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Concordance of macular pigment measurements obtained using customized heterochromatic flicker photometry, dual-wavelength autofluorescence, and single-wavelength reflectance

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ABSTRACT

This study compares *in vivo* measurements of macular pigment (MP) obtained using customized heterochromatic flicker photometry (cHFP; Macular Metrics Densitometer™), dual-wavelength fundus autofluorescence (Heidelberg Spectralis® HRA + OCT MultiColor) and single-wavelength fundus reflectance (Zeiss Visucam® 200). MP was measured in one eye of 62 subjects on each device. Data from 49 subjects (79%) was suitable for analysis. Agreement between the Densitometer and Spectralis was investigated at various eccentricities using a variety of quantitative and graphical methods, including: Pearson correlation coefficient to measure degree of scatter (precision), accuracy coefficient, concordance correlation coefficient (ccc), paired *t*-test, scatter and Bland–Altman plots. The relationship between max MP from the Visucam and central MP from the Spectralis and Densitometer was investigated using regression methods. Agreement was strong between the Densitometer and Spectralis at all central eccentricities (e.g. at 0.25° eccentricity: accuracy = 0.97, precision = 0.90, ccc = 0.87). Regression analysis showed a very weak relationship between the Visucam and Densitometer (e.g. Visucam max on Densitometer central MP: $R^2 = 0.008$, $p = 0.843$). Regression analysis also demonstrated a weak relationship between MP measured by the Spectralis and Visucam (e.g. Visucam max on Spectralis central MP: $R^2 = 0.047$, $p = 0.348$). MP values obtained using the Heidelberg Spectralis are comparable to MP values obtained using the Densitometer. In contrast, MP values obtained using the Zeiss Visucam are not comparable with either the Densitometer or the Spectralis MP measuring devices. Taking cHFP as the current standard to which other MP measuring devices should be compared, the Spectralis is suitable for use in a clinical and research setting, whereas the Visucam is not.

1. Introduction

Macular pigment (MP) is composed of the yellow carotenoid pigments lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (MZ). MP is found at the macula, the specialized part of the retina that mediates fine central and color vision (Hirsch and Curcio, 1989). Its unique anatomic location (Snodderly et al., 1984), short-wavelength (blue) light filtering properties (Bone et al., 1992), and antioxidant properties (Li et al., 2010; Sujak et al., 1999; Wrona et al., 2004), make this pigment important for visual function.

Indeed, in the non-diseased retina (normal subjects), MP has been shown to enhance visual function by reducing the effects of light scatter (thereby reducing glare disability) (Hammond, et al., 2013; Loughman et al., 2012; Stringham et al., 2011; Stringham and Hammond, 2007, 2008; Yao et al., 2013) and chromatic aberration (thereby optimizing contrast sensitivity) (Hammond et al., 2013; Loughman et al., 2010a, 2012; Nolan et al., 2011; Renzi and Hammond, 2010; Richer et al., 2011; Sasamoto et al., 2011; Yao et al., 2013), via its light-filtering (optical) properties (Hammond and Fletcher, 2012; Loughman et al., 2010b; Wooten and Hammond, 2002). Moreover, MP is also postulated to protect against age-related macular disease, particularly age-related macular degeneration (AMD) (Gale et al., 2003; Snodderly, 1995), the developed world's leading cause of age-related blindness (Bressler, 2004; Resnikoff et al., 2004). This putative protection is likely due to the pigment's optical and antioxidant properties (Sabour-Pickett et al., 2011). Of interest, it has been shown that established risk factors for AMD (i.e. age, family history of disease and cigarette

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smoking) (Beatty et al., 2001; Hammond et al., 1996; Kirby et al., 2010; Nolan et al., 2007a) are associated with low levels of MP. However, carotenoid supplementation studies have demonstrated that serum carotenoid concentrations and MP optical density (MPOD) can be increased through dietary modification (Hammond et al., 1997) or supplementation (Bone et al., 2003; Bone and Landrum, 2010; Garcia-Layana et al., 2013; Huang et al., 2013; Koh et al., 2004; Landrum et al., 2012; Murray et al., 2013; Nolan et al., 2011; Richer et al., 2007; Stringham and Hammond, 2008; Tanito et al., 2012; Weigert et al., 2011; Yao et al., 2013), with good results achieved when the formulation used contains all three of the macular carotenoids (L, Z and MZ) (Bone et al., 2007; Connolly et al., 2011; Loughman et al., 2012; Meagher et al., 2012; Nolan et al., 2012).

The typical profile of MP has a central peak, which decreases in concentration with retinal eccentricity (similar to that of a peaked mountain, e.g. Mount Everest). In addition, atypical spatial profiles of MP, containing "ringlike" structures, secondary peaks or plateaus also exist (Berendschot and van Norren, 2006; Delori et al., 2006; Kirby et al., 2009). Little investigation has been done involving MP spatial profiles; however, it has recently been reported that about 12% of the population has an atypical MP profile, characterized by a central plateau or central dip in the pigment profile (e.g. Mount Kilimanjaro). Of note, such atypical central dips have been found to be more common in subjects at increased risk of AMD (Kirby et al., 2010); but it has been recently shown that a central dip in MP's spatial profile can be normalized following supplementation with a formulation containing the centrally dominant macular carotenoid, MZ (Nolan et al., 2012).

Given the importance of MP for vision, and its potential role in preventing and/or reducing risk of AMD development and/or its progression, there is a clear need to measure this pigment with accuracy *in vivo*. Moreover, it is important to be able to measure patient response to supplement formulations containing the macular carotenoids at the target tissue (i.e. the macula).

There are a variety of methods currently in use that claim to measure MPOD. However, researchers have been debating the advantages and limitations of these techniques for over 20 years (Bernstein and Gellermann, 2003; Hammond et al., 2005; Hammond and Wooten, 2006). These methods are divided into psychophysical (sometimes referred to as subjective) and physical (sometimes referred to as objective). The psychophysical techniques available include color matching (Davies and Morland, 2002), motion photometry (Moreland, 2004), heterochromatic flicker photometry (Bone and Landrum, 2004), and customized heterochromatic flicker photometry (cHFP) (Stringham et al., 2008). Of these psychophysical techniques, HFP and cHFP are the most widely used. With HFP, the subject is required to make isoluminance matches between two flickering lights: a green light (not absorbed by MP) and a blue light (maximally absorbed by MP). The log ratio of the amount of blue light absorbed centrally, where MP peaks, to that absorbed at a peripheral retinal locus (the reference point), where MP is assumed to be zero, gives a measure of the subject's MPOD. Customized HFP optimizes the HFP technique by customizing the procedure for each subject (see below). Importantly, HFP has been validated by measuring its absorption spectrum *in vivo* and comparing it to the *in vitro* spectral absorption curve of the macular carotenoids (Bone et al., 1992; Stringham et al., 2008; Wooten and Hammond, 2005), and it is therefore our view that cHFP, an optimized form of HFP, represents a reference standard to which other MP measuring techniques should be compared. Validation of MP measurement techniques has been the subject of lively debate (Gellermann and Bernstein, 2006; Hammond et al., 2005).

Physical techniques currently used for measuring MP include resonance Raman spectroscopy (Bernstein et al., 1998, 2002), fundus reflectance (Berendschot and van Norren, 2004), and fundus autofluorescence (Delori, 2004). However, none of these physical techniques have yet been properly validated (Hammond et al., 2005).

Fundus autofluorescence (AF) uses a confocal scanning laser ophthalmoscope (cSLO) (Delori et al., 2011) or fundus camera (Spaide, 2003). AF exploits the fluorescent properties of lipofuscin present in the retinal pigment epithelium (RPE) (Sparrow, 2007). RPE lipofuscin is a fluorophore that accumulates over time from the phagocytosis of photoreceptor outer segments. Lipofuscin is excited *in vivo* between 400 and 590 nm (peak excitation at 490–510 nm) and emits AF at 520–800 nm (peak emission at 590–630 nm) (Delori, 2004). MP, which is located anterior to the RPE, absorbs light of 400–550 nm (peak absorption at 460 nm). Therefore, AF at the macula is attenuated by MP if the excitation wavelength falls within that of the absorption spectrum of MP.

Fundus reflectance, which quantitatively measures the light reflected from the retina and choroid using a reflectometer (Kilbride et al., 1989), a fundus camera (Chen et al., 2001), or a cSLO (Brindley and Willmer, 1952), has also been widely used for the measurement of MP. The reflectance method calculates MP in one of two ways; either by comparing the light reflected at the macula, some of which will be absorbed by the MP, to the light reflected at the peripheral areas, where there is minimal MP present to attenuate the reflectance; or by a spectral analysis of the reflected light (Berendschot and van Norren, 2004).

This current study was designed to compare *in vivo* measurements of MP obtained using cHFP (Macular Metrics Densitometer™), dual-wavelength fundus autofluorescence (Heidelberg Spectralis® HRA + OCT MuliColor) and single-wavelength fundus reflectance (Zeiss Visucam® 200), and also reports on the intra-session repeatability of these devices.

2. Methods

2.1. Subjects

62 subjects were recruited into the study, of which 49 (79%: mean age = 49 ± 13 years) were included in the final analysis. Eight subjects (13%) were excluded due to presence of ocular disease (e.g. AMD, diabetic retinopathy) and five subjects (8%) were excluded because they were not able to perform cHFP reliably (i.e. sd > 10%). The eye with best corrected visual acuity (BCVA) was selected as the study eye (27 OD and 22 OS).

An ancillary study was conducted to assess concordance between MP spatial profile as recorded on the Densitometer and Spectralis, and this additional study included the 49 subjects from the primary analysis, and a further 15 subjects recruited specifically for this purpose ($n = 64$; mean age = 48 ± 13 years; 38 male, 26 female).

This study was approved by the Research Ethics Committee of the Waterford Institute of Technology and was conducted in accordance with the tenets of the Declaration of Helsinki.

2.2. Order of testing

First, BCVA was measured using a computerized Snellen Chart (Test Chart 2000 Xpert; Thomson Software Solutions). Following this, the first measure of MPOD was performed using cHFP. Subjects were then dilated with one drop of tropicamide 1% (Bausch + Lomb) before MPOD measurements were performed on the Spectralis and Visucam devices.

2.3. Customized heterochromatic flicker photometry

The Macular Metrics Densitometer™ (Macular Metrics, Rehoboth, MA, USA) was used in this experiment to measure MP by