Macular Pigment and Age-Related Macular Degeneration: Longitudinal Data and Better Techniques of Measurement Are Needed

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The past decade has witnessed an upsurge in interest in the carotenoid pigments of the macula. These pigments, collectively known as macular pigment (MP), are constituted by the oxyxarotenoids lutein (L) and zeaxanthin (Z).1 MP possesses certain intrinsic properties, which has made it the focus of study by those interested in the aging macula. MP is not synthesized by the human body and is entirely of dietary origin, and L and Z reach their peak tissue concentrations at the macular retina, far higher than in any other tissue compartment.2 Z predominates in the fovea, whereas L is predominant in the peripheral macula and other body tissues. The oxyxarotenoids are free radical scavengers, and L and Z are more resistant to oxidative breakdown than are some of the other carotenoids.3

Collectively, the spatial distribution and chemical and physiological properties of L and Z, suggests an important role for these pigments in protecting the neural retina from (photo)-oxidative damage and the development of a common visually disabling disorder known as age-related macular degeneration (AMD). Intriguing findings are currently emerging from population-based and large case–control studies supporting the view that higher levels of dietary and serum L and Z are associated with a lower risk of AMD. However, relationships between dietary and serum measures and in vivo estimates of ocular MP concentrations are extremely poor, raising doubts on the validity of such a link. Key questions remain on the methods of measuring MP in vivo, which have inherent and major limitations. In this review, we take a dispassionate look at the evidence so far in MP research and the new developments in this field.

The In Vivo Measurements of MP Are Mostly Indirect and Do Not Reflect Absolute Retinal Levels

Various principles have been explored for measuring MP in vivo, and these may be classified as psychophysical, image-based, and signal-based. Commonly used techniques include heterochromatic flicker photometry (HFP) and color matching, which are both psychophysical tests; fundus reflectometry, and autofluorescence (AF), which are image-based methods; and Raman spectroscopy, which is a signal-based test.

In HFP, the test participant eliminates a flicker in a visual stimulus which alternates between two wavelengths, by adjusting the luminance of one of the wavelengths presented. In this manner, and because one of the wavelengths is maximally absorbed by MP (i.e., 460 nm) whereas the other (typically, 520 nm) lies outside the absorbance spectrum of this pigment, a measure of the optical density (OD) of MP can be obtained.5 HFP has been validated by the generation of spectral absorption curves that match extinction spectra of MP measured ex vivo.4 Advantages to the use of HFP are that the readings are derived from a ratio within a given eye, are not influenced by the optical properties of the preretinal media,5 and do not require pupillary dilation. Disadvantages include the fact that HFP is a subjective technique with a learning curve, and the tests commonly take up to 20 minutes per eye, or even longer when readings are taken at several eccentricities to obtain a spatial profile of MP. Consequently, compliance may be affected, and older people may experience fatigue while taking the test.

Color matching of appropriate monochromatic stimuli represents another psychophysical technique for measuring MP and one that has also provided spectral absorption curves that match the extinction spectrum of xanthophylls.6 However, color matching is not a commonly used technique for measuring MP OD, and few instruments are available for using this method.

Fundus reflectometry, which refers to the quantitative assessment of the amount of light reflected from the fundus, can be used to measure the OD of MP. One such method uses scanning laser ophthalmoscopy (SLO), which yields images at two wavelengths: one well absorbed (488 nm) and one minimally absorbed (514 nm), by MP.7 In this way, dual-pass images are generated, and digital subtraction of one of these two images yields an MP OD map from which the spatial profile can be obtained. However, measuring MP with the SLO requires pupillary dilation, dedicated and expensive special equipment, and technical support.

The AF approach exploits the fluorescent properties of lipofuscin contained within the retinal pigment epithelium (RPE).8 In this method, stimulation of the fluorescence of above 550 nm, where MP has essentially zero absorption, with two wavelengths (one well and one minimally absorbed by MP, respectively) provides a single-pass measurement of the MP OD. Thus, the effects of intraocular light scatter associated with the double-pass procedure of fundus reflectance are avoided. Like reflectometry, digital subtraction of the two images yields an MP OD map with spatial profile, which allows detection of ringlike structures.9 However, the AF method assumes that the relative spectral energy of lipofuscin fluorescence is constant across the central retina and is similar at the two stimulation wavelengths.10 This technique also requires

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expensive specialist equipment, pupillary dilation, and technical support.

The Raman method represents a further technique for quantifying MP. It measures Raman signals from light that is backscattered from the foveal carotenoids. As Raman spectroscopy is not a psychophysical method, its proponents claim that it is objective. Because it does not use an eccentric reference, it provides values that represent absolute amounts of MP. It has the advantage of simplicity of technique and rapidity of signal acquisition. Nonetheless, this technique also requires expensive specialist equipment and technical support. Many also question its robustness and validity, as lens yellowing and small pupil size can reduce the intensity of the Raman signal—parameters that could account for the age-related decline in MP observed in studies using Raman technology that has not been observed in studies based on imaging or flicker photometry.

**SELECTING A METHOD OF MEASURING MP AND ASSESSING THE VALIDITY OF MP MEASUREMENTS**

Present Difficult Choices

Considerations for researchers intent on measuring MP include expense, the ease and speed of acquisition of readings, the need for pharmacologic mydriasis, reproducibility, and inter-session variability of measurements, and the ability to yield a spatial profile of the MP. Most important, in vivo methods of noninvasive measurements of MP should provide spectral absorption curves that match the extinction spectrum of xanthophylls if they are to be deemed valid. The Raman method does not produce spectral profiles but has been calibrated against the concentrations of external standards and through measurements made in postmortem samples. Alternatively, any new technique may be validated through the demonstration of good correlation with a validated technique. However, MP readings obtained by Raman spectroscopy do not exhibit good agreement with HFP, and the relationship between the measured Raman signal and the true amount of MP has been the subject of much debate. Recently though, it has been suggested that acquisition of MP density maps through modifications to the Raman spectroscopy yields improved correlations with MPOD measured by AF (Gellerman W, et al. *IOVS* 2007; 48:ARVO E-Abstract 2130). If additional studies confirm these findings, there will be considerable incentive for the use of Raman spectroscopy. Currently, researchers elect to favor either the psychophysical or the imaging based methodologies depending on the priority ascribed to the variables just mentioned. This may change as improvements are made in existing methods or when new methods become available.

**CORRELATIONS BETWEEN MP AND DIETARY AND SERUM L AND Z ARE POOR**

Correlations between MP and constituent carotenoids in diet and serum are known to be weak. In a study of 1698 women, dietary intake of L and Z explained 5% of the variability in MPOD. Adding measures of body fat, the presence of diabetes, and the serum levels of L and Z resulted in the model explaining some 12% of the variance in MPOD. Likewise, in the LUNA study, supplementation with L and Z, resulted in variable increases of MPOD. The authors comment that in a substantial proportion of the participants, no change in MPOD was detected despite increases in serum concentrations of carotenoids. Although it is recognized that there is imprecision associated with calculation of micronutrient intakes based on dietary questionnaires which are subject to measurement error and recall bias, this is not the case with serum L and Z. Should this lack of correlation between serum and tissue concentrations matter when there are many explanations for the poor associations between serum L (and Z) and MP OD? Adipose tissue is a major storage organ for carotenoids, and it is believed that there may be competition between retina and adipose tissue for uptake of lutein, a hypothesis consistent with the observed preferential uptake of L by body fat. Retinal capture of circulating carotenoids is mediated by specific xanthophyll-binding proteins (XBP), and local tissue expression of these proteins could strongly influence tissue uptake. The RPE-choroid complex may represent an intermediate control and transfer point for L and Z uptake by the neurosensory retina from circulating carotenoids, and this exchange may be influenced by an individual’s lipoprotein and apolipoprotein profiles, creating yet another cause of variation. Stabilization of L and Z within the retina is subject to local oxidant load. Indeed, some investigators have attributed the accumulation of L and Z at the macula to the resistance of these particular xanthophylls to degradation by radical-initiated auto-oxidation. Genetic factors influence MP levels. In a study of 150 twin pairs, in whom MP OD distribution profiles were measured with two-wavelength AF, genetic modeling confirmed that the profiles correlated more highly among monozygotic than among dizygotic twins. From the foregoing, it is clear that many factors have the potential to influence carotenoid levels in the macula, which could account for the observed weak relationship between MP OD and circulating levels of these pigments. Regardless, the lack of robust technology for in vivo measurements of MP remains a major cause of concern. The LUXEIA (Lutein Xanthophyll Eye Accumulation), a double blind, randomized, controlled trial, showed only a small effect on MP peak OD after supplementation with L, Z, or both. MP OD increased by 15% on supplementation with L, or L+Z. Supplementation with Z alone, appeared to result in an augmentation of MP at both the fovea and at the parafovea; consequently, the log ratio of readings taken at these two retinal loci did not change. The trial used a model that assumed that that MP did not increase at the eccentric retinal location after supplementation and a 14% increase in MP OD after supplemental Z was reported. Thus, the topographic distribution of L and Z during supplementation with xanthophylls can act as confounders when using methods that simply rely on point estimates and ratio calculations. Improving the methodology to allow the spatial distribution of L and Z to be accurately plotted should result in more robust estimates of actual in vivo concentrations of these pigments.

**CONCLUSIONS**

The case for a protective effect against AMD for macular carotenoids is based on their antioxidant properties and the belief that chronic and cumulative oxidative stress is a risk factor. Intuitively, in a condition such as AMD which is a late-onset disorder, protection or susceptibility factors should exist for decades before its onset. In this context, the recent demonstration of low MP levels with increasing age, cigarette smoking habit, and family history of AMD in otherwise healthy subjects aged 20 to 60 years are important findings in our search for evidence linking a lack of MP with AMD. A case-control analysis nested within the Age-Related Eye Diseases Study (AREDS) found that high dietary L and Z was independently associated with a decreased likelihood of having neovascular AMD, geographic atrophy, or large or extensive intermediate drusen. However, conflicting reports of an adverse effect have also recently emerged. A longitudinal study has shown that higher intakes of L and Z and fatty acids were...
associated with increased rate of progression to AMD. Thus, additional robust longitudinal cohorts are needed to confirm that either lack of MP is associated with progression to AMD or conversely that supplementation is beneficial. Until such time as the outcomes of ongoing trials (AREDS 2) become known, the question of whether dietary carotenoid augmentation alters the risk for AMD progression remains unanswered.

The limitations of the existing methodologies for in vivo measurement of MP, along with limited longitudinal data, represent a hindrance to our current understanding of the complex relationships between these potentially important molecules and degenerative macular disease in older adults. However, the appreciation that the topographical distribution of MP is a better reflection of overall MP status, along with emerging data from ongoing controlled clinical trials, should help with the elucidation of this pigment’s putative protective role against AMD.

References


