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Interocular agreement in melanin and macular pigment optical density

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Abstract

Macular pigment (MP) and melanin possibly protect the macular area by absorbing blue light and acting as antioxidants. Because little is known about the interocular correlation of melanin, we determined its optical density (MOD) in both eyes of healthy subjects using fundus reflectometry. The measuring method also provided optical densities of MP (MPOD). In addition to evaluating its interocular correspondence we checked its dependency on central retinal thickness as measured with optical coherence tomography (OCT). Spectral fundus reflectance was measured in 69 eyes of 37 healthy participants. Both eyes of 32 subjects (15 males and 17 females, aged 57.9 ± 14.6 years) were used to evaluate interocular correspondences. MPOD data from 35 right eyes of 18 males and 17 females, aged 55.7 ± 15.7 years, was used to evaluate the relation between central retinal thickness and MPOD. MOD was 0.99 ± 0.30 (range: 0.57–2.07) for the left eyes and 1.02 ± 0.28 (range: 0.62–2.07) for the right eyes. The intraclass correlation coefficient (ICC) was 0.89 ($P < 0.001$). MPOD was 0.49 ± 0.19 for the left eyes (range: 0.12–0.81) and 0.47 ± 0.17 (range: 0.14–0.73) for the right eyes. The ICC was 0.91 ($P < 0.001$). Macular retinal thickness (MRT), representing the average macular thickness in the central 1000 μm zone, was 210 ± 28 μm. Foveal retinal thickness (FRT), representing the retinal thickness at the crossing of the 6 radial scan lines on OCT, was 175 ± 34 μm. Pearson's correlation coefficient showed no significant linear association between MRT and MPOD ($r = -0.04$, $P = 0.82$), and between FRT and MPOD ($r = 0.05$, $P = 0.78$). The optical density of melanin showed a high interocular correspondence in healthy white participants. Similar results were found for MPOD. Relative interocular differences of more than 32% in MOD, or more than 34% in MPOD, may point to pathology. No relation between central retinal thickness and MPOD was found.

Keywords: interocular agreement; melanin; macular pigment; reflectometry; retinal thickness; foveal reflection analyzer

1. Introduction

Melanin is present in the retinal pigment epithelium (RPE) and in the choroid. It enhances vision by absorbing straylight and it might also play a minor role in protecting the retina by attenuating harmful short wavelength light. In addition, it might play an antioxidative role (Sarna, 1992). RPE levels of melanin are similar between blacks and whites, but blacks have higher levels of choroidal melanin (Weiter et al., 1986). Macular pigment (MP) is concentrated in the central area of the retina along the axons of the cone photoreceptors (Snodderly et al., 1984a,b). It is entirely of dietary origin. The carotenoids lutein and zeaxanthin have been identified as its major components (Bone et al., 1985). In line with melanin, MP may also protect the macular region against photochemical light damage (Landrum et al., 1997) by acting as a blue light filter, absorbing between 390 and 540 nm (Bone et al., 1992) and scavenging free radicals (Khachik et al., 1997).

Although several studies showed a good interocular correlation of MP or its main components, very little is known about the interocular correlation of melanin. The present study aimed at providing more information on this subject in a healthy white population. Such data may be useful in deciding what level of interocular difference points to pathology in one eye. We used fundus reflectometry to measure the melanin optical density (MOD). Because the optical model with which the data were analyzed also provided macular pigment optical

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density (MPOD) we checked its interocular correspondence too. In addition, we evaluated whether we could also find a positive association between MPOD and central retinal thickness, as shown in a recent study by Liew et al. (2006) The authors postulated that the amount of retinal tissue present may influence the accumulation or storage of MP.

2. Materials and methods

2.1. Participants

Healthy volunteers were recruited among employees of the ophthalmology department of our hospital and via an advertisement in a local newspaper. Persons aged 18 years or older without a known eye disease and with corrected visual acuity of at least 0.8 were eligible to participate.

In total, spectral fundus reflectance was measured in 69 eyes of 37 subjects. The estimation of MOD and MPOD was always based on five single reflectance measurements. Both eyes of 32 subjects (15 males and 17 females) were used to evaluate the interocular correspondence of MOD and MPOD. Mean age was 57.9 ± 14.6 years, ranging from 18.1 to 74.2 years. Reflection data from the right eye of 37 subjects was available for the evaluation of the relation between central retinal thickness and OCT, but OCT data of the right eye was not available for 2 participants (one participant left the study before the OCT scan was performed; the OCT scan of another subject was missing). As a consequence, 35 right eyes of 18 male and 17 female subjects were analyzed (age: 55.7 ± 15.7 years, age range: 18.1–74.2 years). All subjects were Caucasian, except one participant being of Surinam/Portuguese ancestry.

The tenets of the declaration of Helsinki were followed and the study was approved by the local medical ethics committee. Written informed consent was obtained from all participants.

2.2. Measurement of MOD and MPOD

The device used in this study, the Foveal Reflection Analyzer (FRA), is able to measure the foveal spectral reflectance and cone photoreceptor directionality simultaneously in a matter of seconds. A prototype was described by Zagers et al. (2002); the present FRA was used earlier by Berendschot and van Norren (2005).

Briefly, by means of a halogen lamp and a lens system, a light beam was generated forming an entrance pupil of 2.6 × 1.3 mm in the pupil plane. A sharply demarcated light spot of 1.9° diameter was projected on the fovea. Retinal spot intensity was 6.42 log Td. In order to avoid retinal light damage, a maximum retinal illumination time of 15 min had to be taken into account (Health Council of the Netherlands, C.o.o.r, 1993). A heat filter was used (KG3, Schott) to avoid thermal damage. An imaging spectrograph with its entrance slit of 15 × 1 mm conjugate to the pupil plane and below the entrance light beam with a separation of 0.7 mm, was used to capture the light reflected from the central 1.6° of the retinal spot. The image generated by the prism based spectrograph was captured by a CCD camera and covered two dimensions: one directional dimension originating from the 15 mm entrance slit and one spectral dimension (spectral range 400–950 nm). Video observation of pupil plane and retinal plane facilitated proper alignment. A chin rest and temple pads were used to maintain stable head position. The pupils of both eyes were dilated with tropicamide 0.5% and phenylephrine 5%. MOD and MPOD were determined by a full spectral analysis of the reflected light; values were defined at 460 nm (MPOD) and 500 nm (MOD). In short, the incoming light was assumed to reflect at the inner limiting membrane, the disks in the outer segments of photoreceptors and at the sclera. Using known spectral characteristics of the different absorbers in the eye (lens, MP, melanin, blood), the density of the absorbers and the reflectance at the interfaces were optimized to fit the measured data (van de Kraats et al., 1996). The most recent spectral shapes of the lens absorption templates were used for data analysis (van de Kraats and van Norren, submitted for publication). Another change with respect to the original model was the use of a linear blood layer thickness gradient, instead of a homogeneous layer. This thickness gradient varies from 0 to a certain maximum value, found with the model fit procedure. This approach better describes reality in which the path length of light traveling through the blood layer probably depends on the location of incident light (van de Kraats and van Norren, in preparation). Also, participants were not dark adapted prior to the measurements in the current setting. The model did not discriminate between RPE and choroidal melanin. As a consequence, MOD reflected the total amount of both RPE and choroidal melanin. For a more detailed discussion of this technique, see Berendschot et al. (2003) and Berendschot and van Norren (2004).

2.3. Measurement of central retinal thickness

We measured the retinal thickness, indicating the distance between the vitreoretinal interface and the RPE, in the right eye using OCT (Carl Zeiss, Stratus 3000). Central retinal thickness was calculated using the built-in mapping software, using six radial scans (scan length 6 mm) centered on the fovea. Both macular retinal thickness (MRT), representing the average retinal thickness in the central 1000 μm diameter zone, as well as foveal retinal thickness (FRT), representing the retinal thickness at the crossing of the six radial scans, were used in our calculations.

2.4. Statistical analysis

Pearson's correlation coefficient r was computed to evaluate the strength of the interocular linear relationship of MOD and MPOD. In addition we computed an intraclass correlation coefficient (ICC), because besides the strength of the association, this correlation coefficient also takes the interocular agreement into account. A Pearson correlation coefficient was also used to evaluate the strength of the relation between MPOD in the right eye on the one hand, and both MRT and FRT on the other hand.

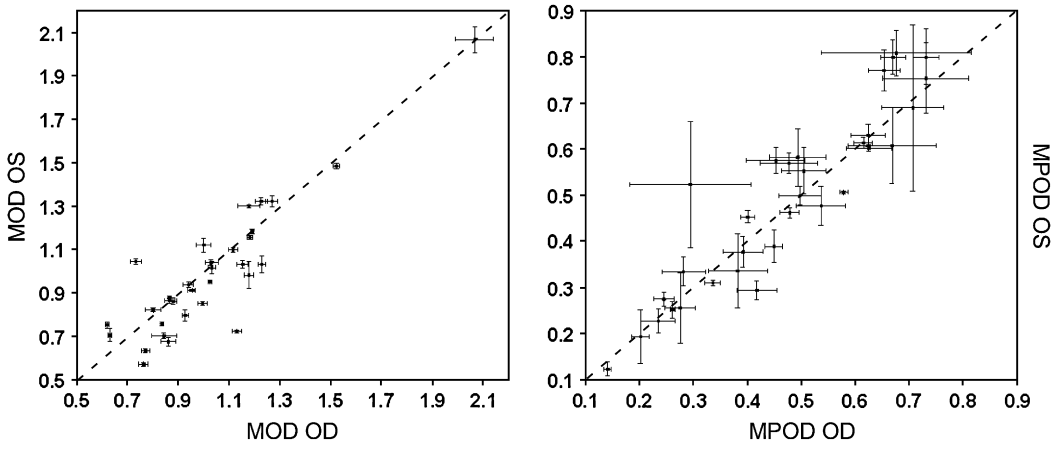

Fig. 1. (Left) Relation of melanin optical density (MOD) between the right eye (OD) and the left eye (OS). Each point represents the mean of 5 single measurements. Pearson's correlation coefficient r was 0.90 ($P < 0.001$). The intraclass correlation coefficient (ICC) was 0.89 ($P < 0.001$). The dashed diagonal line with a slope of 1 represents the line of perfect interocular agreement. The point with the highest MOD value in the right and the left eye, belongs to a 21-year-old healthy woman from Surinam/Portuguese origin. (Right) Relation of macular pigment optical density (MPOD) between both fellow eyes. Pearson's correlation coefficient showed a significant linear relation between MPOD in both eyes ($r = 0.93$, $P < 0.001$). The ICC was 0.91 ($P < 0.001$).

All statistical analyses were performed using SPSS for Windows, release 13.0 (SPSS Inc., Chicago, IL).

3. Results

MOD was 0.99 ± 0.30 (range: 0.57–2.07) for the left eyes and 1.02 ± 0.28 (range: 0.62–2.07) for the right eyes. The interocular MOD data are shown in Fig. 1 (Left).

Pearson's correlation coefficient was 0.90 ($P < 0.001$). The ICC was 0.89 ($P < 0.001$), using the one-way random model. The mean absolute difference in MOD between both fellow-eyes was 0.10 ± 0.09. The mean relative difference in MOD was 0.11 ± 0.11. Thus, 95% of the fellow-eyes differ less than 32% with respect to MOD (mean + 1.96 SD).

MPOD was 0.49 ± 0.19 for the left eyes (range: 0.12–0.81). For the right eyes, MPOD was 0.47 ± 0.17 (range: 0.14–0.73). Fig. 1 (Right) shows the interocular MPOD data. Pearson's correlation coefficient was 0.93 ($P < 0.001$); the ICC was 0.91 ($P < 0.001$). The mean absolute difference in MPOD between both fellow-eyes was 0.06 ± 0.05. The mean relative difference in MPOD was 0.12 ± 0.11. Thus, 95% of the interocular differences in MPOD are less than 34%. The Pearson correlation coefficient showed no significant linear association between MOD and MPOD values, averaged for both fellow eyes: $r = -0.027$, $P = 0.88$.

MRT was 210 ± 28 μm; FRT was 175 ± 34 μm. The relation between MPOD and both MRT and FRT is given in Fig. 2.

The Pearson correlation coefficient showed no significant linear association between MPOD and MRT ($r = -0.04$, $P = 0.82$). Neither was a significant linear association found between MPOD and FRT ($r = 0.05$, $P = 0.78$). As expected on anatomical grounds, no significant linear association was found between MOD and either MRT ($r = -0.26$, $P = 0.13$) or FRT ($r = -0.28$, $P = 0.10$).

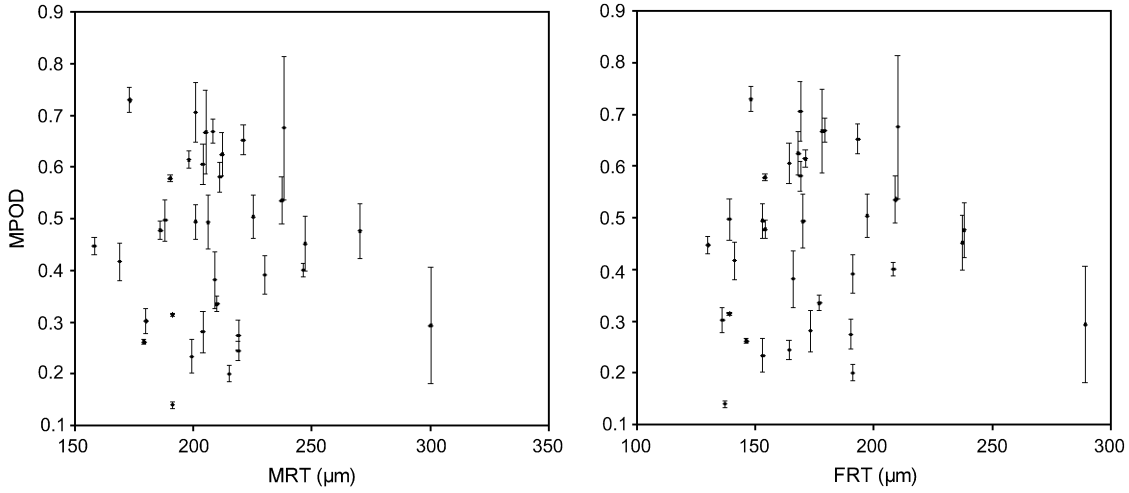

Fig. 2. (Left) Relation of macular retinal thickness (MRT) with macular pigment optical density (MPOD) in the right eye (OD). Each point represents the average of 5 single measurements. Pearson's correlation coefficient showed no significant linear relation between MRT and MPOD ($r = -0.04$, $P = 0.82$). (Right) Relation of foveal retinal thickness (FRT) with MPOD in the right eye (OD), also showing no significant linear relation ($r = 0.05$, $P = 0.78$).

4. Discussion

The results showed that the optical densities of melanin and MP, obtained with fundus reflectometry, do not differ significantly between the two fellow-eyes in healthy white subjects. For melanin, this is in line with an earlier in vitro study (Schmidt and Peisch, 1986). Total RPE melanin concentration was measured biochemically in both (normal) eyes of 12 human donors. In each case, the two values for melanin concentrations were within 20% of each other. In the current study, melanin values of both fellow eyes were within 32% of each other. This higher percentage is probably related to the fact that the technique used in this study also took into account the more individually determined choroidal melanin, in addition to RPE melanin. In a recent study, Keilhauer and Delori (2006) used near infrared autofluorescence imaging for the detection of RPE- and choroidal melanin. They found no significant interocular differences in the ratio of foveal to parafoveal autofluorescence ($n = 36$, paired $t = -1.0$, $P = 0.3$) and the correlation between eyes was significant ($r = +0.55$, $P = 0.001$). For MP or its main components, several studies have also reported a good interocular correlation (Handelman et al., 1991; Hammond and Fuld, 1992; Beatty et al., 2001; Gellermann et al., 2002; Davies and Morland, 2004; Neelam et al., 2005; Liew et al., 2006), in line with our findings.

The present data open the possibility to check whether pathology in one eye has led to a significant change in either parameter. A difference of more than 32% in MOD between the eyes, as measured with the present technique, may point to pathology in either eye. For example, central RPE changes in early stage AMD may lead to an increase as well to a decrease of central MOD. On the other hand, a choroidal melanoma located underneath the foveal retina probably leads to a higher central MOD in the diseased eye when compared to the fellow eye. Although probably of less clinical importance, this is 34% for MPOD.

Liew et al. (2006) recently reported a significant and positive relationship of MPOD with central retinal thickness as measured by OCT in about 300 eyes. The observed relationship ($r \sim 0.30$) was independent of the technique used to measure MPOD (heterochromatic flicker photometry and 2-wavelength autofluorescence). Although we used fewer subjects and did not correct for factors that might have influenced MPOD, our results emphasize that the anatomical factor plays at most a very weak role in setting the density of macular pigment.

In the optical model utilized in this paper a light-absorbing melanin layer (RPE + choroidal melanin) is placed before an absorbing blood layer representing the choriocapillaris and the choroid. When looking for covariance between model estimates, we found a linear inverse relation between the OD of the blood layer and MOD, averaged for both eyes (slope $= -0.001$; $P = 0.02$; $r^2 = 0.18$). In other words, high MOD estimates correlate with low blood OD estimates. This is probably the result of the model being not advanced enough regarding deep blood layers. In line with the absence of an a priori rationale we found no significant linear association between MOD and MPOD. We also found no significant covariance between MOD and any other model parameters used. The same holds for MPOD.

In conclusion, we provided firm evidence regarding a strong interocular association of MOD in healthy white people. The same holds for the earlier found interocular association of macular pigment. In addition, this study emphasizes the possibility to use fundus reflectometry together with a model fit, to obtain an in vivo assessment of MOD. Interocular differences more than 32% in MOD or 34% in MPOD, may point to pathology. Only very recently autofluorescence was proposed as another means to monitor melanin. Central retinal thickness assessed with OCT proved not very valuable as a predictor of MPOD.

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