Macular pigment and its measurement

Dr Hannah Bartlett and **Dr Frank Eperjesi** review the various ways that macular pigment may be measured

INTEREST HAS BEEN RAISED into the protective role of the oxygenated xanthophylls group of carotenoids in the eye, particularly the retina. This group includes lutein, zeaxanthin, and meso-zeaxanthin, and they are the only carotenoids present in the lens[1] and retina.[2-5] Within the retina they are also known as macular pigment (MP), and the proposed specific function of xanthophylls at the macula is supported by the fact that macular levels are several thousand times higher than serum levels.[6]

MACULAR PIGMENT STRUCTURE

In the central macula, lutein, zeaxanthin, and meso-zeaxanthin are found in equal quantities, but the ratio of meso-zeaxanthin to zeaxanthin decreases with increasing eccentricity.[7] Meso-zeaxanthin has been found in the human macula, retina and retinal pigment epithelium (RPE), but not in the plasma or liver.[8] This forms the basis for the assumption that meso-zeaxanthin is formed via isomerisation of lutein,[7] and is not obtained directly through the diet. The conversion mechanism is thought to be concentrated at the macula.[7] A putative lutein-binding protein has been found in the retinae of human eyes,[9] which binds with high affinity and specificity to lutein and other xanthophylls. It has been suggested that people who are less responsive to xanthophyll supplementation may be so because of genetic differences that result in reduced or less efficient binding proteins.[10] This protein may also act as an enzyme for the conversion of lutein to meso-zeaxanthin.

In human retinae the xanthophylls are concentrated mainly in the inner and outer plexiform layers. The ratio of lutein to zeaxanthin and meso-zeaxanthin within 0.25mm of the fovea is approximately 1:2.4,[11] but the situation reverses at the retinal periphery, where the ratio is 2:1[11] (Figure 1).

Xanthophylls have also been isolated in the rod outer segments[12,13] where there is a high concentration of polyunsaturated fatty acids that are particularly prone to oxidative attack. Within the rod outer segments, their highest concentration is found perifoveally, where it is 2.5 times higher than in the peripheral retina.[13]

The MP may prevent light-initiated oxidative damage to the retina and therefore protect against subsequent age-related deterioration.[14] The presence of MP in the inner retinal layers[15] supports a photoprotective role. The absorbance spectrum of MP peaks at 460nm and it is purported to act as a broadband filter, reducing the sensitivity of the macular region to short wavelength light which is most damaging in the 440 to 460nm range.[16,17] Lutein is reported to be a superior filter,[18] given it is orientated both parallel and perpendicular to the plane of the membrane.[19] Zeaxanthin is orientated perpendicular to the membrane plane only, and so may not be able to absorb the excitation beam from all directions (Figure 2). Zeaxanthin however, is reported to be a superior photoprotector during prolonged light exposure; the shorter time-scale of protective efficacy of lutein has been attributed to oxidative damage of the carotenoid itself.[19]

Carotenoids are also able to quench singlet oxygen (a potent oxidant),[20] scavenge reactive oxygen species,[21] limit peroxidation of membrane phospholipids,[22] and reduce lipofuscin formation.[23] The presence of MP in the rod outer segments and RPE[12,13] is suggestive of a reactive oxygen species (ROS) – quenching function. The fact that lutein and zeaxanthin have been found in higher concentration in the rod outer segments of the perifoveal retina than the peripheral retina, lends support to their proposed protective role in age-related macular disease (AMD).[12] The negative effect of oxidative processes in the retina is clearly demonstrated in Stargardt's disease (a macular condition reminiscent of AMD that occurs in the second or third decade), in which there is a genetic defect that results in lack of control of oxidative processes.[24,25]

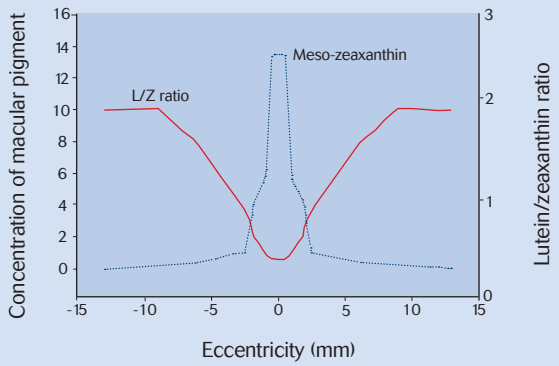
IN VIVO MEASUREMENT OF MACULAR PIGMENT OPTICAL DENSITY

The term macular pigment optical density (MPOD) refers to the amount of macular pigment in the retina. MPOD in the central 1-2 degrees of the macula lies in the range 0.1 to 0.9 for most people.[26,27] For a person with MPOD at the low end of this range, structures posterior to the MP will be exposed to approximately six times the blue light flux compared to a person with MPOD at the higher end of the range.[28] It follows that there is a suspected increased risk of AMD development for those with low MPOD levels. It has also been noted that geographic atrophy tends to spare the very central macula, where MPOD peaks, at least until the disease is well advanced.[29,30]

Psychophysical methods

The psychophysical approach to MPOD measurement is based on the fact that

FIGURE 1



Adapted from Landrum & Bone 2001

FIGURE 2. Structure of macular pigment

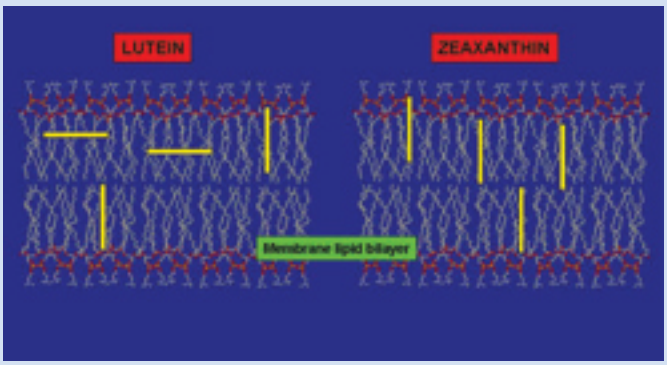
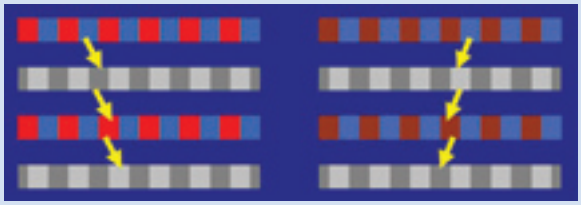


Figure 3. Apparent motion photometry (AMP)

The four gratings are presented sequentially. Each is phased 90° (quarter of a cycle) ahead of the previous



When the red bars are brighter than the blue, there is apparent motion to the right

When the blue bars are brighter than the red, there is apparent motion to the left

Based on Anstis & Cavanagh (1983) A minimum motion technique for judging equiluminance

Figure 4. MPOD measurement technique developed by Cambridge Research Systems

the MPOD acts as a broadband filter in to 440 to 460nm range. In heterochromic flicker photometry,[31-33] a blue reference light, close to the optical peak density of MP (450nm) is alternated with a light of variable wavelength. This is set to a value which is not absorbed by the MP, such as 560nm.[34] While viewing this flickering stimulus, the luminance of one of the lights is altered until the perceived flicker is minimised. At the minimum flicker point, the perceived luminance of the two lights is equalised. The perceived intensity of the blue reference light will be relatively low when viewed at the fovea (where MP is relatively high), compared with a point outside the fovea (where there is less MP). The difference between the ratios of the luminance of the two lights obtained at foveal and para-foveal points is used to derive the MPOD. Although this technique is reproducible and exhibits good test-retest reliability,[35] it is difficult for the subject to perform,[36,37] and requires good visual acuity. It is also associated with high variability in subjects with low levels of MPOD.[38] A commercial instrument that employs this technique for measurement of MPOD is the MacuScope and is available from Birmingham Optical Group. (See page 25).

Apparent motion photometry

A more recent development in MPOD measurement is based on an apparent motion phenomenon reported by Anstis and Cavanagh[39] for matching the luminance of different colours. This technique has the advantage of simplicity when used for adjusting colour luminance on television displays. If a red/green square-wave grating is suddenly replaced with a dark yellow/light yellow square-wave grating which is displaced by one-quarter of a cycle to the right, then the grating will appear to jump to the left if the green bars are lighter than the red bars, or to the right if the reverse is true.[39] But if the red and green bars are made equiluminous, no consistent apparent motion is seen.

An MPOD measurement technique developed by Cambridge Research Systems (www.crsltd.com) uses a stimulus made up of four consecutively presented square wave gratings, each 90 degrees out of phase with the next (Figure 3). The first grating is a chromatic grating of red and blue bars. The luminance of the blue is fixed, while the red luminance can be varied.

The second grating is a purely luminance modulated grating, modulated around the mean luminance of the blue/red chromatic grating. If the luminance of the red component in the chromatic grating is greater than the blue, the observer correlates that with the brighter of the bars of the luminance grating when it is presented. However if the luminance of the red is less, then it is correlated with the darker bar in the luminance grating. This continues in successive grating presentations, so that the sequence of gratings appears to move in one direction or the other, the direction being solely dependent upon the relative luminance of the two components in the chromatic gratings. The subject is simply required to decide which way the grating is drifting in a two alternative forced choice (2AFC) weighted up/down staircase procedure (Figures 4 and 5).

The Apparent Motion Photometer (AMP) uses a parafoveal point as a reference point and can be programmed to take MP measurements at various locations. This has the advantage of permitting a profile of MP to be built up, but has the disadvantage of longer testing times. Good contact lens visual acuity is required.

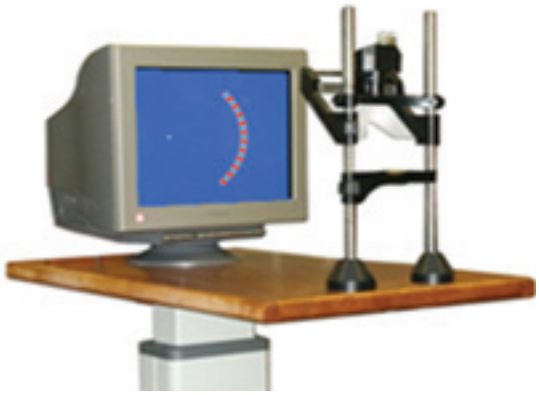


Figure 5. The Apparent Motion Photometer

Raman spectroscopy

This technique is based on the Raman effect, which is the inelastic scattering of photons by the molecules under investigation. In other words, the wavelength of a small fraction of the radiation scattered by certain molecules differs from that of the incident beam, and the shift in wavelength depends on the chemical structure of the molecules responsible for the scattering. This phenomenon has been used in assessment of MPOD because when carotenoids are excited with a monochromatic laser beam, they exhibit characteristic wavelength shifts of the back-scattered light. A blue/green argon laser is used to excite the electronic absorption of carotenoid pigments.[40] The resultant Raman signals generated are recorded and analysed by a spectrometer. This technique has the advantage that it can be used to assess MPOD in AMD-affected eyes. This technique is reported to be highly reproducible and not subject to meaningful test-retest variability,[41] although it has only been used in a research setting.

Imaging techniques

Fundus reflectometry involves measuring the reflectance of short wavelength light (462nm) that has passed through pigment-containing layers of the retina twice.[42] A digitised image obtained at an illuminating wavelength of 559nm is subtracted from one taken at 462nm to correct for the absorptive effects of melanin and

oxyhaemoglobin. This provides the spatial variation of the MP.

Scanning laser ophthalmoscopy (SLO) can also be used to produce fundus reflectance maps, and this method is reported to be more resistant to light scatter than conventional fundus reflectometry.[43] Digital subtraction of the maps at 488 and 514nm, with adjustments made for absorption of the lens, provides a mean value of MPOD.[44] A major advantage of this technique is its objectivity. A disadvantage of this technique is that it requires a normal retinal structure, and therefore is not suitable for use in patients with advanced AMD.

CONCLUSION

Evidence suggests that xanthophylls may have antioxidant and photoprotective effects within the retina. The ability to measure macular xanthophylls is of importance for further investigation of these roles, as well as for dietary supplementation studies. Psychophysical methods have dominated macular pigment measurement in recent trials, but are difficult to perform. Future development of a clinical instrument is likely to involve objective imaging techniques.

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◆ *Dr Bartlett is a post-doctoral research optometrist in the Ophthalmic Research Group in the School of Life and Health Sciences at Aston University and Dr Eperjesi is director of undergraduate studies for Optometry in the School of Life and Health Sciences at Aston University and a research optometrist in the Ophthalmic Research Group in the School of Life and Health Sciences at Aston University*