Macular Pigment Optical Density Assessment in Monozygotic and Dizygotic Twins

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Introduction. Macular pigment (MP) acts as a pre-receptor filter that selectively absorbs short wavelengths [1]. A low density of macular pigment may represent a risk factor for age-related macular degeneration (AMD) by permitting greater blue light damage [2]. MPOD is a measurement of the attenuation of blue light by macular pigment and is linearly related to the amount (concentration×pathlength×area) of lutein and zeaxanthin in the macula if integrated over the region where macular pigment is deposited [3].

Woo and Lee found that the differences in macular pigmentation between Europeans and Asians significantly influenced the results of the F-M 100 hue test [4]. MPOD in blacks, for example, has been reported to be 41% lower than that in whites [5], although another study did not find such a difference [6]. However, others authors also pointed out that the difference was notable between Asians and brown-eyed Europeans [7].

The level of macular pigments varies among different populations [7]. It should be noted, however, that comparison of MPOD levels among studies are difficult or even impossible because different studies may employ different methodologies or study protocols.

Twin studies are important because they allow us to estimate the overall influence of genes. Twin method assumes that DZ twins are influenced by largely similar environmental differences as MZ twins, but MZ twins share the same genes whereas DZ twins on average share only half their genes [8].

The aim of our research is to determine macular pigment optical density (MPOD) within pairs of MZ and DZ twins.

Methods. In this research, visual acuity as well as the transparency of the cornea and lens, and the fundus was investigated in the patients. Biomicroscopy was performed in order to assess the corneal and lenticular transparency. Non-corrected and the best-corrected visual acuity (measured in decimals from 0.1 to 1.0) was evaluated using Landolt's rings (C optotypes) by Snellen test types at a 5 meter distance from the chart. The lens was evaluated by biomicroscopy. The lens was examined using a slit-lamp, positioning the illumination source at a 45 degree angle and the light beam being set to 2 mm width. Pupils of the

subjects were dilated with tropicamide 1%. After dilation of the pupils, funduscopy was performed with an ophthalmoscope of the direct monocular type and the slit-lamp, using a double aspheric lens of +78 dioptres. Stereoscopic colour fundus photographs of the macula were obtained: centered at 45º to the fovea for a detailed macula analysis with Visucam NM Digital camera (Carl Zeiss Meditec AG, Germany).

MPOD was measured centered at 30° to the fovea. The optional macular pigment density module for the Visucam 500 used the reflectance of a single 460-nm wavelength based on a single blue-reflection fundus image to determine MPOD and its spatial distribution. A shading correction is used that approximates the reflectance of the fundus in absence of MP. It is based on a three-dimensional parabolic function automatically fitted to fundus reflectance at peripheral locations. The subject was positioned in front of the fundus camera and instructed to look at a target inside. The fundus was illuminated by a monochromatic blue light. Four MPOD parameters were automatically calculated: maximum optical density (MPOD measured at the peak); mean OD (mean MPOD within the measurement area); area (area where macular pigment could be detected); and volume (sum of all optical densities, as recommended by the manufacturer), but only the mean optical density was included in our research with hypothesis that if the mean correlates with F-M 100 hue test, then the other parameters can be included.

Statistical analysis was performed using the computer program SPSS / W 19.0 (*Social sciences statistical package program for Windows, Inc., Chicago, Illinois, USA*). The data are presented as real numbers (percent), the average values and standard deviations (SD). T test and the Mann-Whitney U test were used for the comparison of the two groups. A statistically significant difference was considered if $P<0.05$.

Table 1. Demographic characteristics of the study population

Characteristic	**Results**	
	MZ twins	**DZ twins**
Men, n (%)	8 (26.7)	4 (28.6)
Women, n (%)	22 (73.3)	10 (71.4)
Age, (min./ max. median)	25-54 (31)	19-59 (38)
Macular pigment optical density		
(min./ max. median)	0.071- 0.157 (0.102)	0.080-0.132 (0.104)
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Macular pigment optical density was evaluated in MZ and DZ, there were no statistical significant difference between zigosity, $p=0.541$. Also, macular pigment optical density was compared between women and men, there were no statistical difference between spreads across gender, $p = 0.312$

But, there was statistically significant difference between the right and the left eye MPOD values, p=0.015. Also the results did not reveal any difference according to age, p=0.559. (Table 2).

Table 2. Results of macular pigment optical density (MPOD)

Parameter	MPOD min/max median	
	MZ twins	**DZ twins**
Males	0.075-0.125 (0.102)	0.090-0.118 (0.104)
Females	0.071-0.157 (0.103)	0.080-0.132 (0.108)
Right eye	0.071-0.146 (0.101)	0.086-0.131 (0.107)
Left eye	0.079-0.157 (0.103)	0.08-0.132 (0.101)
40< years	0.795-0.1300 (0.1015)	0.084-0.1125 (0.100)
40≥ years	0.091-0.1515 (0.1035)	0.101-0.1275 (0.1159)

Discussion. Our results revealed, there were no statistical significant difference between zigosity between gender and age.

There are only few studies analysing MPOD in twins [Hammond et Liew]. Hammond BR Jr study revealed that macular pigment is not completely determined genetically and allows the possibility that macular pigment density may be modified for the protective purposes. This data suggest that dietary fat, iron, and fiber may influence macular pigment levels (perhaps through their influence on carotenoid metabolism. Other study investigated one hundred fifty twin pairs (76 monozygotic [MZ] and 74 dizygotic [DZ]), aged 18 to 50 years. MP optical density was measured psychophysically with heterochromatic flicker photometry (HFP) and also with an imaging method involving fundus autofluorescence (AF). This twin study demonstrates that genetic background is an important determinant of MP optical density, reflected in heritability estimates of 0.67 and 0.85 for HFP and AF measures, respectively.

The yellow MP is mainly located in the ganglion cell layers and inner plexiform layers of the retina [9]. Typically, the concentration of the MP is maximal at, or near, the fovea and rapidly decreases with eccentricity [10-12]. Because of its pre-receptorial location, MP is thought to shield the retina from deleterious effects of high-energy blue light (λ ~320 to 450 nm), by partly absorbing it [13-15]. Macular pigments act as a pre-receptor filter and are believed to contribute a variety of potentially beneficial properties for vision, including improvement of spatial vision and contrast enhancement [16], increased photopic increment sensitivity [17], reduced glare sensitivity in some studies [18]. The role of retinal carotenoids lutein and (meso-)zeaxanthin, together forming the macular pigment (MP) in the human retina, has been a topic of interest in ophthalmologic research for many years [19-22].

Macular pigments are mainly made up of two oxycarotenoids, lutein and zeaxanthin. Many factors are known to influence MPOD: age, gender, obesity, smoking and genes involved in the transportation of lutein along the lipid pathway [23]. Results of our investigated twins showed no significant

associations between gender, age, zigosity. But it was detected significant differences between left and right eyes in monozygotic and dyzigotic twins.

Humans cannot synthesize MPs and dietary intake – i.e., fruits, vegetables and egg yolk – is the only source for the body [24]. As it functions as an anti-oxidant, MP may protect the retina by scavenging of free radicals formed by oxidative stress [25-27].Consequently, MP might protect against degenerative eye diseases, such as age-related macular degeneration. Accurate assessment of the amount of MP, expressed as MP optical density, is therefore necessary to investigate the role of carotenoids and their assumed protective functions. High repeatability and reliability are especially important to monitor patients in studies investigating the influence of diet and/or nutritional (lutein and (meso)zeaxanthin) supplements, or disease processes on MPOD.

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The purpose of the study. To determine macular pigment optical density (MPOD) within pairs of monozygotic (MZ) twins and dizygotic (DZ) twins.

Thirty MZ twins and fourteen DZ opthalmologically healthy twins were tested. MZ and DZ twins were matched by age and gender. The optional macular pigment density module for the Visucam 500 used the reflectance of a single 460-nm wavelength based on a single blue-reflection fundus image to determine MPOD and its spatial distribution. The median of macular pigment optical density were 0.102 for MZ twins vs 0.104 for DZ twins, respectively, p=0.541.

Our results revealed that there were statistically significant difference between left and right eyes in monozygotic and dyzigotic twins.