Macular Pigment and Melanin in Age-Related Maculopathy in a General Population

Tos T. J. M. Berendschot,1 Jacqueline J. M. Willemse-Assink,2,5 Mieke Bastiaanse,1 Paulus T. V. M. de Jong,2,5,4 and Dirk van Norren1,5

PURPOSE. It has been suggested that macular pigment (MP) and melanin may protect against age-related maculopathy (ARM). To check this, MP and melanin optical density were measured in a random population-based sample of subjects 55 years of age or older.

METHODS. Spectral fundus reflectance of the fovea was measured in one eye per subject in a 2.3° detection field with a fundus reflectometer. The sample consisted of 199 men and 236 women. Analysis with a fundus reflectance model yielded individual estimates for the MP and melanin optical density. Diagnosis of ARM was based on grading of standardized fundus transparencies. Eyes were stratified in four exclusive stages of ARM.

RESULTS. MP optical density (at 460 nm) was 0.35 ± 0.15 in eyes without ARM (n = 289) and 0.35 ± 0.16 in eyes at any stage of ARM (n = 146). Melanin optical density (at 500 nm) was 1.18 ± 0.19 in eyes without ARM and 1.20 ± 0.21 in eyes at any stage of ARM. We found no gender differences for either MP or melanin optical density.

CONCLUSIONS. No differences in MP and melanin optical density were found between eyes with and without ARM or between the various ARM stages. (Invest Ophthalmol Vis Sci. 2002;43: 1928–1932)

A ge-related maculopathy (ARM) is a disorder that occurs frequently in older persons.1–4 Its end stage, age-related macular degeneration (AMD), is the leading cause of irreversible vision loss among the older population in Western countries.5 Macular pigment (MP) is concentrated in the central area of the retina along the axons of the cone photoreceptors.6,7 It possibly protects the macular region by its ability to scavenge free radicals.8 MP is composed of the carotenoids lutein and zeaxanthin.9,10 A significant association has been found between MP optical density and lutein concentrations in serum and adipose tissue,11,12 and there are significant differences between men and women.13–15 MP optical density can be changed either by a dietary modification16 or by supplements of lutein.11,17 Cross-sectional studies have resulted in controversial associations: In some studies a high content of the carotenoids lutein and zeaxanthin in the serum or diet resulted in a lower prevalence of AMD,18–21 whereas other studies found no relation with AMD.22 Melanin in the retinal pigment epithelium (RPE) and choroid may also protect the macular region by its antioxidant capability.23 Two reports mentioned a significant association between light iris color and AMD, which was attributed to a possible correlation with melanin.24,25 However, in pooled data from three large eye studies (Beaver Dam Eye Study, Blue Mountains Eye Study, Rotterdam Study, n = 12,486) no association was observed between iris color and AMD.26 Others showed that sensitivity to glare and poor tanning ability are markers of increased risk of AMD.27 This could be due to differences in melanin optical density, although comparisons of AMD prevalence between black and white persons are controversial.28,29 Both MP and melanin may protect the macular region by their capability to attenuate blue light.30,31 thereby decreasing photochemical light damage.20,32 However, epidemiologic evidence to support this assumption is inconclusive. Cumulative ocular exposure to blue light has been associated with an increased prevalence of severe macular degeneration.33 Data from the population-based Beaver Dam Eye Study suggest that exposure to sunlight may be associated with AMD.34 In contrast, in a case–control study there was no association between recreational or occupational exposure to sunlight and AMD35 and in a case–control study, sun exposure was even greater in control subjects than in patients with AMD.36 The purpose of this population-based study was to look for differences in MP or melanin optical density in eyes with no ARM or at different stages of ARM.

METHODS

Subjects

The present study involved a subset of the participants in the Rotterdam Study, a population-based cohort study among residents 55 years of age and older, in a suburb of Rotterdam.3,35 The study was conducted according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Erasmus University Medical School. Written informed consent was obtained from all participants. All participants who visited the research center between March 1999 and July 1999 for interview and medical examination for the were eligible for our study. They can, for our purpose, be seen as a nonselext group. All subjects underwent a full ophthalmic examination that included indirect ophthalmoscopy and stereoscopic photography of both eyes in mydriasis.

Measurement of MP and Melanin Optical Density

Spectral fundus reflectance was measured with the Utrecht Retinal Densitometer.36 A chin rest and temple pads were used to maintain
head position. MP optical density was measured in the right eye, if possible. A 5.8 log troland bleaching light in the densitometer bleached all visual pigments. The illumination field was 2.7° centered at the fovea. Light reflected from the fundus was measured in a detection field of 2.3° centered on the fovea, concentric within the illumination field. A relatively large field width was chosen to improve signal-to-noise ratio in the elderly population. We used a specific optical model of foveal reflection to arrive at individual parameter values of densities of the lens, MP, melanin (i.e., in this analysis the sum of the RPE and choroidal melanin optical density) and blood.37 In short, in this model, the incoming light is assumed to reflect at the inner limiting membrane (ILM), at the discs in the outer segments of the photoreceptors, and at the sclera. The spectral characteristics of the different absorbers within the eye (lens, MP, blood, melanin) were taken from the literature. The optical densities of these absorbers were optimized to fit the measured data at all wavelengths. Also, the reflectance at the ILM and the outer segments of the photoreceptors were optimized. The sclera reflectance was held constant at 50%.38 For more details see Van de Kraats et al.37

The influence of drusen is neglected in this model, which may be wrong in eyes with ARM. Drusen are located at the level of the RPE. Delori and Burns39 showed that the log reflectance is higher with drusen than in the absence of drusen. A rough estimate from their results suggests drusen reflectance without any wavelength dependence. If so, inclusion of drusen would only result in an apparent increase in the reflectance at the discs in the outer segments of the photoreceptors. All other parameters would be similar, including the MP and melanin optical density. We also tried to derive the actual spectral reflectance by including a reflector at the RPE level in the model and adapting its spectral fingerprint such that the change in log reflectance would resemble the result found by Delori and Burns. We found a very slight wavelength dependency. The omission of this wavelength-dependency would result in a maximal overestimate in MP optical density of 0.01.

Grading of ARM

The screening for presence of ARM has been described in detail elsewhere.35 In brief, 35° stereo color transparencies were made, centered on the fovea. The diagnosis of ARM features was based on grading of the transparencies according to the International Classification System.40 Inter- and intragrader agreement on each fundus feature was regularly assessed, and consensus training was initiated when \( k < 0.6 \). Eyes were stratified in four exclusive stages of disease (Table 1), with presumed increased risk of development of AMD in each successive stage.55-41-45 The stage classification was based on the eye in which MP had been measured.

<table>
<thead>
<tr>
<th>Stage of ARM</th>
<th>Criteria</th>
<th>( n )</th>
<th>Age (y)</th>
<th>Male/Female</th>
<th>Macular Pigment</th>
<th>Melanin Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ARM</td>
<td>No features or only drusen ≤63 ( \mu \text{m} )</td>
<td>289</td>
<td>68 ± 5</td>
<td>139/150</td>
<td>0.33 ± 0.15 (0.05-1.20)</td>
<td>1.18 ± 0.19 (0.59-1.94)</td>
</tr>
<tr>
<td>ARM Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>Soft, distinct drusen only</td>
<td>119</td>
<td>69 ± 6</td>
<td>48/71</td>
<td>0.33 ± 0.16 (0.04-0.68)</td>
<td>1.21 ± 0.21 (0.78-2.12)</td>
</tr>
<tr>
<td>1b</td>
<td>Pigmentary irregularities only</td>
<td>8</td>
<td>69 ± 7</td>
<td>4/4</td>
<td>0.27 ± 0.12 (0.04-0.41)</td>
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</tr>
<tr>
<td>2a</td>
<td>Soft, indistinct or reticular drusen</td>
<td>4</td>
<td>74 ± 9</td>
<td>1/3</td>
<td>0.45 ± 0.16 (0.30-0.68)</td>
<td>1.13 ± 0.08 (1.01-1.19)</td>
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<td>2b</td>
<td>Soft, distinct drusen with pigmentary irregularities</td>
<td>13</td>
<td>75 ± 7</td>
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<td>146</td>
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Density of macular pigment was determined at 460 nm and that of melanin at 500 nm, in one eye of 435 subjects, stratified according to the different stages of presumed ascending severity of ARM. All ARM represents pooled measurements in all subjects. Optical density data are expressed as the mean ± SD, with the range in parentheses.

**Results**

In a 4-month period, 449 subjects visited the center, and all were measured. For 11 subjects, photographs for the grading of ARM were missing. One eye had AMD. Because the model analysis of its reflectance may have been unreliable because of scarring of the retina, its data were omitted. In two subjects, measurement of fundus reflectance failed, because of technical difficulties with the setup. The final study group consisted of 435 subjects, 199 men, aged 69 ± 6 years, and 236 women, aged 69 ± 6 years (\( P = 0.39 \)). Because of difficulties in the right eye (e.g., ptosis and amblyopia) in 84 subjects, the left eye was measured instead of the right. These measurements were included in the analyses, because there is a good correlation of MP optical density between both eyes.44 MP (at 460 nm) was 0.32 ± 0.16 in men and 0.34 ± 0.15 in women (\( P = 0.23 \)). Melanin optical density (at 500 nm) was 1.20 ± 0.21 in men and 1.18 ± 0.19 in women (\( P = 0.31 \)). Table 1 shows the gender distribution and means (±SD) for age, MP, and melanin optical density at different stages of ARM. As expected, age differed significantly between the different stages of ARM (\( P < 0.001 \)). Gender distribution (\( P = 0.73 \)) and MP and melanin optical density (0.39 and 0.40, respectively) were similar for the different stages of ARM. Comparison of all pooled ARM cases with those without ARM also resulted in no differences in gender distribution (\( P = 0.17 \), MP (\( P = 0.92 \)), and melanin optical density (\( P = 0.38 \)). MP optical density showed a slight but significant increase with age (Pearson correlation, \( r = 0.15 \), \( P = 0.002 \), \( \beta = 0.0041 \) year), whereas melanin optical density showed a similar decrease (\( r = -0.14 \), \( P = 0.004 \), \( \beta = -0.0049 \) year). Although unlikely, this could influence the association between ARM stage, MP, and melanin optical density. Therefore, we applied a GLM analysis with MP and melanin optical density as dependent variables, age as a covariate, and ARM stage as a factor. We found no increase to a significant effect for ARM stage in the MP analysis (\( P = 0.30 \)) or in the melanin analysis (\( P = 0.42 \)).

To estimate the reliability of the measurements in this population of elderly subjects, fundus reflectance was measured twice in the same eye of 17 random subjects (7 men, aged 67 ± 6 years, and 10 women, aged 68 ± 5 years). The repeat measurements were performed in the same session, and sub-

### Table 1. Single-Pass Optical Density (mean ± SD, range) of Macular Pigment and Melanin

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Discussion

Macular Pigment

We did not find differences in MP optical density between normal eyes and those with different stages of ARM. Although several studies support the hypothesis that MP protects against AMD, there is also evidence against it. Homozygous twins showed 100% concordance of AMD in a study of a select group of twin pairs, although MP optical density is highly variable between homozygous twins. MP possibly protects the macular region by its ability to scavenge free radicals. However, Beatty et al. reviewing the role of oxidative stress in AMD, found no evidence of a causal link. Further, if MP protects against AMD by its ability to filter blue light, a positive association between cumulative ocular exposure to blue light and prevalence of ARM is expected. Epidemiologic evidence to support this is inconclusive.

Differences in MP optical density were observed between donor eyes from subjects with AMD and subjects without AMD. However, these differences may be due to the destruction of the cones and their axons, where MP is normally concentrated, as a result of AMD itself. To tackle this problem, Bone et al. compared differences between the lutein and zeaxanthin content in AMD and control eyes, in different concentric regions centered on the fovea. Although a model that attributes loss of lutein and zeaxanthin to the destructive effects of AMD was less likely, it could also explain their result within the experimental error. To our knowledge, there are no studies of MP optical density in donor eyes with different stages of early ARM.

We found MP optical density to be the same in men and women. Some studies using a smaller test field that was centered on the fovea found significantly lower MP optical density in women than in men. However, others showed no gender effect in a test field. Two studies measured the MP optical density in a test field, centered on the fovea. They found no or only minimal and insignificant gender differences, in line with our results with a test field.

In pooled data from three large eye studies (Beaver Dam Eye Study, Blue Mountains Eye Study, Rotterdam Study, n = 12,486) no gender differences in risk for AMD were found. In a review of the risk for AMD between men and women in all population-based studies, only a few studies demonstrated unequivocally an increased risk for AMD in women. Overall, a small increased risk for AMD was found in women than in men, although correction for age effects was not completely possible.

In our study a slight, but statistically significant, positive age effect on MP optical density was found contrary to another study of 217 subjects, in which a small significant negative age effect was found. This may be due to sample size: the larger the study group, the smaller the differences that are statistically significant. Others found no age effects, which could also be due to cohort or dietary effects. We found large variances in MP optical densities, similar to findings in other studies. Absolute values of MP optical density differ between different measurement techniques, as a result of different field sizes and/or the different weighting of the MP optical density across the measured field.

It has been shown that MP optical density can be increased by lutein supplementation. Therefore, if subjects with ARM used lutein supplements more often than subjects without ARM, possible differences may have been reduced. However, subjects with ARM did not receive the diagnosis during the data collection and did not have symptoms and thus were not encouraged to use any supplements. Moreover, overall use of supplements in the Rotterdam Study was low. The possible use of supplements was recorded in 418 of the 435 subjects in this study. Only 20 (4.8%) used any kind of supplement: 12 without ARM, 5 with stage 1a, 1 with stage 1b, 1 with stage 2a, and 1 with stage 2b. There was no significant difference in use between the different stages (Pearson χ², P = 0.15) or between the use with or without the presence of ARM (Pearson χ², P = 0.47).

The reflectance model for foveal reflection does not include possible reflectance at drusen. Adjusting for drusen reflectance (see the Methods section), we found a maximum overestimation of MP optical density of 0.01. Only the reflectance at the discs in the outer segments of the photoreceptors changed significantly. Further, Delori and Burns found only significant changes in reflectance for drusen if they occupied more than 50% of the sampling area. When drusen occupied less than 50% of the sampling area, they found a small insignificant increase in reflectance. Therefore, we feel that the possible presence of drusen cannot explain the absence of a difference in MP optical density between the different stages of ARM.

In our setup, the coefficient of repeatability for the MP optical density was 0.11 and the mean relative difference between two measurements was 10%. The minimum change that could have been detected depends on the distribution of the MP optical density in the population. Taking the number of participants in our groups (n = 289 for no ARM and n = 146 for ARM) and an MP optical density of 0.35 ± 0.15 for the no ARM group, a minimum change of 15% could have been detected. Thus, our method is accurate enough to determine differences in MP optical density of 30% between control subjects and AMD patients, as found by others. In a former study with the same apparatus, we measured the influence on MP optical density of lutein supplementation and were able to monitor a linear 4-week increase of 5%. In that study, we also used reflectance maps, made with a scanning laser ophthalmoscope, to measure MP optical density. This method provided similar results.
A more definite proof of the influence of MP optical density on ARM may be obtained, by using the present results as baseline data for a longitudinal study and comparing incidence of AMD between eyes with low and high MP optical density.

**Melanin**

We did not observe differences in melanin optical density between the different stages of ARM, in line with recent epidemiologic studies. As mentioned, the evidence that AMD is the result of oxidative damage and thus the hypothesis that melanin may protect the macular region by its antioxidant capability may be questionable. Some studies, however, have shown an increase in the prevalence of AMD in white compared with black subjects. The RPE melanin content is similar between black and white persons, whereas black persons have almost twice the amount of choroidal melanin than do white persons. The spatial distribution of melanin has been measured in different races. The melanin optical density in the RPE was 0.40 ± 0.15 in white subjects and 0.40 ± 0.14 in black subjects (the results for an effective spectral range of 500–600 nm of that study were scaled to match the optical density at 500 nm, as defined in this study). The choroidal melanin optical density was 0.96 ± 0.67 in white subjects and 1.98 ± 1.03 in black subjects (Student’s t-test, \( P = 0.001 \)). In our analysis, the melanin optical density is the sum of the RPE and choroidal melanin optical density. The total melanin optical density of 1.19 ± 0.20 found in this study, compares well with the earlier results in white subjects. In the present study, conducted in a suburb of a city in The Netherlands, we measured melanin optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight optical density in only a few black subjects. The race was recorded of 421 of the 435 subjects in this study. Only eight.

The small negative age effect on melanin optical density found in this study is in line with an earlier study showing a decrease in RPE melanin optical density but no change in choroidal melanin with age. The reflectance at drusen only slightly varied with wavelength at wavelengths more than 600 nm. Because only changes in this wavelength region can modify the melanin optical density, the effect of drusen on model levels of melanin optical density is negligible. One of the strong advantages of the present study was the population-based design. In contrast to clinical-based studies, we had less bias due to referral and selection.

In conclusion, this population-based, cross-sectional study with meticulous grading of the various ARM stages, did not show any differences in MP and melanin optical density between eyes with and without ARM.

**Acknowledgments**

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**References**


