Macular Pigment Optical Density in a Central European Population

Antonios Pipis, MD; Eftychia Touliou, MD; Albert J. Augustin, PhD

BACKGROUND AND OBJECTIVE: The purpose of this study is to measure the macular pigment optical density and study its spatial profile as well as identify its determinant factors in a Central European population.

PATIENTS AND METHODS: The macular pigment optical density (MPOD) and its distribution were assessed in 228 eyes of 129 subjects using fundus reflectometry with the Visucam 500 (Carl Zeiss Meditec, Jena, Germany).

RESULTS: A statistically significant positive association between a diet rich in xanthophylls and all MPOD values was found. A positive monotonic relationship was demonstrated between an increasing degree in pigment distribution eccentricity and age, as well as all MPOD values except for area.

CONCLUSION: Assuming that macular pigment is protective against age-related macular degeneration, our study highlights the role of nutritional counseling and intervention in preventing this disease. Furthermore, MPOD appears to increase with age, and the distribution of macular pigment appears to form more eccentric profiles.

INTRODUCTION

Macular pigment is composed of chemical substances commonly known as xanthophylls, which include three isomers: lutein, zeaxanthin, and meso-zeaxanthin.1 The macula lutea, or yellow spot, is the region in and around the fovea where lutein and zeaxanthin are concentrated.2,3 Xanthophylls are not synthesized in the human body and therefore must be obtained from the diet. Lutein and zeaxanthin are widely distributed in plants, green leafy vegetables, and yellow to orange fruits and vegetables.4 A diet rich in lutein and zeaxanthin leads to high levels of these xanthophylls in serum. However, not all individuals respond the same way to the dietary intake of lutein and zeaxanthin.5 Macular pigment (MP) serves as a potent blue-light filter and has significant antioxidant properties. Consequently, it is believed that macular pigment may protect against macular diseases attributable to oxidative stress, such as age-related macular degeneration (AMD).6 Of particular interest is the possibility that nutritional counseling or intervention might reduce AMD incidence or retard AMD progression.5,7

The study of human macular pigment and its distribution in vivo is now possible through determination of macular pigment optical density (MPOD). The methods used to measure MPOD can be categorized into two groups: psychophysical methods and objective methods. The first group consists of heterochromatic flicker photometry and minimum motion photometry. Objective methods are fundus reflectometry, fundus autofluorescence, and resonance Raman spectroscopy.8 MPOD measurement...
Figure 1. (A) Color fundus image in which the yellow pigment of the macula and its distribution can hardly be recognized (blue arrow), and small hard drusen can be seen. (B) Using single wavelength fundus reflectometry, macular pigment can easily be detected. (C) The MPOD module provides measurements of macular pigment parameters and the macular distribution profile.
using the Visucam 500 (Carl Zeiss Meditec, Jena, Germany) belongs to the second group, and uses the method of single wave blue-reflection fundus imaging. This process involves the measurement of the reflectance of short wavelength light near the maximum absorption of macular pigment at 460 nm. This methodology produces a three-dimensional distribution of MP, thus allowing for extrapolation of four parameters from the distribution: maximal optical density, mean optical density, macular pigment volume, and macular pigment area.\(^9\) Visucam shows a certain variation of the above four parameters. The mean standard deviation of measurements is 9% for macular pigment density volume, 7% for macular pigment density area, 4% for maximal optical density, and 3% for mean optical density.\(^10\) Schweitzer et al showed that the reproducibility of the above parameters in a one-wavelength reflection method determined for three subjects (47, 61, and 78 years old) was as follows: \(\text{maxMPOD}_x = 6.3\%\), \(\text{meanMPOD}_x = 4.6\%\), volume = 6%, and area = 6% before stray-light correction.\(^11\)

**PATIENTS AND METHODS**

We retrospectively analyzed data that were gathered during the regular examination of our macula and retina patients in both the outpatient and inpatient clinic. Approval was granted by the institutional review board.

Patients were asked to provide a medical and ophthalmological history. Characteristics such as tobacco use and body mass index (BMI) and details such as iris color were documented.

**Food Frequency Questionnaire**

Our patients were routinely informed about macular diseases such as AMD and the role of macular pigment and were asked to complete a questionnaire concerning their nutritional habits. This food frequency questionnaire was based on the weekly and monthly consumption of specific foods with proven high concentration in xanthophylls.\(^12\)

O’Conell et al used a food frequency questionnaire to assess the dietary intake of key nutrients in relation to the risk for developing of age-related maculopathy in a healthy Irish population.\(^13\) Using our food frequency questionnaire and taking into account
the concentrations of lutein and zeaxanthin in each nutrient, we calculated a nutritional score for each subject. The nutritional score is a number that could provide us with a raw estimation of dietary intake of xanthophylls for each individual.

Measuring Macular Pigment Optical Density
After measuring the intraocular pressure and instillation of tropicamid 0.5 mg/mL, photographs of both eyes were obtained using the Zeiss Visucam 500 system in combination with the MPOD module. All fundus reflectance photographs were obtained by the same observer, under the same light conditions, using the same flash level, and under pharmacological mydriasis. The MPOD analysis provided measurements of MPOD volume, area, maximal MPOD, and mean MPOD, as well as a colored map and a three-dimensional pigment distribution profile (Figure 1A-C).

Subjects
A total of 228 eyes of 116 subjects (72 women and 44 men) were selected out of 129 subjects for further analysis. Exclusion criteria were the presence of ocular disease interfering with the picture quality and measurement results such as dense cataract or corneal opacity, advanced AMD with macular scarring, geographic atrophy or drusen, and any other macular pathologies such as macular pucker or edema. Subjects taking supplements containing lutein, zeaxanthin, or meso-zeaxanthin were also excluded.

Spatial Profile of Macular Pigment
The distribution of MP in humans has been studied in numerous investigations. Using flicker photometry, Hammond et al observed an individual variation in the spatial profile of the MP in humans with the existence of minor flanking peaks or shoulders. Kirby et al studied a “central dip” appearing in the macular pigment spatial profile (MPSP). In contrast, Berendshot et al and Dietzel et al, using fundus autofluorescence, showed that the distribution of macular pigment forms ring-like structures around a central peak.

Studying the spatial profiles of our sample as depicted by the MPOD-Module (Figure 1), we observed certain patterns with many similarities to those described in previous studies. Those patterns were repeated throughout our sample in such a manner that we could divide the MPSP of all subjects into five distinct patterns or types of distribution with increasing degree of eccentricity as described in Figure 2A-E.

Statistical Analysis
We used Analyse-it statistical software (Analyse-it Software, Leeds, United Kingdom) designed as an add-on to Microsoft Excel for Windows (Microsoft Corp., Redmond, WA). We performed a series of Pearson correlations and regression analysis to investigate the relationship between continuous variables (eg, age, body mass index, MPOD parameters) as well as t tests to analyze the relationship between a continuous variable and a categorical one (eg, sex, iris color, tobacco use). In addition, we took our observations on the MPSP one step further and tried to identify correlations or connections between the various types (I through V) and the variables mentioned above. This was done investigating monotonic relationships using Spearman correlation. The level of statistical significance was set to $P < .05$.

RESULTS
MPOD volume, maximum and mean correlated strongly with each other, the strongest correlation demonstrated between mean and maximum values ($r = 0.90, P < .0001$). The mean values of these MPOD parameters for the study population are shown in the Table.

Patient Characteristics
There was no difference in MPOD values (volume, area, maximum, mean) between subjects with dark or light iris color. There was no statistically significant difference found for volume as well as for the other MPOD values between the two sexes. There was a weak but significant negative correlation between BMI and all MPOD values except for MPOD area. The strongest negative correlation was with MPOD volume ($r = -0.18, P = .0072$).

The mean age of the subjects was 56.6 years ($\pm 2$ years, SD: 14.7) (Figure 3). We found a medium positive correlation between age and MPOD mean and MPOD maximum ($r = 0.40$ and $r = 0.35, P < .0001$, respectively) but only a weak one between age and volume ($r = 0.18, P = .0074$) and a negative correlation with MPOD area ($r = -0.23, P = .0003$).

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### Table: Mean Values and Standard Deviation for Macular Pigment Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOD volume</td>
<td>8550 ± 314</td>
</tr>
<tr>
<td>MPOD area</td>
<td>68202 ± 1477</td>
</tr>
<tr>
<td>MPOD max</td>
<td>0.353 ± 0.009</td>
</tr>
<tr>
<td>MPOD mean</td>
<td>0.126 ± 0.004</td>
</tr>
</tbody>
</table>

For all values, CI = 95%.

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Lifestyle Characteristics

No significant difference in MPOD values was demonstrated between the smokers and non-smokers for MPOD volume and area; however, there was a small statistical significant superiority for the non-smoker group in maximum (\(P = .0144\)) and mean (\(P = .0058\)) values.

Nutritional score showed a weak to medium correlation with all MPOD values, the weakest being for MPOD area (\(r = 0.19, P = .0047\)) and the strongest for MPOD volume (\(r = 0.30, P < .0001\)). Linear regression analysis provides the linear equation to define MPOD mean using nutritional score (Figure 4). Nutritional score was not related to age.

Eccentricity in Macular Pigment Distribution

Using Spearman correlation, a clear positive monotonic relationship between age and increasing degree in pigment distribution eccentricity (MPSP type I to V) was demonstrated (\(rs = 0.47, P < .0001\)) showing a tendency to more eccentric distribution profiles such as type III, IV, or V in the older subjects, something not seen at all in our sample in participants below the age of 40 years (Figure 5). The degree of eccentricity in pigment distribution was positively correlated with all MPOD values except MPOD area (no correlation), with MPOD mean yielding the strongest coefficient (\(rs = 0.37, P < .0001\)) and MPOD maximum the weakest (0.19, \(P = .0041\)). Using multiple regression analysis incorporating all MPOD values and age in relation to MPSP type (dependent value), the regression coefficient of age and that of MPOD mean remained positive and statistically significant (\(P < .0001\) and \(P = .0019\), respectively). The coefficient of MPOD volume was positive but insignificant (\(P = .31\), while that of MPOD area was negative and insignificant (\(P = .704\)); the coefficient of MPOD maximum was negative and significant (\(P < .0001\)), and the adjusted \(R^2\) was 0.37 for this linear regression. A weak positive correlation between nutritional score and MPSP type was also demonstrated (\(rs = 0.14, P = .034\)). No statistically significant difference in pigment distribution among men and women or the other groups described above and a relationship to BMI was found.

**DISCUSSION**

Our study did not identify any difference between MPOD values among men and women. Other studies have shown controversial results with no difference in MPOD\(^{24,25}\) or higher MPOD for men\(^{26}\) or women.\(^{27}\)

In this study, BMI was negatively associated with all MPOD values except for area. Dietzel et al have shown that up to 80% of the total carotenoids in the body are found in adipose tissue and could theoretically result in a smaller storage in the retina as a possible explanation for such a finding.\(^{24}\) However, this interpretation is very speculative.

We found a very small but statistically significant superiority in MPOD mean and maximum for the non-smokers. However, we did not differentiate cur-
rent smokers according to number of cigarettes. We also did not analyze for former smokers who have now quit. Other studies found either no significant difference or reported lower MPOD levels for the smoker group as well.\textsuperscript{24,26}

In our study, MPOD values were positively associated with age, except MP area, which showed a negative correlation. In studies with a similar age range of participants such as Dietzel et al, a positive age effect on MPOD was also demonstrated.\textsuperscript{24} Other studies have found no age dependency of MPOD,\textsuperscript{28} an increase,\textsuperscript{24,25} or a decline.\textsuperscript{26} A possible explanation for the increase in measured MPOD with age is provided by Delori et al, who suggest that changes of fluorescence at Bruch’s membrane affecting the fluorescence spectra of the outer retinal layers with age could affect MP estimates using autofluorescence.\textsuperscript{24,25} It has also been suggested that lipofuscin found in the retinal pigment epithelium, having a similar light absorption spectrum as MP, could interfere with the measurement of MPOD using fundus reflectometry and autofluorescence. Since lipofuscin is known to accumulate with age, this could explain the increase in MPOD observed with aging.\textsuperscript{29} The above phenomena, however, cannot explain our finding of a decrease in macular pigment area with age.

The present study showed that dietary intake of lutein and zeaxanthin, as measured by the subject’s nutritional score, positively correlates with all MPOD values. Regression analysis suggests that an increase in nutritional score results in an increase in MPOD values such as volume and mean (Figure 4). Other studies have demonstrated that the enhancement of the dietary intake of these substances elevates their serum levels and their macular concentration except for in a group of “non-responders.”\textsuperscript{5,24,30} Taking this into account as well as the results of our statistical analysis, we see that nutrition plays an important but nevertheless small part in defining the MP level of a subject ($R^2 = 0.09$ for MPOD volume in linear regression). On the other hand, this shows that subjects with a high consumption of xanthophylls could still have low MPOD values and thus be at greater risk for development or progression of AMD, a hypothesis supported by a number of studies.\textsuperscript{6,31-33} The above findings highlight the possible role of MPOD measuring as a screening method in everyday praxis together with the use of a food frequency questionnaire as part of nutritional counseling for patients, helping to identify those who may be at high risk of developing AMD and encouraging them to adopt dietary habits to increase their macular pigment.
Research concerning macular pigment distribution has yielded interesting results. Distributional patterns described vary from minor flanking peaks or shoulders of macular pigment to central dips or the formation of ring-like structures around a central peak. Using autofluorescence, Sharifzadeh et al depicted MP distribution in three-dimensional patterns and divided it into five distinct categories. This differs from our approach. We divided the spatial profiles according to morphologically observed increasing degree of eccentricity (Figure 2A-E). Furthermore, Sharifzadeh et al did not investigate the relation between these profiles and factors such as age or gender.

Some researchers have sought associations between MP distribution and foveal architecture or pathology. Sharifzadeh et al suggested that pathological changes in the retina and/or the vitreoretinal interface may contribute to the appearance of these ring-shaped MP distributions. Pocock et al demonstrated that the eccentricity in MP distribution is positively correlated with foveal width. Our study does not directly provide data supporting or opposing these findings, but our results demonstrated that the width of the area in which MP is detected (MPOD area) is correlated negatively or not at all with the degree of eccentricity of MPSP.

Kirby et al found that the prevalence of a central dip in MP spatial profile increases with age. Berendschot and van Norren, on the other hand, found no age effect in MP distribution. The present study demonstrated a clear positive correlation between age and degree of eccentricity of the MPSP (Figure 5). Furthermore, MPOD values increase with age (except for MPOD area, which demonstrates a shrinking effect), and MPOD values except for area correlate positively with eccentricity in pigment distribution.

We believe that this evidence may suggest a dynamic redistribution or remodeling of macular pigment that takes place with aging and is affected by the MP quantity parameters as well as other yet unknown factors. In addition, the possible role of different forms of MP distribution in macular diseases

Figure 5. Scatter plot depicting the relationship between macular pigment distribution type and age. Spearman analysis shows a positive correlation (rs = 0.47, P < .0001)
such as AMD could be further investigated. Interestingly, Dietz et al found that ring-like structures in the MPSP appeared to be inversely related with age-related maculopathy.14

The exact mechanisms underlying this phenomenon are unknown, but the role of age-related changes in foveal architecture and vitreoretinal pathology cannot be excluded. Furthermore, the possibility of minor artifacts such as opacities of the usually transparent ocular media influencing the variety of MP distribution patterns documented in this study is plausible. More research in this field is certainly required.

REFERENCES