(RESEARCH ARTICLE)

Macular pigment optical density measurement by heterochromatic flicker photometry: Challenging a basic premise

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Abstract

Measurement of macular pigment optical density (MPOD) in the human eye is important due to the protection it provides against certain eye diseases such as age-related macular degeneration. A commonly used psychophysical technique is heterochromatic flicker photometry (HFP). This technique assumes a uniformity in the retinal spectral sensitivity from the central fovea to the surrounding parafovea, areas of the retina employed in HFP. We show here that the MPOD spectrum obtained by HFP is characteristic of the carotenoids of which the macular pigment is composed, but only in the blue part of the spectrum. At longer wavelengths, in the red part of the spectrum, the measured MPOD was found to rise with increasing wavelength. We propose that this artefact is due to different contributions to retinal spectral sensitivity in the fovea and parafovea from long- (LWS) and medium-wavelength-sensitive (MWS) cones. By adjusting the LWS/MWS ratio, we could calculate potential retinal spectral sensitivities for the fovea and parafovea that would account for the apparent MPOD in the red part of the spectrum. An LWS/MWS ratio of 4.15 in the parafovea, relative to the fovea, provided the necessary correction, and had the additional effect of providing an increase in the measured peak MPOD at 460 nm. Caution is therefore warranted when using HFP to measure only the peak MPOD, as is commonly the case.

Keywords: Macular Pigment; Heterochromatic Flicker Photometry; Retina; Fovea; Spectral Sensitivity

1. Introduction

Macular pigment is a characteristic of human and other primate retinas, and its concentration at the center of the retina is why this area is referred to as the macula (or macula lutea, meaning yellow spot). This critically important part of the retina, where the density of cones increases dramatically, is responsible for our ability to discern fine detail, i.e. where our visual acuity is highest. It is also the area that deteriorates in age-related macular degeneration (AMD). The macular pigment is comprised of three carotenoids, lutein and zeaxanthin which we obtain through our diet, and meso-zeaxanthin which is not normally of dietary origin, but is believed to be converted from lutein in the retina [1,2]. All three carotenoids absorb light in the blue region of the visible spectrum, from about 400 to 520 nm, with peak absorption at about 460 nm [3]. At wavelengths longer than 520 nm, they are completely transparent [4]. Interest in the macular pigment centers on its ability to protect the central retina from the potentially damaging effects of excessive blue light. Progression of AMD has been found to be lower in patients having higher levels of lutein and zeaxanthin in their diet [5]. Protection is two-fold. Firstly, the pigment is found in the cone axon layer, or "Henle fiber" layer, which is anterior to the cones themselves [6]. On average, the pigment blocks about 40% of incident blue light which might otherwise damage the cone cells through photooxidation [7]. Secondly, all three carotenoids are anti-oxidants and, as such, are capable of quenching reactive oxygen species such as singlet oxygen [8]. Because of these important properties, knowledge of an individual's macular pigment optical density (MPOD) is desirable so that dietary recommendations can be made. For example, a person with a low MPOD could be advised to eat more green, leafy

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vegetables, which are rich in lutein, or to take a dietary supplement that contains the carotenoids. Both options are known to increase MPOD.

The most commonly used method of measuring a person's MPOD is heterochromatic flicker photometry (HFP) [9,10]. A small circular visual stimulus that subtends a visual angle of 1 to 2 degrees is presented to the subject. To determine the peak MPOD, the stimulus alternates between a test wavelength of 460 nm and, for example, a reference wavelength of 540 nm (zero MPOD), at a frequency of around 20 Hz. The stimulus appears turquoise in color due to additive color mixing and, in addition, appears to flicker unless the luminances of the blue (460 nm) and green (540 nm) components are equalized. The subject's task is to adjust the blue luminance until the stimulus appears steady with no flicker. Typically the frequency is fine-tuned for each subject so that the range of luminances for no flicker is very small. The test is divided into two parts. Initially the subjects are instructed to direct their gaze at the center of the stimulus while making the luminance adjustment. The stimulus is imaged in the central, foveal region of the retina. After several repeat measurements, the subjects direct their gaze to a small fixation mark, typically 8° to the side of the stimulus, and again adjust the blue luminance to minimize flicker. The stimulus is now imaged in the peripheral retina (parafovea) where there is negligible macular pigment. Consequently a lower blue luminance is required to minimize flicker since it is no longer partially blocked by the macular pigment. The luminance of the green component should be the same when imaged in the foveal and parafoveal regions of the retina, hence we refer to it as the reference wavelength.

We can show that the MPOD is given by the log ratio of intensity settings for the central (fovea) and peripheral (parafovea) measurements:

$$MPOD = \log_{10}\left(\frac{I_{FOVEA}}{I_{PARAFOVEA}}\right)\dots\dots\dots\dots(1)$$

In a more sophisticated version of the procedure, the test wavelength can be varied over a range of wavelengths and the complete MPOD spectrum generated [3].

In principle, the reference wavelength could have any value from about 530 to 700 nm since the macular pigment has essentially zero optical density in this range [4]. We have discovered, however, an apparent MPOD that increases with wavelength in the red region of the spectrum. Here we present an alternative explanation and examine its impact on the actual MPOD spectrum.

2. Materials and Methods

The flicker photometer used in these experiments was a modified version of that described by Landrum et al [9]. Test wavelengths at 10 nm or 20 nm intervals were obtained using a set of interference filters with a half-width of 7 nm in conjunction with a 75 W xenon arc lamp as the source. The 10 nm interval was used in the 410 to 500 nm range, and the 20 nm interval was used in the 500 to 680 nm range. The reference wavelength was obtained using a 540 nm interference filter (half width 7 nm) in conjunction with the same source. A rotating semicircular mirror allowed the subject to view the test and reference lights in an alternating manner at an adjustable frequency. The optical system provided Maxwellian view with the stimulus being limited to 1.5° diameter by means of a screen with a circular aperture. The front of the screen was illuminated with white light of essentially the same luminance as the stimulus. The purpose was to maintain photopic conditions (cone mediated) for the retina. Cross-hairs facilitated central viewing while a small red LED provided a fixation point at 8° eccentricity for parafoveal viewing. The luminance of the test wavelength was adjustable by the subject using a calibrated neutral density wedge.

Subjects in the study signed an informed consent form approved by the Institutional Review Board at Florida International University. The complete MPOD spectrum was obtained for the right eye of a single subject, subject 6. For each test wavelength, the subject sought to minimize flicker by adjustment of the wedge while looking first at the center of the cross-hairs (foveal measurement) and then at the eccentric fixation point (parafoveal measurement). Five repeat measurements were taken for each and averaged. From equation 1, we can show that the MPOD is just the difference between the optical densities, D, of the wedge,

$$MPOD = D_{PARAFOVEA} - D_{FOVEA}$$

Because of the discovery of an apparent and unexpected MPOD in the red part of the spectrum, five additional subjects were recruited but MPOD measurements were made at 620 nm only in both eyes. Additionally, measurements were made for the left eye of subject 6.

3. Results and Discussion

The MPOD spectrum obtained for subject 6 is shown in Fig. 1. For wavelengths below approximately 580 nm, the spectrum looks normal and resembles spectra of the carotenoids, obtained by spectrophotometry, that peak at around 460 nm [4]. The apparent MPOD at 620 nm in the left and right eyes of all six subjects is shown in Table 1. Apart from subject 1, all other subjects had apparent MPODs at this wavelength that were significantly greater than zero, and this occurred in both eyes.
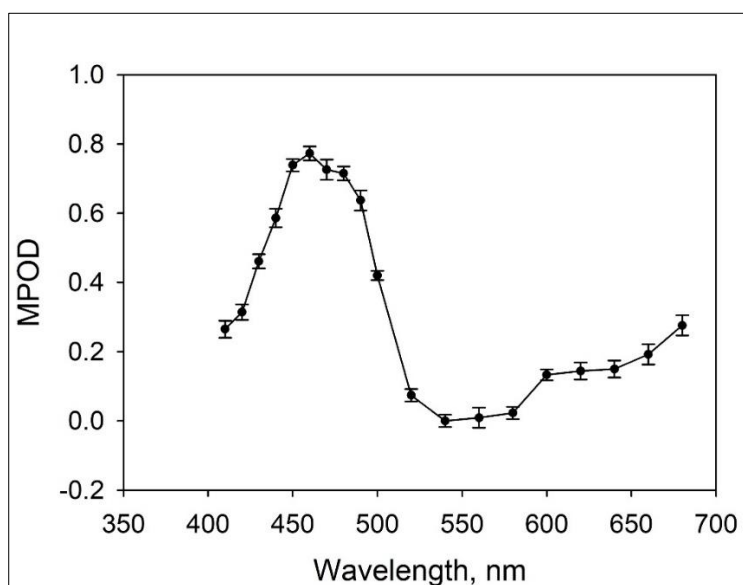

Figure 1 Macular pigment optical density (MPOD) spectrum obtained by heterochromatic flicker photometry for subject #6

Table 1 Apparent MPOD ± SEM measured at 620 nm in the left and right eyes of six subjects. These values are the averages of five foveal and five parafoveal measurements

Subject #	Left Eye	Right eye
1	-0.029 ± 0.023	0.017 ± 0.025
2	0.037 ± 0.014	0.084 ± 0.013
3	0.093 ± 0.022	0.188 ± 0.020
4	0.147 ± 0.031	0.135 ± 0.018
5	0.125 ± 0.033	0.209 ± 0.017
6	0.278 ± 0.016	0.270 ± 0.008

Analysis of pigments extracted from the human retina reveals the presence of lutein, zeaxanthin and meso-zeaxanthin with absorbances occurring in the blue part of the visible spectrum ($\sim$ 400 to 520 nm) only [11]. From 520 to 700 nm, these carotenoids have zero absorbance [4]. Now heterochromatic flicker photometry is based on the premise that the spectral sensitivity of the cone mechanisms is the same in the fovea and parafovea, and that measured differences in sensitivity are due to a pre-receptor absorbing pigment that differentially affects the fovea and parafovea. The apparent MPOD at the longer wavelengths cannot be due to any of the carotenoids, and no other pre-receptor candidate pigment has been identified in the retina that could explain this effect. It is quite possible that the effect could be an artifact resulting from different cone spectral sensitivities in the fovea and parafovea. For example, if the ratio of long-wavelength-sensitive (LWS) cones to medium-wavelength-sensitive (MWS) cones is higher in the parafovea compared

with the fovea, then the fovea would be less sensitive than the parafovea to the longer wavelengths [12]. This would indeed mimic the effects of a long-wavelength-absorbing pigment in the fovea.

Using the detailed data and modeling provided by Stockman and Sharpe [13], it is a straightforward exercise to construct photopic luminosity functions (i.e. retinal spectral sensitivity functions) based on different contributions from LWS and MWS cones. It should be noted that short-wavelength-sensitive (SWS) cones contribute little, if any, to these luminosity functions. For different LWS/MWS ratios, we constructed the corresponding luminosity functions. Since our hypothesis was that these ratios might be higher in the parafovea than in the fovea, we then calculated a spectral function obtained by dividing a range of parafoveal functions with different LWS/MWS ratios by a foveal function with, arbitrarily, an LWS/MWS ratio of 1. This function, plotted as a log, would represent the apparent MPOD spectrum for a subject having no macular pigment. The results are shown in Fig.2. As expected, the curves for the different LWS/MWS ratios in the parafovea, indicate an apparent MPOD rising in the red part of the spectrum and consistent with Fig. 1. They also reveal a negative apparent MPOD from 420 to 550 nm, the region where macular pigment absorbs.
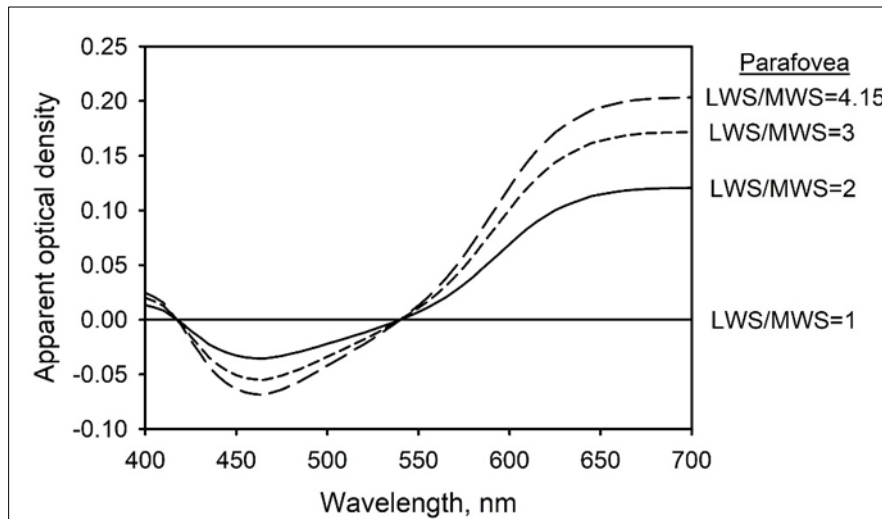

Figure 2 Apparent optical density spectra for hypothetical subjects with no macular pigment. The curves are based on an LWS/MWS cone ratio of 1 in the fovea, and various other ratios in the parafovea
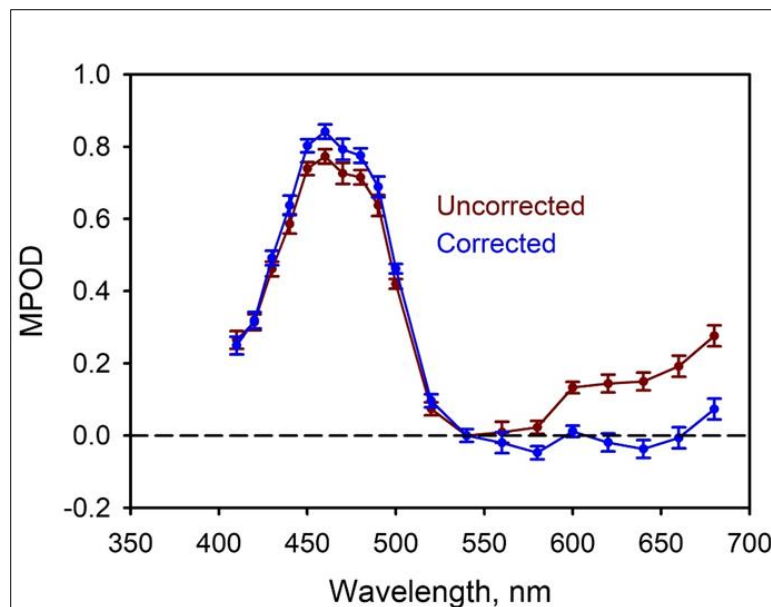

Figure 3 MPOD spectrum for subject #6 showing the measured curve (Fig. 1) and the corrected curve based on an LWS/MWS cone ratio of 4.15 in the parafovea. (See Fig. 2)

The data in Fig. 2 were then used to provide a correction for the MPOD spectrum shown in Fig. 1. To do so, the LWS/MWS ratio in the parafovea was adjusted to obtain a least squares fit between the curves of Figs. 1 and 2 in the wavelength range 540 to 680 nm. The corresponding LWS/MWS ratio that provided the best fit was found to be 4.15 and is indicated in one of the curves of Fig. 2. Using this curve as a correction curve, the corrected MPOD spectrum could be calculated. It is shown, together with the uncorrected spectrum (Fig. 1) in Fig. 3 and shows an MPOD that does not differ significantly from zero above 540 nm, consistent with the carotenoid spectra obtained by spectrophotometry. It also shows that the peak MPOD at 460 nm is adjusted upwards.

We also used the data from Fig. 2 to determine the correction factor to apply to subjects' peak MPOD at 460 nm based on their apparent MPOD at 620 nm. These corrections, which would be added to the subject's apparent MPOD at 460 nm, are shown in Fig. 4, and indicate a linear relationship.
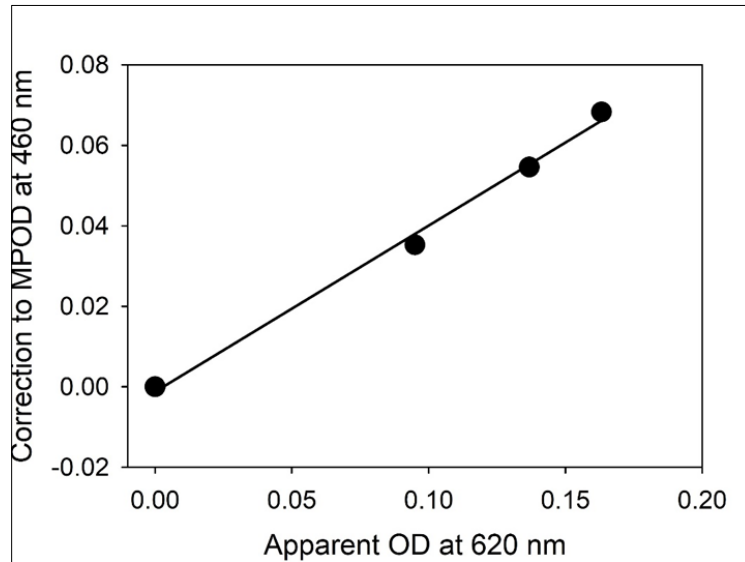

Figure 4 Corrections that should be applied to the peak MPOD at 460 nm based on the apparent OD measured at 620 nm.

4. Conclusion

The premise of heterochromatic flicker photometry that, absent the macular pigment, the spectral sensitivity of the retina is the same in the foveal and parafoveal regions, is challenged by the data presented here. It would appear that retinal sensitivity to the red part of the visible spectrum is higher in the parafovea than in the fovea, and that this leads to an underestimation of the measured, peak MPOD at 460 nm. Caution is therefore warranted when using heterochromatic flicker photometry for MPOD measurements. Other physical methods, such as resonance Raman spectrometry [14], retinal reflectometry [15,16,17] or autofluorescence spectrometry [18], which do not involve the retina as the detector, might have the potential for more reliable measurements.

Compliance with ethical standards

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Statement of informed consent

Informed consent was obtained from all individual participants and approved by the Institutional Review Board at Florida international University

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