaration of Helsinki. Healthy Chinese volunteers were recruited to the study by poster and oral advertisement in the local community of downtown Beijing. All study participants underwent a standard ophthalmic examination, including visual acuity, intraocular pressure, slit-lamp biomicroscopy, and fundus examinations through dilated pupil. Color fundus photographs centered on the fovea of both eyes were taken for each subject. Body mass index (BMI, kg/m²) was calculated according to the height and weight of the participant. Personal and medical information was obtained using a questionnaire. Only those subjects with self-reported healthy status and normal fundus in both eyes were included in the study. Exclusion criteria included history of diabetes or any life-threatening disease such as myocardial infarction or cancer, or subjects with ocular disease including corneal scar, dense cataract, glaucoma, uveitis, vitreous hemorrhage, chorioretinal and optic nerve diseases, or any maculopathy.

### Measurement of Macular Pigment Optical Density

MPOD was measured psychophysically in both eyes using heterochromatic flicker photometry (HFP) in a dark room. The examiner familiarized the subject with the apparatus and showed an instructional video that was designed to provide a uniform introduction to the task (www.macularmetrics.com). Four foveal test configurations at 0.25°, 0.5°, 1.0°, and 1.75° eccentricity points were measured. The former two are solid disks and the latter two are annuli. The parafoveal reference test target is a solid disk set at 7° eccentricity. Flicker frequency of the two test wavelengths (460 nm and 530 nm) usually began with 14 Hz for the foveal and 12 Hz for the parafoveal measurement. The frequency would be decreased if the subject could not recognize sensation of flickering or increased if the subject could not find a no-flicker zone.

The subjects were asked to affix their gaze on the exact center of the test target and perceive the flickering. For the parafoveal test, the fixation target is a small red light-emitting diode located to the left of the background field. The task for the participant was to identify whether the target is flickering or not. The examiner adjusted the knob from the flicker zone to the no-flicker zone, clockwise first and then counterclockwise to obtain two values indicating the border of the flicker and no-flicker zones (minimum and maximum intensity of blue light). The exact mathematical average of the two values, which stands for the middle of the no-flicker zone, was calculated. Measurement at each eccentricity point was repeated five times, and the average was used to calculate MPOD.

Throughout testing, the subject was reminded to gaze at the fixation point. They were also advised to blink frequently or to close the testing eye for a short while to minimize the influence of fatigue. Both eyes were measured in a randomized order to avoid the potential order effect.

### Statistical Analysis

Data analyses were performed using the open-source R statistical software package. Mean and standard deviation of MPOD at each eccentricity were calculated. Paired t-test was performed to compare MPOD levels between the right and left eyes at each eccentricity. MPOD levels between males and females or between smokers and nonsmokers were compared using t-test for two independent samples or Wilcoxon rank sum test for non-normal distributed data. Multiple comparisons were made using ANOVA followed by Tukey’s test for normal distributed data or Kruskal-Wallis test for non-normal distributed data. Correlations between continuous variables were evaluated using Pearson’s correlation coefficient (r). Relationships between MPOD and age, BMI, sex, and smoking status were analyzed by multiple linear regressions. Statistical significant level was set as P values less than 0.05.

### Results

A total of 281 healthy subjects aged from 17 to 85 years participated in the study. Ninety-six were male and 185 were female. Characteristics of the study subjects are given in Table 1. None of the study subjects reported the use of lutein/zeaxanthin supplementation. A comparison of MPOD levels between right and left eyes at 0.25°, 0.5°, 1.0°, and 1.75° eccentricities is shown in Figure 1. Substantial interocular agreement was obtained at 0.25°, 0.5°, 1.0° and 1.75° eccentricities (r = 0.81, 0.82, 0.74, and 0.69, respectively). The average MPOD level of right and left eyes of each study subject was thus calculated and used for subsequent analysis.

Table 2 shows the MPOD measurement among study subjects. The measurement was defined as successful if the subject could complete the task at test point of 0.25°, 0.5°, 1.0°, or 1.75°, respectively. The rate of successful measurement for MPOD was 79% (222/281) at 0.25°, 96.8% (272/281) at 0.5°, 1.0°, or 1.75°,
100% (281/281) at 1.0°, and 83.6% (235/281) at 1.75° eccentricities. Mean MPOD level and standard deviation from study subjects of successful measurement was 0.56 ± 0.19 at 0.25°, 0.49 ± 0.18 at 0.5°, 0.36 ± 0.15 at 1.0°, and 0.19 ± 0.12 at 1.75°.

As shown in Figure 2, a significant age-related decline in MPOD levels was observed at 0.25° eccentricity point (r = -0.165, P = 0.014). However, there was no significant association between MPOD and aging at 0.5° (r = -0.025, P = 0.68), 1.0° (r = -0.053, P = 0.38), or 1.75° (r = 0.094, P = 0.15) eccentricities.

A comparison of MPOD levels between males and females is shown in Figure 3. Females tended to have relatively lower MPOD level than males at 0.25°, 0.5°, and 1.0° eccentricities. This sex difference was statistically significant at 0.5° (P = 0.025) but was not observed at 0.25° (P = 0.21) and 1.0° (P = 0.16). No correlation was observed between smoking status and MPOD (P = 0.68 at 0.25°, 0.21 at 0.5°, 0.68 at 1.0°, and 0.67 at 1.75°) or between BMI and MPOD (P = 0.84 at 0.25°, 0.71 at 0.5°, 0.79 at 1.0°, and 0.19 at 1.75°) among study subjects.

The results from a multiple linear regression model are given in Table 3. Age was found to be the only factor showing statistically significant inverse correlation with MPOD, but only at 0.25° eccentricity point (multiple R² = 0.063, P = 0.003). The effect of sex on MPOD became insignificant in the model of multivariate analyses.

### Table 3. Macular Pigment Optical Density Measured by Heterochromatic Flicker Photometry among Study Subjects

<table>
<thead>
<tr>
<th>Degree of Eccentricity</th>
<th>Subjects</th>
<th>0.25°</th>
<th>0.5°</th>
<th>1.0°</th>
<th>1.75°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td>0.61 ± 0.20 (43)</td>
<td>0.51 ± 0.19 (54)</td>
<td>0.38 ± 0.14 (54)</td>
<td>0.19 ± 0.10 (44)</td>
</tr>
<tr>
<td>≤30</td>
<td></td>
<td>0.63 ± 0.17 (45)</td>
<td>0.53 ± 0.17 (48)</td>
<td>0.39 ± 0.14 (48)</td>
<td>0.20 ± 0.12 (45)</td>
</tr>
<tr>
<td>31–40</td>
<td></td>
<td>0.51 ± 0.18 (41)</td>
<td>0.44 ± 0.16 (45)</td>
<td>0.30 ± 0.13 (46)</td>
<td>0.15 ± 0.09 (45)</td>
</tr>
<tr>
<td>41–50</td>
<td></td>
<td>0.52 ± 0.16 (53)</td>
<td>0.47 ± 0.15 (61)</td>
<td>0.33 ± 0.13 (64)</td>
<td>0.19 ± 0.10 (56)</td>
</tr>
<tr>
<td>≥60</td>
<td></td>
<td>0.55 ± 0.20 (40)</td>
<td>0.50 ± 0.20 (64)</td>
<td>0.37 ± 0.18 (69)</td>
<td>0.23 ± 0.15 (47)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0.56 ± 0.19 (222)</td>
<td>0.49 ± 0.18 (272)</td>
<td>0.36 ± 0.15 (281)</td>
<td>0.19 ± 0.12 (235)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>0.59 ± 0.20 (75)</td>
<td>0.52 ± 0.20 (92)</td>
<td>0.37 ± 0.17 (96)</td>
<td>0.19 ± 0.13 (80)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>0.55 ± 0.18 (147)</td>
<td>0.47 ± 0.17 (180)</td>
<td>0.35 ± 0.14 (185)</td>
<td>0.19 ± 0.11 (155)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤23</td>
<td></td>
<td>0.57 ± 0.19 (96)</td>
<td>0.49 ± 0.17 (119)</td>
<td>0.36 ± 0.13 (121)</td>
<td>0.18 ± 0.10 (102)</td>
</tr>
<tr>
<td>23–27</td>
<td></td>
<td>0.56 ± 0.19 (99)</td>
<td>0.49 ± 0.19 (116)</td>
<td>0.35 ± 0.16 (123)</td>
<td>0.20 ± 0.13 (106)</td>
</tr>
<tr>
<td>≥27</td>
<td></td>
<td>0.56 ± 0.19 (27)</td>
<td>0.47 ± 0.18 (37)</td>
<td>0.35 ± 0.16 (37)</td>
<td>0.22 ± 0.13 (27)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td></td>
<td>0.56 ± 0.19 (181)</td>
<td>0.48 ± 0.18 (219)</td>
<td>0.35 ± 0.15 (226)</td>
<td>0.19 ± 0.12 (191)</td>
</tr>
<tr>
<td>Smokers</td>
<td></td>
<td>0.57 ± 0.18 (41)</td>
<td>0.51 ± 0.19 (53)</td>
<td>0.36 ± 0.17 (55)</td>
<td>0.20 ± 0.13 (44)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. The number of subjects with successful measurement is given in parentheses.
DISCUSSION

This study determined the effects of age, sex, BMI, and smoking status on MPOD in a relatively large sample of the Chinese population. We found that the MPOD levels decrease with aging in the center of the retina (at the 0.25° eccentricity point). Because MPOD plays an important role in blocking harmful blue light and in quenching reactive oxygen species, this age-related decline in MPOD may partially explain the age-related increase in the risk for macular degeneration. It should be noted, however, that only 6.3% of the decline in MPOD in the central fovea could be attributed to aging in the multiple linear regression model (multiple $R^2 = 0.063$). Thus, the hypothesized relationship between MPOD and the risk of AMD may be relatively weak.

The age-related decline in MPOD was also reported in several previous studies. However, there were studies that did not detect this age-related difference in MPOD. The discrepancy in the age–MPOD relationship between different studies may be related to differences in sample size, subject selection, or methods of measurement. In a comparative study that used different methods to investigate the possible effect of age on MPOD levels, a small but statistically significant age-related decline of MPOD was detected by the method of HFP, but this age-related difference in MPOD was not observed when the fundus reflectance spectroscopy or scanning laser ophthalmoscope was used. Among all of the available methods for noninvasive measurement of MPOD, including HFP, autofluorescence, Raman spectroscopy, fundus reflectance spectroscopy, and motion photometry, the HFP method has been the most commonly used in research studies. The advantages of the HFP method include the relatively inexpensive instrument, easy training of research staff to operate the machine, and no requirement for pupil dilation. In addition, HFP remains the only validated technique uniquely unaffected by changes in the ocular media with aging. Thus, robust to patients with mild age-related cataract. It should be noted, however, that this method of HFP, as well as other techniques except Raman spectroscopy, uses peripheral points of the retina as a reference. If there were an age-related diffusion of the macular pigment from macula to the periphery, the use of HFP technique may result in an age-related decrease of MPOD because of the increase of MPOD at the parafoveal reference point. Future studies using complementary methods of measurement of macular pigment optical density (MPOD) measured by heterochromatic flicker photometry in various published studies.

### Table 3. Multivariate Analyses for the Association of Macular Pigment Optical Density Measured at 0.25° with Variable Factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>$-0.005$</td>
<td>$0.001$</td>
<td>$0.005^*$</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>$0.003$</td>
<td>$0.005$</td>
<td>$0.52$</td>
</tr>
<tr>
<td>Sex (reference=male)</td>
<td>$-0.024$</td>
<td>$0.034$</td>
<td>$0.48$</td>
</tr>
<tr>
<td>Smoking status (reference=nonsmoker)</td>
<td>$0.017$</td>
<td>$0.042$</td>
<td>$0.68$</td>
</tr>
<tr>
<td>Light exposure</td>
<td>$0.008$</td>
<td>$0.009$</td>
<td>$0.40$</td>
</tr>
</tbody>
</table>

* Multiple $R^2$: 0.063.

### Table 4. Comparison of Macular Pigment Optical Density (MPOD) Measured by Heterochromatic Flicker Photometry in Various Published Studies

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study Population</th>
<th>Study Subjects</th>
<th>Sample Size</th>
<th>Age Range, y (mean ± SD)</th>
<th>Foveal Eccentricity</th>
<th>Parafocal Reference</th>
<th>MPOD (mean ± SD or range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iannaccone et al. (2007)</td>
<td>U.S.</td>
<td>Healthy</td>
<td>222</td>
<td>69-86 (79.1 ± 3.2)</td>
<td>0.5°</td>
<td>7°</td>
<td>0.37 ± 0.19°</td>
</tr>
<tr>
<td>Snodderly et al. (2006)</td>
<td>U.S.</td>
<td>CAREDS</td>
<td>54</td>
<td>50-79 (66)</td>
<td>0.5°</td>
<td>7°</td>
<td>0.42 ± 0.22</td>
</tr>
<tr>
<td>Hammond et al. (2000)</td>
<td>U.S.</td>
<td>Healthy</td>
<td>217</td>
<td>17-92 (41.5 ± 19.7)</td>
<td>0.5°</td>
<td>4°</td>
<td>0.22 ± 0.13</td>
</tr>
<tr>
<td>Hammond et al. (2002)</td>
<td>U.S.</td>
<td>Healthy</td>
<td>680</td>
<td>29.6 ± 13.1</td>
<td>0.5°</td>
<td>4°</td>
<td>0.18 ± 0.12°</td>
</tr>
<tr>
<td>Ciulla et al. (2001)</td>
<td>U.S.</td>
<td>Healthy</td>
<td>280</td>
<td>18-50</td>
<td>0.5°</td>
<td>4°</td>
<td>0.21 ± 0.13</td>
</tr>
<tr>
<td>Ciulla et al. (2001)</td>
<td>U.S.</td>
<td>Cataract</td>
<td>24</td>
<td>48-82 (68.7 ± 9.5)</td>
<td>0.5°</td>
<td>4°</td>
<td>0.21 ± 0.13</td>
</tr>
<tr>
<td>Beatty et al. (2001)</td>
<td>European</td>
<td>Healthy</td>
<td>46</td>
<td>21-81 (51 ± 18)</td>
<td>0.475°</td>
<td>6°</td>
<td>0.29 ± 0.16</td>
</tr>
<tr>
<td>Nolan et al. (2007)</td>
<td>Irish</td>
<td>Healthy</td>
<td>828</td>
<td>20-60</td>
<td>0.5°</td>
<td>5,5°</td>
<td>0.30 ± 0.17</td>
</tr>
<tr>
<td>Lam et al. (2005)</td>
<td>Chinese (Hong Kong)</td>
<td>Healthy</td>
<td>92</td>
<td>16-85 (59.1)</td>
<td>0.5°</td>
<td>7°</td>
<td>0.30 ± 0.60</td>
</tr>
<tr>
<td>Tang et al. (2004)</td>
<td>Chinese (Hong Kong)</td>
<td>Healthy</td>
<td>67</td>
<td>18-23</td>
<td>0.5°</td>
<td>4°</td>
<td>0.48 ± 0.23</td>
</tr>
<tr>
<td>Present study</td>
<td>Chinese (Mainland)</td>
<td>Healthy</td>
<td>272</td>
<td>17-85</td>
<td>0.5°</td>
<td>7°</td>
<td>0.49 ± 0.18</td>
</tr>
</tbody>
</table>

* Caucasian.
† African American.
‡ Subjects with BMI > 29 kg/m².
¶ Patients with cataract before cataract exaction.
© Patients with cataract after cataract exaction.
CAREDS, Carotenoids in Age-Related Eye Disease Study.
MPD measurement are warranted to verify the age-related decline in MPD in this Chinese population.

It has been reported that body fat and smoking status affect the level of macular pigment. However, the present study did not detect an association between MPD and BMI or smoking status. The association between MPD levels and sex has been reported in several previous studies, with females having relatively lower levels of MPD than males. A similar trend of sex-related difference in MPD was observed in this study. However, the impact of sex on MPD became insignificant in the model of multivariate analyses. Given the fact that several studies that did not find this sex-associated difference in MPD, the authors' data indicate that the sex-related difference in MPD is marginal. The level of MPD and its distribution in retina may be affected by factors such as genetic background, demographies, or lifestyle characteristics. It has been reported, for example, that females tend to have higher levels of macular pigment than males. Females therefore are more likely to have higher residual pigment density at the parafoveal reference point, which could lead to a relatively lower MPD measurement. Moreover, it is thought that females tend to have higher percentage of body fat and adipose tissue than males, which may compete with the retina for uptake of lutein and zeaxanthin. If the relatively higher body fat in females contributes to the association between sex and MPD in the previous publications, the lack of association between BMI and MPD may partially explain the weak sex-MPD association in this study. It is plausible that the higher dietary intake of lutein and zeaxanthin in Chinese population may saturate the adipose tissue with these carotenoids and attenuate the competition with the retina for lutein and zeaxanthin, thus negating the effects of BMI and body fat on MPD. The level of macular pigments varies among different populations. MPD in blacks, for example, has been reported to be 41% lower than that in whites, although another study did not find such a difference. It should be noted, however, that comparison of MPD levels among studies are difficult or even impossible because different studies may employ different methodologies or study protocols. MPD measured by HFP at the same eccentricity point with a 4° reference point, for example, would be expected to be lower than that with a 7° reference point because of the relatively higher residual pigment at 4° reference point, which could lead to underestimation of MPD levels.

MPD levels measured by HFP at a 0.5° eccentricity point are reported to be 0.18 to 0.22 log units in the U.S. population with a 4° parafoveal reference point. The relatively higher measurement of MPD levels reported in the Chinese population in the present study were in agreement with the data obtained from a study in Hong Kong Chinese. However, the MPD levels reported in the present study were higher as compared with studies in other populations that used the similar parameters (Table 4). The relatively higher measurement of MPD levels reported in the Chinese population in the present study and in Hong Kong Chinese is in agreement with the reports that the Chinese population consumes more lutein and zeaxanthin and may have relatively higher serum concentration of carotenoids. The lack of dietary information, however, is a limitation of this current study.

In summary, the authors reported measurement of MPD levels using a method of HFP in a relatively large sample of healthy Chinese population and observed a significant age-related decline in MPD levels at the fovea. Approximately 6.5% of the decline in MPD in the central fovea could be attributed to the age. No significant association of MPD levels was found with BMI and smoking status. This age-related decline in MPD may contribute to the increased risk of AMD with aging. The Chinese population has relatively higher dietary intake of lutein and zeaxanthin and higher MPD, which may partially explain the relatively lower prevalence of AMD in the Chinese population. Future studies to verify the age-related decline in MPD and to elucidate the underlying mechanisms are warranted.

References


