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Progress in Retinal and Eye Research 23 (2004) 533–559
Progress in RETINAL AND EYE RESEARCH
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Macular pigments: their characteristics and putative role

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Abstract

The macular pigments (MP) absorb light in the blue–green region of the visible spectrum and comprise two carotenoids, lutein and zeaxanthin. In humans the concentration of MP varies widely across the normal population. There are two (not mutually exclusive) proposed roles for MP: to improve visual function and to act as an antioxidant and protect the macula from damage by oxidative stress. In this article we review the origin, spectral characteristics and ocular distribution of MP and also discuss the effect MP has on central visual function and the techniques available for measurement of MP optical density in vivo. Finally, we review the evidence for both proposed physiological roles of MP. Considering the first of these, we conclude that although MP might improve visual function in theory, to date there is no firm evidence that higher levels of MP are correlated with enhanced measures of visual performance. There is a growing body of evidence that has highlighted associations between macular disease and low levels of MP, most particularly with age-related macular degeneration (AMD) and with risk factors for AMD. However, all findings to date are associative only and there is no direct evidence for high MP levels conferring a protective effect. Increased dietary intake of MP gives rise to increased levels of serum and retinal MP. This, taken together with the associative evidence of low MP levels in disease, indicates that a potential, and perhaps serendipitous, therapeutic strategy for macular disease exists. We conclude, however, that the potential protective properties of MP will only be fully evaluated by undertaking longitudinal studies that follow initially healthy participants through to the development of macular disease.

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doi:10.1016/j.preteyeres.2004.05.004

1. Introduction

The first documentation of a yellow colour in the centre of the retina was made by Buzzi (1782) and a few years later Soemmering (1799) was of the opinion that the yellow spot represented a central retinal hole. Maxwell (1856) made the observation that large, spatially uniform coloured stimuli were often perceived to have a central dark region. It was also noted that the spot was particularly marked when stimuli included short-wavelength (SW) light (Maxwell, 1856). Schultze (1866) hypothesized that such SW absorption may reduce the consequences of chromatic aberration and may also play a protective role.

Although a phenomenon consistent with macular pigmentation had been documented behaviourally, Gullstrand felt that the yellow colour was a post mortem change and not present in vivo (Gullstrand, 1907). In 1945, Wald showed that the absorption spectrum of the yellow pigment was characteristic of a carotenoid (Wald, 1945). Since Wald's study, there has been an explosion in efforts to chemically identify the macular pigment(s) (MP), develop methodology to measure and characterize MP in vivo, and understand the physiological role of MP. More recently, great interest has developed in the potential role that MP may play in macular disease. In this article, we will concentrate on the work undertaken in the last 50 years that has shed light on these questions. Despite the large body of work, significant issues remain unresolved that may bring into question the validity of the two current working hypotheses for the role of MP: firstly, to improve visual function, and secondly, to protect the macula from oxidative stress.

2. What are the MPs?

Wald (1945) identified MP as belonging to the Xanthophyll family. The first separation of the carotenoids from the macula was made much later by Bone et al. (1985) using high-performance liquid chromatography (HPLC). Chromatograms obtained in this study consistently showed the presence of two components. Bone et al. (1985) labelled these components with the neutral terms MP1 and MP2 and used experimental procedures to determine the chemical identity of these constituents. Firstly, the chromatograms were to all

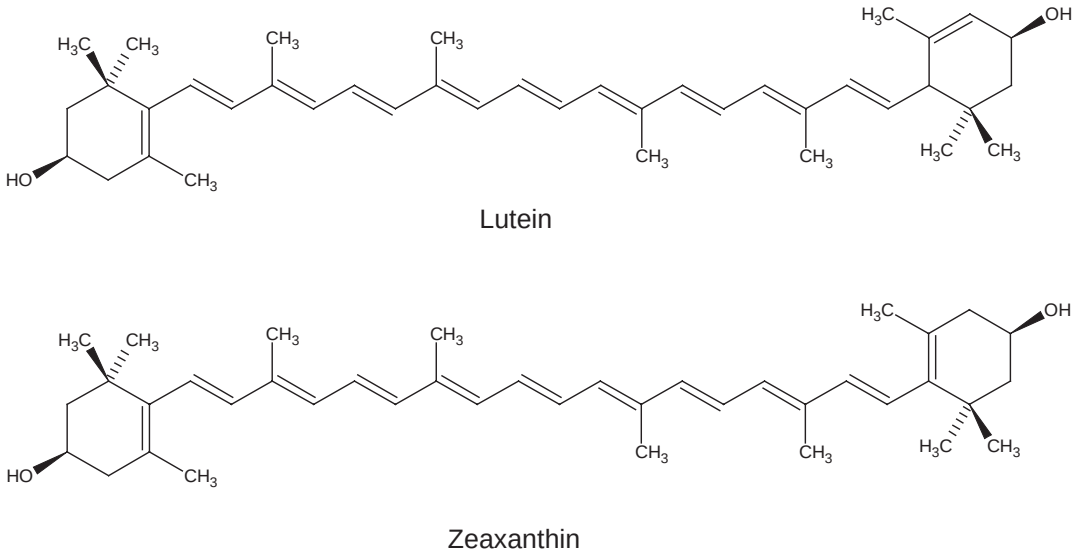

Fig. 1. The chemical structures of lutein and zeaxanthin, the principal constituents of the macular pigmentation.

intents identical to chromatograms obtained from lutein (L) and zeaxanthin (Z) standards. Secondly, the absorbance spectra of MP1 and MP2 could also be identified with those of L and Z. Thirdly, evidence of the identification of MP1 with lutein and MP2 with zeaxanthin was made based on the subtle difference in chemical structures of L and Z. L has an allylic hydroxyl group which can be converted to a methyl ester. Z, however, does not undergo the same reaction. Performing this chemical reaction on MP1 and L standard, followed by HPLC, gave two new compounds with identical elution times on the HPLC column. The same procedure was conducted on MP2 and the Z standard. The HPLC results showed a single compound only, for both MP2 and Z, and this peak was identical to that of untreated zeaxanthin. The feature that the MP comprised two components was later confirmed by Handelmann et al. (1988). It is very important to note, therefore, that the pigmentation of the macula does not arise from the presence of a single carotenoid, but rather the presence of two major carotenoid constituents. The two carotenoids (members of a 40 strong family of carotenoids) are depicted in chemical form in Fig. 1.

3. Where do the MPs come from?

L and Z are not synthesized within the body and therefore the MPs have to be provided by dietary intake. Wald (1945) originally identified the MPs as having the spectral properties of a xanthophyll, which originate in the leaves of green plants. More recent work has allowed the relative concentrations of the MPs in foodstuffs to be assessed more thoroughly. Khachik et al. (1992) used HPLC to assess levels of carotenoids in fruits and vegetables. These studies had the disadvantage of not separating the individual concentrations of L and Z. As L and Z are found in different distributions in the retina, Sommerburg et al. (1998) used HPLC to analyse the contents of different fruits and vegetables for each MP constituent. Their findings are presented in Table 1, reproduced from the original paper. The results agreed with those from a previous paper collating data from 1971 to 1991 (Mangels et al., 1993), but quantify the distribution of L and Z in the different foodstuffs.

An important development in recent years has been the analysis of carotenoid concentration in the human plasma in vivo (Khachik et al., 2002) and how concentration is influenced by diet. Primates fed a carotenoid-free diet had no detectable yellow pigmentation in the macula (Malinow et al., 1980) and levels of MP in humans (measured using heterochromatic flicker photometry) can be raised by dietary supplementation (Bone et al., 2003; Hammond et al., 1997a; Landrum et al., 1997). The underlying mechanisms of incorporation of MP into retinal tissues are poorly understood. The increase in optical density of MP with oral supplementation is slow, rising steadily and reaching a plateau after 140 or more days of supplements containing 30 mg of L (Landrum et al., 1997). Following cessation, supplement levels fall at a slower rate and have been shown to remain raised for a period of at least 6 months in some individuals (Hammond et al., 1997a).

It is also now possible to buy lutein and zeaxanthin concentrated in tablet form, either alone or in combination with other health products. A simple Internet search reveals a large number of retailers offering such preparations.

Table 1
Macular carotenoid content of fruits and vegetables given in mol%

Vegetable/fruit	Lutein and zeaxanthin	Lutein	Zeaxanthin
Egg yolk	89	54	35
Maize (corn)	86	60	25
Kiwi	54	54	0
Red seedless grapes	53	43	10
Zucchini squash	52	47	5
Pumpkin	49	49	0
Spinach	47	47	0
Orange pepper	45	8	37
Yellow squash	44	44	0
Cucumber	42	38	4
Pea	41	41	0
Green pepper	39	36	3
Red grape	37	33	4
Butternut squash	37	37	0
Orange juice	35	15	20
Honeydew	35	17	18
Celery (stalks, leaves)	34	32	2
Green grapes	31	25	7
Brussels sprouts	29	27	2
Scallions	29	27	3
Green beans	25	22	3
Orange	22	7	15
Broccoli	22	22	0
Apple (red delicious)	20	19	1
Mango	18	2	16
Green lettuce	15	15	0
Tomato juice	13	11	2
Peach	13	5	8
Yellow pepper	12	12	0
Nectarine	11	6	6
Red pepper	7	7	0
Tomato (fruit)	6	6	0
Carrots	2	2	0
Cantaloupe	1	1	0
Dried apricots	1	1	0
Green kidney beans	0	0	0

4. Where are the MPs located?

The MPs, as the name suggests, are most dense within approximately the central 7 mm^2 of the human retina. To the first approximation, the overall distribution of the pigments peaks in the fovea and gradually decreases with increasing eccentricity, a feature that can be readily observed in fundus photographs. The nature of the spatial distribution of the human MPs has also been the focus of quantitative evaluation. Psychophysical techniques have revealed the spatial profile of the MPs in human in vivo (Hammond et al., 1997a,c; Moreland and Bhatt, 1984). It has also been noted that the decrease in MP density with eccentricity is not necessarily monotonic (Moreland and Bhatt, 1984; Robson et al., 2003). This feature could arise from the two principal chemical constituents varying in concentration differently across the retina (Bone et al., 1988). It also seems clear that the spatial extent of the MP distribution varies between subjects (Hammond et al., 1997c; Moreland and Bhatt, 1984; Robson et al., 2003) and the extent also appears to be influenced by age (Chang et al., 2002). The psychophysical evaluations of the spatial profile of MP density are painstaking and lengthy measurements. Also, the resolution of such measurements is limited to the size of the visual stimulus that is presented (most often circa $1^{\circ 2}$).

Imaging techniques, which can obtain information over a larger area of the retina, overcome many of the shortcomings of behavioural assessments of the spatial distribution of MP in the human retina. The pioneers of this technique (Kilbride et al., 1989) revealed that their data were best fit by a Gaussian function of eccentricity centred on the fovea, measurements being made at every 0.1°. More recent use of imaging techniques has allowed even greater spatial resolution to be achieved, but not all have reproduced Kilbride et al.'s finding that the distribution is Gaussian (Robson et al., 2003). Robson et al. (2003) also highlighted an interesting dissociation, where the overall amount of macular pigmentation does not correlate with the peak density. It remains to be seen whether the peak density or total amount represents the most prudent measurement to quantify macular pigmentation if its enhancement as a treatment for disease is to be assessed.

In addition to the work undertaken on the overall topography of macular pigmentation in humans, other studies on non-human primates have been able to reveal the distribution of the pigments in the retinal layers. Snodderly et al. (1984b) used photographic and microspectrophotometric techniques to evaluate the distribution of MP in the layers of the primate retina. A companion paper used microdensitometry to investigate the spatial distribution of the MP across the retina (Snodderly et al., 1984a). Microphotographs of prepared sections of foveal tissue obtained from *Macaca fascicularis*, *M. mulatta* and *M. nemestrina* were taken using a 460 nm spectral primary and also with a primary of 525 nm. The bands of high absorption of the SW light were seen in the photoreceptor axon layer and in the inner plexiform layer. To identify whether these absorption bands in the different retinal layers contained the same or different pigments, the absorption spectrum of single retinal layers was measured. The measuring beam had a cross-sectional diameter of 10 μm and absorbance values at 5 nm intervals from 400 to 500 nm and at 10 nm intervals from 500 to 600 nm were taken. Using a computational technique and a series of template spectra derived by taking the difference in absorption between two nearby locations in the specimen under study, they were able to show that the majority of yellow pigments in the photoreceptor axon layer and the inner plexiform layer of the fovea were MPs, with a peak absorbance of 460 nm. They also identified two other SW filters, with peaks of absorbance

at 410 and 435 nm, which were named P410 and P435, respectively. Spectra taken at increasing eccentricity from the centre of the fovea showed a rapid decrease in the density of MP in the photoreceptor axons, to reach the levels found in the other retinal layers by 400 μm eccentricity.

The P410 filter showed an increase with eccentricity, whilst the P435 filter showed a decrease with eccentricity. The P410 filter levels in the receptor axon layer decreased with increasing eccentricity. In the inner retinal layer, the levels of P410 were higher and the net effect of this overall was to lead to a small but steady increase in the level of P410 with eccentricity. The P435 levels showed a steady increase in the receptor layer, but always existed in low levels in the inner layers. The increasing volume of the inner layers with eccentricity lead to an overall reduction in P435 density with eccentricity. The P410 and P435 pigments have spectra that can be identified with the haemoproteins reduced cytochrome C and oxidized haemoglobin, respectively.

In the second paper (Snodderly et al., 1984a), the spatial distribution of MP was studied using two-wavelength microdensitometry. Foveal tissue from primates was prepared and scanning microdensitometry was performed at two wavelengths, 460 and 525 nm. The spatial profile of difference in absorption between the two wavelengths was overlaid on images of the retinal layers traced from microphotographs. This method allows the peaks of absorbance in the different fibre layers of the retina to be seen. Interestingly, the relative amounts of the MP in the receptor axons and the inner plexiform layer varied considerably between specimens. In some specimens the peak density of pigment in the receptor axons exceeded that of the inner plexiform layer and the IPL density decreased rapidly with increasing eccentricity. In other specimens, the pigment density in the inner plexiform layers declined less rapidly and hence exceeded the receptor axon pigment density at eccentric locations.

The relative distribution of L and Z across the macula has also been investigated (Bone et al., 1988). In adult retinae Z is clearly dominant in the centre of the fovea, with the amount reducing with eccentricity. There is a concomitant rise in the relative level of L with increasing eccentricity. The L:Z ratio changes from 1:2.4 centrally to 2:1 peripherally. The reason for this change in distribution is unclear, although the authors offered a tentative explanation. A plot of L:Z ratio against the rod:cone ratio obtained in another study from one individual showed a linear relationship. The suggestion was made that Z may be associated with cones and L with rods. This is, however, an inductive step from the two data sets and the direct association of different carotenoids with different receptor types remains unconfirmed. However, since that study, both L and Z have been shown to be associated with rod outer segments (ROS). Sommerburg et al. (1999) demonstrated that about 25% of the total retinal carotenoid is found in the ROS. Rapp et al. (2000) has also measured the L and Z concentrations in ROS following the argument that receptor outer segments are the sites most prone to damage from oxidative stress. The results again showed the presence of both L and Z in the membranes of ROS, representing approximately 10–15% of the total retinal amount of the MP. The ratio L:Z also varied with eccentricity, increasing from 1.8 in the perifovea to 2.68 in the peripheral sample.

Although the carotenoids L and Z are concentrated in the macula, they are also present in non-central retina and in other ocular structures. Several studies to date have investigated the presence of the MP carotenoids in ocular tissues. Bernstein et al. (2001) used human donor eyes to identify and quantify carotenoids in the ocular tissues, using HPLC. The aim of the study was to characterize the complete carotenoid profile of the eye; here we present the results for the macular carotenoids only. Globes were obtained within 24 h of death and corneas harvested for transplantation. Corneas rejected for transplantation on the basis of serum antigenicity were used in the study. The remaining ocular tissues were dissected and separated to give irides, ciliary body, lens, vitreous, retina, and RPE/choroid. The retinae were divided into macular retina (5 mm trephine), four samples of mid-peripheral retina (superior, inferior, nasal and temporal) and the remaining peripheral retina. The RPE/choroid samples were peeled from the retinal samples after trephination. Both individual and pooled tissue samples were prepared, the latter to aid detection of low levels.

The levels of L and Z obtained from pooled extracts and individual ocular tissues are given in Table 2. The results clearly show that although L and Z are concentrated in the macular retina, they are also present in the majority of ocular tissues, with the exception of the vitreous. Trace levels only were found in cornea and sclera.

5. Spectral properties of the MP

The spectral absorption properties of macular pigmentation have been subject to study for over 50 years. The studies can be divided into in vivo and in vitro measurements. The early in vivo measurements were derived from spectral sensitivity measurements (Brown and Wald, 1963; Stiles, 1953; Wald, 1949), colour matching (Ruddock, 1963), and visualization of Haidinger's brushes (de Vries et al., 1953; Naylor and Stanworth, 1954). The spectra derived in some of these studies were brought together by Wyszeski and Stiles (1982) in order to generate a 'preferred mean curve'. The mean curve was a weighted mean of the spectra derived

Table 2
Lutein and zeaxanthin levels in human ocular tissues

Ocular region	Eyes examined	Area (mm^2)	Lutein (ng per tissue ±SD)	Zeaxanthin (ng per tissue ±SD)	L/Z ratio
Macular retina	14	20	13.98±3.58	19.06±4.5	0.7
Peripheral retina	19	≈1000	64.18±30.10	34.11±16.83	1.9
Superior retina	78	20	1.68±0.88	0.80±0.62	2.1
Inferior retina	78	20	1.46±0.71	0.63±0.26	2.3
Nasal retina	7	20	1.76±1.01	0.81±0.53	2.2
Temporal retina	7	20	1.42±0.90	0.65±0.42	2.2
RPE/choroid	17	Whole	11.58±5.99	5.89±4.13	2.0
Superior RPE/choroid	78	20	0.63±0.26	0.19±0.09	3.3
Inferior RPE/choroid	78	20	0.53±0.26	0.16±0.09	3.3
Submacular RPE/choroid	25	20	0.77±0.50	0.32±0.20	2.4
Ciliary body	20	Whole	12.72±7.90	5.98±3.50	2.1
Iris	21	Whole	4.03±1.98	1.54±0.98	2.7
Lens	18	Whole	1.66±1.09	1.43±1.20	1.2
Cornea	3	Whole	Trace	Trace	—
Sclera	5	20	Trace	Trace	—
Vitreous	3	0.5 ml	Not detected	Not detected	—

from the studies, with weights being proportional to the ease of the measurements. This curve has been used extensively since its publication. However, there have been more recent in vivo measurements that indicate departures from the adopted standard. Pease et al. (1987) derived a spectrum psychophysically and reported the spectrum of the MPs having considerable absorption beyond 535 nm. The extent of the long-wavelength (LW) absorption was, however, not nearly as great when Sharpe et al. (1998) derived a spectrum from sensitivity measurements of isolated cone mechanisms (some MP spectra are compared in Fig. 2).

The chemical identification of the MPs as L and Z has allowed the absorption spectra of these constituents to be determined. In general, the constituents absorb most strongly in the blue–green region of the spectrum (circa 460 nm). Significant absorption in the pigments also occurs at wavelengths beyond the lower limit of visibility (<380 nm). The chemical constituents do not appear to absorb light strongly beyond wavelengths of 530 nm. It should be noted, however, that the chemical environment of L and Z might play some role in modifying the absorption characteristics of macular pigmentation. To address this issue, Bone et al. (1992) mixed L and Z in appropriate quantities and chemically mimicked the conditions under which L and Z would be found in the eye. The resultant spectrum of the L and Z mixture, modified by the presence of a bilipid membrane, showed very good agreement with psychophysical measurements made in the same study. It should also be noted that a more recent psychophysical investigation (Sharpe et al., 1998) indicated that the in vitro spectrum derived by Bone et al. (1992) explained better the spectral data derived by Sharpe et al.

A noteworthy feature of the spectrum for the L and Z mixture derived by Bone et al. (1992) is the light

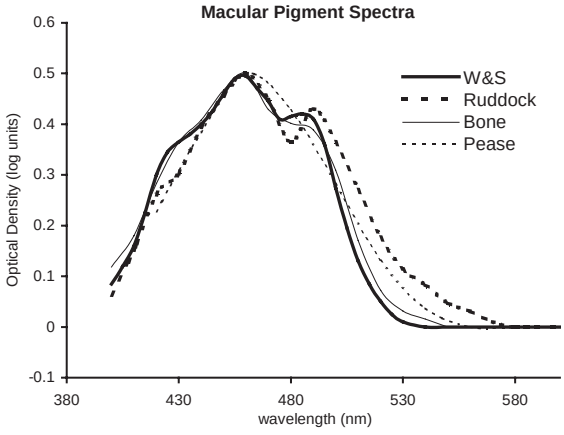

Fig. 2. MP density spectra. Data are given for studies by Ruddock (1963), Bone et al. (1992) and Pease et al. (1987). The thick solid line labelled W&S is the spectrum derived from different studies summarized by Wyszeski and Stiles (1982).

absorption at wavelengths beyond 530 nm. This feature differentiates the spectrum from those put forward by Wyszecki and Sitles (1982), which indicates insignificant absorption beyond 530 nm (Fig. 2).

Is it now time to advocate a standard spectrum? It might be argued that Bone et al.'s (1992) spectrum should be adopted as it represents the most precise measurement of the absorption characteristics of a likely combination of L and Z. It is worth noting that no single spectral template is likely to be appropriate for each individual. The principal reason for this is that the relative concentrations of L and Z, pigments that have different absorption spectra, will vary from one observer to another (perhaps due to diet) and also with retinal location in each individual (Bone, 1976). It should also be noted that the oxidation products of L and Z may

exist in significant concentration in the macula and their presence may perturb further the spectral absorption due to the overall macular pigmentation. It is feasible, therefore, for two subjects with the same absorption of light at 460 nm to have differing absorption of light at other wavelengths because of the different proportions of the constituents of MP. As Sharpe et al. (1998) point out, it may be impossible to reach a consensus about the spectrum of the MPs. However, if one template is required, it is the view of the authors that Bone et al. (1992) provide the best spectrum at present.

6. The effect of macular pigmentation on visual responses

The MPs, as explained earlier, exhibit selective absorption of light in the visible spectrum. The majority of this absorption occurs before light is incident on the photoreceptors. The effect of the pigments therefore is to change a known spectral distribution of light incident on the cornea, the external stimulus, to an unknown spectral distribution at the macular photoreceptors, the internal stimulus. It should also be noted that other spectrally selective absorptions occur to the external stimulus, the most significant being that of the lens. In changing the external stimulus, the MPs can have profound effects on spectral responses that are specified in terms of the physical properties of the external light stimulus. In fact, this very effect is relied upon to estimate the density of the MP with psychophysical techniques in vivo.

Because of inter-observer variations in macular pigment optical density (MPOD) (Bone and Sparrock, 1971), visual responses can be profoundly different between subjects, particularly at wavelengths where the MPs absorb most light in the blue–green region of the visible spectrum. If underlying mechanisms of human vision need to be quantitatively evaluated, the variability in visual responses associated with macular pigmentation needs to be minimized or corrected for. In other words, an estimate of the spectral distribution of light incident on the photoreceptors is required if the function of the photoreceptors and mechanisms beyond them need to be probed quantitatively.

In order to illustrate how spectral responses can be modified by inter-observer variations in macular pigmentation, we briefly present a study of the LW sensitive cone spectral response for 10 deuteranopic observers. Such measurements are essential for generating spectral primary functions for the specification and reproduction of coloured stimuli and have been documented by a number of groups with different methods (Smith and Pokorny, 1972, 1975; Vos and Walraven, 1971; Walraven, 1974). Here we measure the spectral responses using a colour-matching method devised by Maxwell and used in a study by Alpern and Pugh (1977), who fully elaborate on the method used. The data derived from the colour-matching procedure can be plotted as a spectral response for each subject (Fig. 3A).

The responses have been normalized to the mean of sensitivities acquired at wavelengths beyond 600 nm, where the MPs do not absorb light. The variance in the responses over the shorter wavelengths is suggestive of variations in the density of macular pigmentation between subjects. A model of the effect of the MP on an ideal spectral response is shown in Fig. 3B. The ideal response displays the highest sensitivity over shorter wavelengths, and the effect of screening by macular pigmentation of densities 0.4 and 0.8 is shown by the systematic reduction of sensitivity over shorter wavelengths in the other two responses. Qualitatively, the model reflects the observed variations across subjects in Fig. 3A. The model can therefore be adapted to determine quantitatively how much screening due to macular pigmentation is required to align an ideal spectral response to the response of an individual subject. This is equivalent to calculating the MP density for each subject.

A useful procedure is to then remove the effects of the estimated macular pigmentation from each subject's spectral response to determine what remaining variance exists between subjects. This procedure results in the plot shown in Fig. 3C, which indicates that the variance over the shorter wavelengths is reduced considerably once the effects of inter-subject variability in macular pigmentation are accounted for. Although the correction of the spectra for variations in macular pigmentation provides a self-consistent way of reducing inter-subject variability over shorter wavelengths, it is also desirable to determine whether the magnitude of the corrections made to the spectral data correlate with a more direct estimate of macular pigmentation. In Fig. 4, we show how the estimate of macular pigmentation evaluated from the spectral responses relates to another measure that we derived from Ruddock's (1963; also see below) colour-matching method. The correlation between the estimate and measurement is very high and allows the modelling to be verified.

The data presented are shown as an illustration of how much inter-subject variation in visual response can be caused by macular pigmentation. However, the illustration we have used only indicates how macular pigmentation causes changes a spectral response when data were obtained with a stimulus that extended over the central 1.3°. Macular pigmentation is known to show a decrease in density with retinal eccentricity and can therefore cause problems if spectral responses need to be compared at different retinal locations *within* a subject.

A consequence of spatial variations in pigment density is that the luminous efficiency function, V_λ, will be different at different retinal locations in an

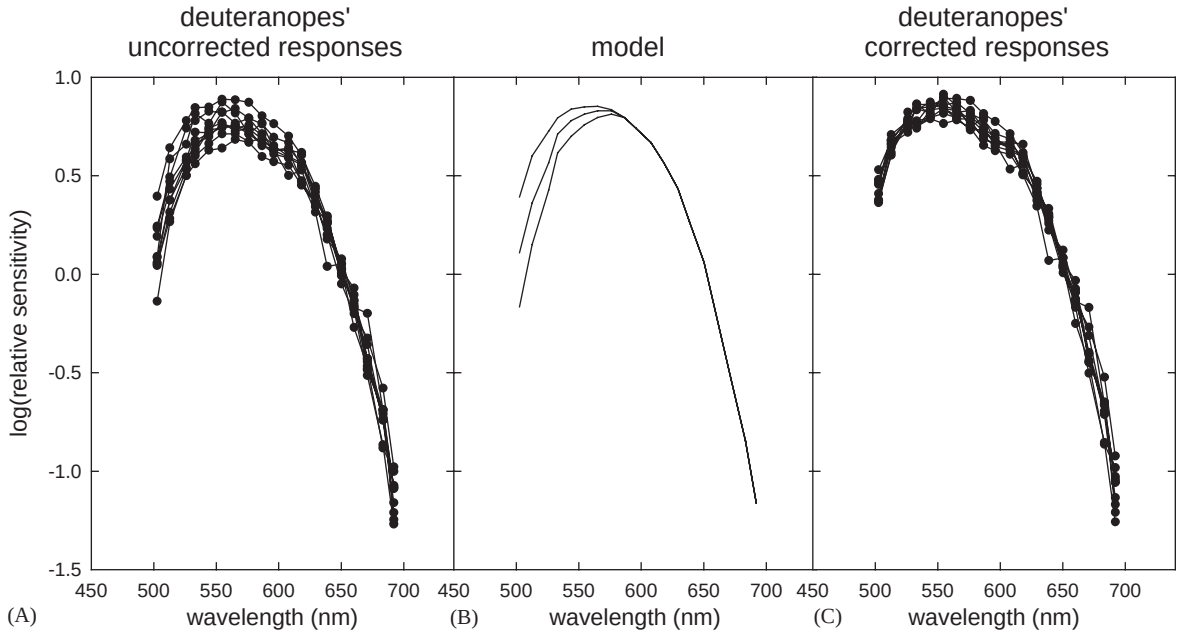

Fig. 3. (A) Spectral responses reflecting the sensitivity of the long-wavelength cone mechanism obtained for 10 D. Responses were normalized to the mean sensitivity obtained at wavelengths $\geqslant$600 nm. (B) An ideal spectral response (the uppermost curve) and responses derived from that curve by modelling the effects of light absorption in the (MPs) due to optical densities of 0.4 (middle curve) and 0.8 (lower curve). (C) Spectral responses of 10 D that have been modified in line with light absorption in the MPs (see text for details).

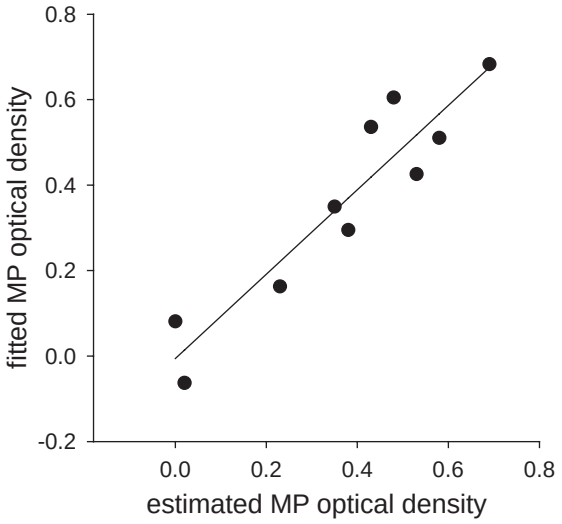

Fig. 4. MP density derived from the spectral responses shown in Fig. 3 (fitted MP) plotted as a function of the MP density (estimated MP) derived from the colour-matching method described by Ruddock (1963). Note that the estimated MP density was measured at 460 nm and that the fitted MP density was derived from measurements made at 500 nm and above. The line of best fit (solid line) is described by fitted MP = 0.988 × estimated MP + 0.006 ($R^2 = 0.872$). The gradient is essentially unity, which indicates that the estimate of MPOD at 460 nm is an excellent predictor of variations in the spectral responses caused by macular pigmentation for wavelengths greater than 500 nm. The spectral absorption characteristics of the MP used in the modelling were those derived by Ruddock (1963).

individual. The knock-on effect of this variation is that specification of equiluminant stimuli has to vary with retinal eccentricity in an individual. This causes problems for investigators who are interested in determining the properties of SW sensitive mechanisms over an extended region of retina. Such investigations are prone to having spatially varying luminance signals in stimuli, which do not allow the action of SW chromatic mechanisms to be disambiguated from responses of achromatic mechanisms. At first, this may appear only to be of interest to the few psychophysicists who investigate retinal processing of colour in humans, but objective measurements of clinical relevance can also rely on isolating chromatic from achromatic mechanisms. For instance, the blue-cone ERG measurement needs to record a signal that principally reflects the response of blue cones. Unfortunately, the MP absorbs strongly at wavelengths that would be best used to stimulate blue cones. Methods have been developed that can overcome these issues. For instance, the use of very bright LW background lights helps suppress luminance detection mechanisms, whilst leaving the sensitivity of blue-cone mechanisms little changed (Chiti et al., 2003).

7. Measuring the macular pigmentation in vivo

7.1. Psychophysical techniques

Because the MP has profound effects on human perception of light, it has been relatively straightforward to use psychophysical techniques to assess MPOD in individuals. In fact, psychophysical measurements preceded the chemical identification of the MPs by many

decades. We will describe the contemporary psychophysical techniques that are used to determine the properties of the MPs and provide a critique of each. We will not describe methods that involve visualization of Haidinger's brushes, but note here that early evaluations of MPOD did implement such a technique (de Vries et al., 1953; Naylor and Stanworth, 1954).

As we have already outlined, the MPs modify the spectral content of a light stimulus incident on the macular photoreceptors. In order to assess the extent to which the spectral content of the stimulus has been modified by the MPs, psychophysicists have frequently compared foveal with extrafoveal responses. In taking this approach, two conditions need to be met: (1) different concentrations of MPs need to be present at the foveal and extrafoveal locations and (2) the response measured is influenced only by the difference in MP concentration at the two locations. As we will describe, the first condition is generally met, but the extent to which the second is met varies considerably between different techniques. It could also be argued that the second condition may never be met in full.

The human luminous efficiency curve, V_λ, can be derived from absolute threshold measurements, heterochromatic flicker photometry (HFP) or motion photometry. From quantitative evaluations of V_λ, it was clear that for foveal targets considerable inter-subject variability in sensitivity existed and was particularly pronounced for SWs (Stiles, 1953; Wald, 1949). These measurements indicated that macular pigmentation could be quantified if a comparison of the sensitivity achieved in the fovea were compared with a response obtained from the same observer at a more eccentric retinal location. The difference between the two responses as a function of wavelength provides an estimate of the spectral absorption of the MPs.

The accuracy of the estimate would, in this case, depend on at least three factors: (1) the photopigment density in the cones at the two locations, (2) rod intrusion to the response at extrafoveal locations and (3) the relative numbers of each cone class at the two retinal locations. The change in photopigment density with retinal location is a very difficult issue to overcome. Moreover, photopigment density may also undergo pathological modification in disease, which in turn could affect estimates of MPOD in patients. The second effect of rod intrusion can be reduced by selecting an extrafoveal location that is not too eccentric, and by using high-luminance stimuli.

The remaining problem of relative numbers of cones varying in different locations is overcome when sensitivity is mediated by only one detector (cone class). Stiles (1949) used large, bright background stimuli to selectively reduce the sensitivity of some cone mechanisms over others, and thereby render the detection threshold dependent on one cone mechanism alone. By using such a technique, Pease et al. (1987) successfully derived macular pigmentation estimates for 27 observers and also derived spectra for the MPs in 12. This method offers, therefore, an advantage over measuring overall sensitivity because of the fewer assumptions involved. The most commonly adopted method of HFP can also be adapted to suppress the sensitivity of the S-cone mechanism (Hammond et al., 1998). Although sensitivity still remains a product of the M and L cone mechanisms under these conditions, it is particularly desirable to exclude the spatially varying effects of the S-cones on sensitivity measures (Hammond et al., 1998). It should be noted that there has been some recent debate concerning the effects of isolating different cone mechanisms on the estimates of MP density spectra (Sharpe et al., 1998).

An elegant method to estimate the spectral absorption due to macular pigmentation was pioneered by Ruddock (1963, 1965) and later developed by Moreland (e.g. Moreland and Kerr, 1978). The colour-matching protocol requires that the observer adjust the energy of three monochromatic light stimuli to match a single monochromatic test stimulus. The monochromatic test stimulus receives no spectral change due to the absorption of MPs. However, the triplet of matching stimuli receives differential absorption by the MPs. For instance, the MPs will absorb an SW more than an LW stimulus. The effect of the differential absorption results in more SW light being needed for a colour match measured at the fovea than for one established at a more eccentric location. Colour matches can be performed with the choice of different wavelengths and a full spectral characterization of the MPs can therefore be derived (see Ruddock, 1963 and Fig. 2).

The quantification of the colour match readily reveals the magnitude of the MP density (Ruddock, 1963). Equipment and efficient methods have been established to measure MP density with colour matching (Moreland, 1980). Moreover, selection of matching stimuli wavelengths that are equally absorbed by the MPs allow the effects of macular pigmentation on colour matches to be negated (Moreland and Kerr, 1979; Ruddock, 1963). The so-called Moreland match uses such stimuli and is a useful tool to probe the function of the SW sensitive cones and therefore deficiencies of this cone mechanism (Moreland and Kerr, 1979). One problem with all psychophysical procedures is that a 'MP-free' measurement has to be made at an eccentric stimulus location, which is demanding for the observer. Some studies (Moreland and Bhatt, 1984; Moreland et al., 2003) have used large annular targets, which are less demanding on the observer, and standard errors of matches are reduced for such a stimulus arrangement (Morland, 1992).

The measurements of MP density with colour matching are not subject to changes in the relative

number of cones with retinal location, but in common with sensitivity measurements, rod intrusion could play a role in modifying matches made at eccentric locations. To overcome rod intrusion, bright stimuli can be used. Again, variation in photopigment density with retinal location is the most difficult obstacle to overcome. However, it is possible to model what effect it might have on results. We have undertaken such modelling (Davies and Morland, 2002) and find that for photopigment density variations that might be expected in the healthy retina, the change in MP density estimated from colour matching varies little for the wavelengths used in our study.

Up to now, we have focussed on how sensitivity measurements and colour matching have been used to obtain a detailed picture of the spectral characteristics of the MPs. There is perhaps a far greater demand now for obtaining a quick estimate of peak MP density for a large number of individuals, because of the putative role that MPs may play in protecting the retina. This demand can be met by employing both of the methods we have outlined. A selection of just two wavelengths at which sensitivity is measured at two retinal locations can provide an estimate of macular pigmentation. For example, measuring spectral sensitivity at a LW, say 600 nm, where no absorption due to macular pigmentation occurs, provides a useful point to normalize the foveal and parafoveal sensitivities. The other measurement of sensitivity should be made at a wavelength where MPs absorb strongly, for example 460 nm. In this case, only a few measurements are necessary, a feature that makes the method reasonably quick to implement and applicable to large numbers of observers. Many groups have used such techniques extensively in their studies of the factors that influence macular pigmentation (see Section 8). Similarly, just two colour matches using appropriately selected wavelengths can provide an estimate of peak MPOD with reasonable speed. It is interesting to note that colour matching has not been used to survey large samples.

Many variants of psychophysical techniques have been employed to assess MPOD. We now consider which techniques represent the most promising approaches and how they can be optimized to allow comparison across studies. The ideal technique needs to conform to three demands: (1) it needs to be readily implemented in a reduced form so that data from a large group of observers can be readily obtained; (2) it needs to reduce the effects of confounding variables as much as possible; and (3) in an extended form, it should provide a viable method for determining the absorption spectrum of the MPs in vivo. The third demand could perhaps be met by specialized laboratories where the extended form of the test may be possible. The first two demands should be met and can be done by implementing colour-matching methods or by determining the sensitivity mediated by a single-cone mechanism. Both techniques offer the same sort of advantages, so it would not seem appropriate to recommend one over the other.

Recommendations can be made to increase the likelihood that the data derived from both techniques could be compared. Standardizing the wavelength, locations and size of target stimuli would allow psychophysical measurements to be compared with the fewest assumptions. These three factors are of undeniable importance if a comparison of MP density across different studies is required. It may be useful to implement a choice of these parameters, which has some backward compatibility with studies that have already been performed on large groups of subjects. Unfortunately, many previous studies have used an eccentric reference stimulus that is presented at 4°. This choice has the consequence of introducing errors in estimates of peak MPOD that are due to intersubject variations in the spatial profile of MP concentrations (Moreland and Bhatt, 1984; Robson et al., 2003). Recent work indicates that a choice of 7° for the location of the eccentric target is more appropriate (Robson et al., 2003). The other stimulus features of previous studies have fewer problems associated with them. It would appear reasonable to recommend the following choices: (1) a central stimulus of 1° diameter, (2) wavelengths of 460 and 600 nm, (3) an eccentric reference location of 7°. To underscore the importance of size of the central stimulus, it is worth quoting the change in estimated MPOD that Bone et al. (1997) reported in an observer when targets with different diameters were used: for targets of 0.25°, 0.5°, 1.0°, 1.6°, the 'peak' MPOD was estimated at 0.92, 0.80, 0.75 and 0.57. The effect of spatial configuration of stimuli on estimates of MPOD can also be readily seen in the data presented in Fig. 5, where two stimulus types are compared. Also of note is the relatively high mean MPOD of 0.77 recorded by Pease et al. (1984) in their study, which used a 40 min target.

Although the psychophysical techniques we have described can be optimized for estimating MPOD, there is one problem that may be insurmountable and of some considerable relevance if measurements need to be made on elderly observers or those with disease. This problem concerns unstable fixation and eye movements, which result in the experimenter not knowing exactly the retinal locus of the stimulus (Abadi and Cox, 1992). Objective measures that image the retina have the distinct advantage of not being subject to such uncertainties and will be reviewed next.

7.2. Objective measures

It may well be apparent to the reader that the psychophysical techniques leave a lot to be desired most particularly because there are confounding variables and

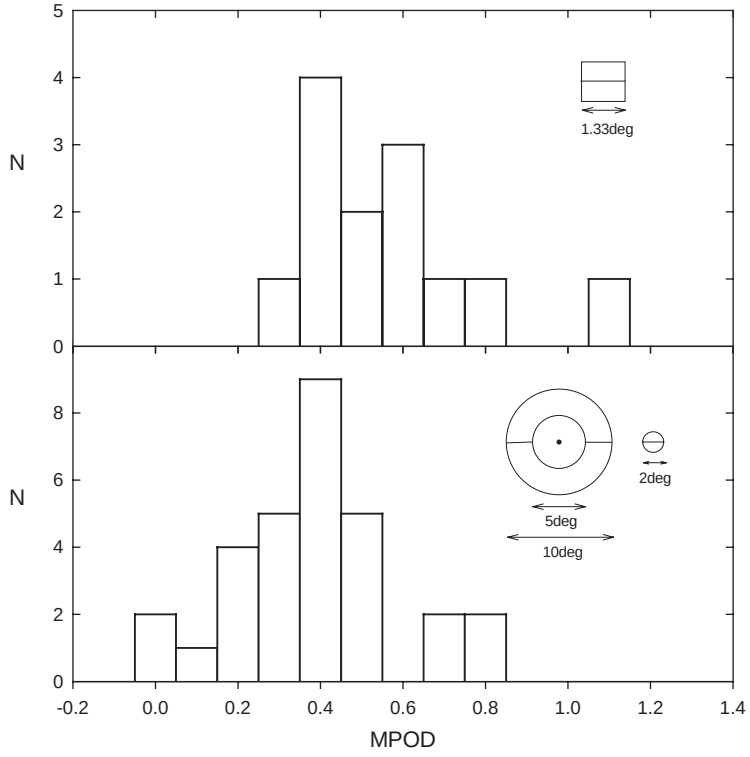

Fig. 5. MPOD measured at 460 nm with colour matching (Ruddock, 1963). The upper panel shows the frequency distribution of MPOD for a group of 13 normals measured with a 1.33° square bipartite field (see inset to the upper panel) presented foveally and 6° extra foveally. The lower panel shows the MPOD frequency distribution for 30 normal subjects, but in this case the foveal bipartite field was a circle of 2° diameter and the extra-foveal filed was annular (see inset to the lower panel). The mean values of the distributions of 0.56 (upper panel) and 0.38 (lower panel) were significantly different (independent t test, $t = 2.63$, $p = 0.006$ (two-tailed)).

no standards have been established to develop a measurement protocol. An important issue is also neglected by all psychophysical techniques, namely, that a portion of the macular pigmentation may also exist adjacent to the photoreceptors, rather than in front of them. This portion of the macular pigmentation would not be documented by psychophysical studies as it would not affect the light incident on the photoreceptors. It is desirable, therefore, to have an objective measurement that could be routinely applied in the clinical setting, particularly if enhancing MP levels becomes a therapeutic strategy. There are a few objective techniques that have emerged and it is our belief that some of these, perhaps with modification and standardization, could become very useful tools in the future, most particularly for the ophthalmologist.

A digitized image of the fundus (Fig. 6) shows absorption of blue light that is characteristic of macular pigmentation. The image shown was derived from a digitized fundus image obtained from a Topcon 3 chip CCD camera. The image was spatially filtered using a 2d Gaussian and the log of each pixel for the blue and red channels was taken and the difference calculated. The subject underwent light adaptation at high luminance in order to bleach the photopigments. Inspection of the image shows a characteristic, circularly symmetric pattern of macular pigmentation. This demonstration is entirely consistent with the images that Kilbride et al.

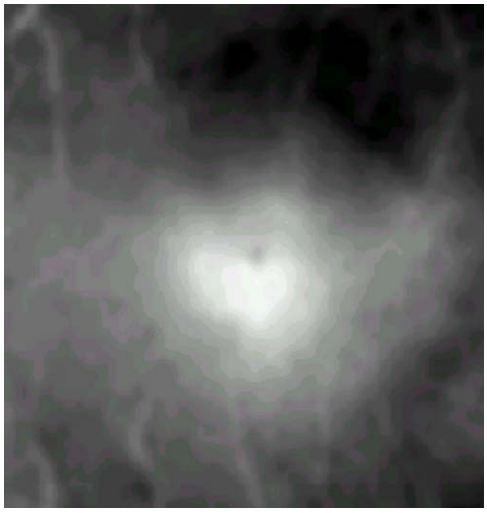

Fig. 6. An image of the fundus of one of the authors obtained by taking the log difference of the blue and red channels of a digitized fundus image taken with a Topcon camera. The data have also been spatially filtered to increase the signal to noise.

(1989) obtained in their pioneering measurements of this type. However, the image does not offer a precise way to quantify MPOD because of the use of broad-band detectors in standard fundus cameras. The introduction of a narrow band filter in a fundus camera can yield reliable correlates of MPOD and with this technique it has been demonstrated that human albinos have little or no MP (Abadi and Cox, 1992) and has also been used in pediatric subjects (Bour et al., 2002).

Spectral fundus reflectometry represents a technique that is capable of quantifying MPOD from fundus images (Kilbride et al., 1989). However, there are some issues that can detrimentally affect MPOD estimates derived from this type of imaging technique. Firstly, it is not possible to assert that the only spatially varying light absorption at a SW is due to L and Z (Kilbride et al., 1989). Both haemoglobin and melanin display spatially varying light absorption, and unless a multiple regression analysis of spectral data is performed the relative contributions of these absorptions cannot be accounted for. It is also worth noting that such multiple regression analysis is also dependent on an absorption spectrum of the MPs (see Section 5 and also Bone et al., 1992) and that this may vary between observers depending on the relative amounts of the L and Z constituents. Secondly, the measurements are not derived from a single pass of light through the MP. To the first approximation, the measurements are double pass, but this is undoubtedly a poor approximation, because reflection of light does not only occur at the RPE, but also the internal limiting membrane (Berendschot et al., 2000). Scatter will also play a role in perturbing estimates of MPOD, and this will be a particularly significant problem in imaging older eyes. Thirdly, autofluorescence of the lens and lipofuscin can contribute to the light reflected from the eye and hence cause problems with measurements of MPOD.

Do these issues limit the accuracy of the spectral fundus reflectometry measurements? The answer is yes, but on the whole they are only likely to prevent an absolute measurement of MPOD. Spectral fundus reflectometry is, therefore, measuring a correlate of MP. It has been found that this correlate is systematically lower than the value of MPOD determined by psychophysical means (Delori et al., 2001; Kilbride et al., 1989), but when a sophisticated model that accounts for the light reflected from the inner limiting membrane is used estimates of MPOD increase (Berendschot et al., 2000) to levels that are similar to those derived from psychophysics.

Scanning laser ophthalmoscopes (SLO) can also be used to determine correlates of MPOD (Berendschot et al., 2000). Images obtained at two wavelengths are subtracted in a way similar to a standard image of the fundus (as described earlier). Because the choice of imaging wavelengths is restricted by the lasers employed, estimates of MPOD require scaling in line with MP spectral absorption. However, the precision of these measurements is higher than spectral fundus reflectometry measurements (Berendschot et al., 2000). The accuracy of the SLO measurements may not however be high, as systematically lower values of MPOD were documented with this technique (Berendschot et al., 2000).

More recently autofluorescence (AF), originating from lipfuscin (Delori et al., 1995), has been used to estimate MPOD. The advantage this technique offers is an estimate of a single-pass absorption through the MP, because the fluorescence can be recorded at wavelengths that are not absorbed by MP. The disadvantage is the low signal that is recorded and the specialized equipment that is required. The method can be implemented with (Robson et al., 2003) or without imaging the retina (Delori et al., 2001). Delori et al. (2001) found that the AF estimates of MPOD more closely match those derived from psychophysics (HFP) than the estimates derived from fundus reflectometry. The imaging study by Robson et al. allowed additional information concerning the spatial profile of MP to be obtained. It should also be noted that AF generated more reproducible results than fundus reflectometry and HFP (Delori et al., 2001).

One recent development has been to use resonant Raman spectroscopy to evaluate MPOD in vivo (Gellermann et al., 2002). This technique has generated promising results but further work is required before the method can be properly compared with established procedures.

The objective techniques we have described certainly offer advantages over psychophysical procedures: most particularly, the subject does not need to maintain fixation nor undergo extensive periods of observations. It is worth noting that the objective measures of MPOD considerably underestimate those derived from psychophysics.

8. Factors affecting MPOD within normal populations

The presence of MP in different amounts in different individuals is interesting, particularly if the MP has a role in the continued health of the macula or for maximization of foveal visual function. In this section, we review the data available to date regarding any systematic variations in MP density that there may be in different physiological and geographical situations.

8.1. Comparison between eyes

Hammond and Fuld (1992) performed a specific study on both eyes of 10 subjects. Using HFP the subjects had

their MPOD assessed for each eye. The results showed that in all eyes the difference in MPOD was less than 0.1 log units. Interestingly, there was some variability in the measurements made over a period of several days (i.e. on some days the measurements showed a difference between eyes, but on other days not) but overall, there was no consistent difference between eyes.

Colour matching has also revealed the correlation between the MPOD in one eye with that in the other (Morland, 1992). The data derived in that study are reproduced in Fig. 7. Differences between the values obtained from the two eyes exceeded 0.1 log units in two of the 12 subjects, but overall there was no significant difference between the values obtained from the left and right eyes for the group (paired t test, $t = 1.2$, $p > 0.05$ (two-tailed)).

Two subjects were involved in the study by Landrum et al. (1997) to assess the effect of an oral lutein supplement. The MPOD was measured in both eyes and in one subject the levels were essentially the same, whereas in the other, the MPOD in the left eye was consistently about 0.1 log unit greater than in the right eye.

Beatty has also measured MPOD in both eyes in a group of healthy subjects as part of a study for risk of AMD (Beatty et al., 2001). The MP density was 0.289 ± 0.156 for the right eye and 0.299 ± 0.159 for the left eye.

From these studies, it would appear that the MP quantities present in the eyes of individuals are, in the main, similar. This has important consequences for the study of large numbers of eyes; data from right and left eyes should not be pooled but treated separately.

8.2. Twin studies and genetics

Another important issue is to try to establish whether MP levels in the retina are genetically determined. The mechanisms of incorporation into retinal tissue are not understood at present. The existence of a specific transport mechanism seems likely, given the observation that there are approximately 40 dietary carotenoids and only two in the macula. It has been shown that the macular carotenoids are bound to tubulin both in bovine and human retinae (Bernstein et al., 1997). A transport system requires the production of the necessary apparatus and this requires the genetic code for its manufacture.

Hammond et al. (1995) used HFP to measure the MPOD in 10 pairs of monozygotic twins. Serum levels of L and Z were measured using HPLC and dietary intake assessed using a food frequency questionnaire. This study found significant differences in MP levels in 5 out of the 10 pairs of twins. There was no relationship between dietary intake of carotenoid or serum levels with MPOD. It was shown, however, that the differences in MPOD in the 5 pairs were related to the differences in intake of dietary fat, iron, linoleic acid and fibre. The conclusions drawn were that the levels of MP in the macula are not completely genetically determined and that there are likely to be multiple factors involved in the deposition of MP in the retina.

8.3. Gender differences

The possibility of a consistent difference in average levels of MP between males and females has also been

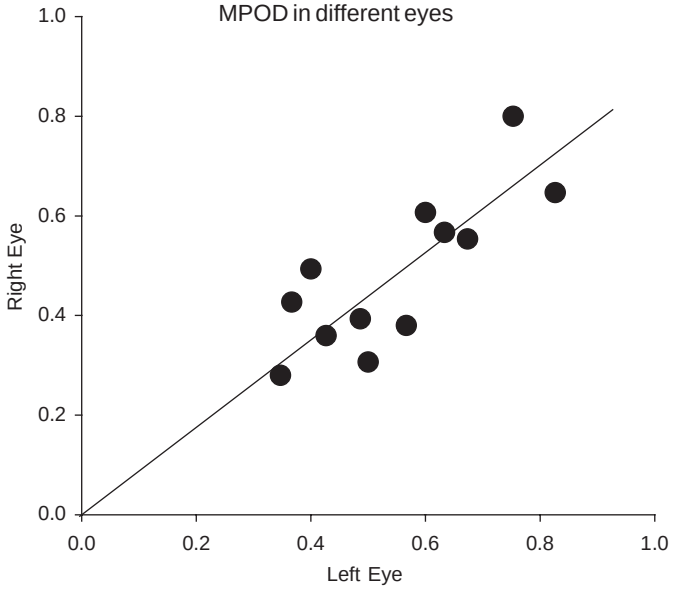

Fig. 7. MPOD measured with a 1.33° field at 460 nm with a colour-matching technique (Ruddock, 1963). Data for the left eye are plotted against data for the right eye. The straight line is the least-squares fit of the data with a line passing through the origin with the equation right = 0.88 left ($r = 0.79$).

investigated (Hammond et al., 1996a) (this was done as part of the associative studies of MPOD with age-related macular degeneration, following the observation that AMD is more common in females).

Hammond et al. studied MPOD in a group of males and females and found that males had MPOD 38% higher than females. The study measured MPOD in 48 women and 40 men. Geographically, 45 were from New Hampshire and 43 of the participants were from the Boston area. The mean MPOD for the men was 0.38 ± 0.216 and for the women 0.24 ± 0.159. Another study on a population from the Southwest USA on 79 men and 138 women gave mean MPOD for the men as 0.24 ± 0.15 log units and for the women 0.21 ± 0.12 (13% lower in women).

Interestingly, a large study performed in the Netherlands using fundus reflectometry on 199 men and 236 women did not show any gender difference in MPOD (Berendschot et al., 2002a). A study based on 280 volunteers from the Midwest USA also showed no gender difference in MPOD (Ciulla et al., 2001).

From these studies, it does not appear clear whether there is a systematic difference between MPOD in men and women.

8.4. Age

The results of many studies will be included in this section, some of which were designed to investigate the variation of MPOD with age and others where age analysis was performed as part of the study protocol, even if age variation was not the primary aim of the study. Unfortunately, in only one of these studies was the testing longitudinal (Hammond et al., 1997c) and in that study the data from only ten persons was available over time span ranging from 1 to 16 years.

The remaining studies are cross-sectional in nature and one is left making the assumption that if MPOD shows an age-related change in a sufficient number of people this represents a true measure of the tendency of MPOD to change with age during an individuals' lifetime. A prospective, truly long-term study would be extremely difficult to perform for obvious reasons and although the above assumption is likely to be flawed the results of large cross-sectional studies may be the closest we can get to answering the question of whether MPOD does change with age. Disappointingly, the results to date on studies of age dependence in adults have been variable. Below we review the evidence for and against age dependence in adults.

The HPLC studies by Bone et al. (1988) and by Handelman et al. (1988) on adult maculae and retinae did not show any age-related decline in MPOD. Using two-colour fundus photography, Chen et al. (2001) separated the results of 54 subjects into three age categories with mean ages 24.8, 40.2 and 67.5 years. They did not find any association of MPOD with age, but did find a change in the spatial profile of the distribution, with older subjects having a broader distribution. In a paper comparing the measurement of MPOD using an autofluorescence technique with both fundus reflectometry and HFP, a total of 159 subjects were studied and no relationship with age was found (Delori et al., 2001).

The findings of the more objective methods above agree with those of several psychophysical studies by Ruddock (1965), Werner et al. (1987), Hammond's group (Hammond and Caruso-Avery, 2000; Hammond et al., 1997c), Mellerio et al. (2002) and Davies and Morland (2002). Of note, only one of these studies contains longitudinal data (Hammond et al., 1997c) and this was in ten subjects. The data were collated from studies performed in several laboratories, using the same stimulus conditions. Importantly, no age-related change was seen in any of the subjects. The study by Mellerio used HFP and the MPOD of 124 eyes of 124 subjects was measured. Davies and Morland (2002) used colour matching and measured MPOD in 34 control subjects, again finding no age-related change, although investigation of MPOD as a function of age was not the main aim of the study. Two hundred and seventy-one subjects took part in the study by Ciulla in the Midwest United States and a multivariate analysis was performed on a total of 58 variables (Ciulla et al., 2001). The outcome of this analysis did not show age as a significant factor.

Age-related decline in MPOD has, however, been reported in other studies. Using resonance Raman spectroscopy, Bernstein et al. (2002) measured MP levels in 220 eyes of 138 subjects in the age range 21–84 years. The results clearly show an age-related decline in MP, measured as spectrometer counts. Calibration showed a linear relationship of spectrometer counts with MP optical density. The results from left and right eyes were not separated, however. Beatty et al. (2001) measured MPOD in 46 healthy controls, aged 21–81 years. They found a small age-related decline of approximately –0.05 log units per decade (this figure was calculated by us from the best fit line given in Beatty et al., Fig. 4). Another psychophysical study by Hammond in the Southwest United States, as a part of the studies investigating the geographical variation of MPOD in North America, also showed a small but significant decline in MPOD with age of -0.01 log units per decade (Hammond and Caruso-Avery, 2000). There is a large spread in the data and the correlation coefficient was low ($r = -0.14$).

Drawing conclusions from these studies is difficult, as different techniques were used, different populations studied and different levels of analysis were performed on the data. We feel at present that the data available indicate that there is probably no cross-sectional change in MPOD with age once adult levels have been achieved.

However, it is apparent that further study is required across all age ranges in a large number of subjects in order to provide a more definitive answer to the question of age dependence.

8.4.1. Children and young adults

Recently, Bour et al. (2002) published a technique of two wavelength fundus photography to measure MPOD in children. Twenty-three subjects were studied, in the age range 6–20 years. The mean value of MP found was 0.13 ± 0.04 log units. The results are lower than those obtained using a similar technique in older subjects published in two other studies (Chen et al., 2001; Elsner et al., 1998). These latter studies used different calculations on the images and there could therefore be systematic differences between them, which make direct comparison of the results difficult.

Bour et al. (2002) did not present any results of the use of the technique to measure MPOD in adults, nor were any psychophysical investigations to measure MP performed on the young subjects. This lack of comparison leaves some doubt as to whether the low values observed represented a true finding or an underestimation of the MPOD because of the method used.

8.4.2. Prenatal, neonatal and infants

In his review from 1981, Nussbaum noted that at that time there were no data regarding the presence of MPs in the neonate or infant. In the absence of any further information, it would also seem reasonable to hypothesize that MP may play a role in the development of the macula lutea and that the levels seen in adulthood simply represent an embryonic remnant. Levels of lutein in foetal cord blood are significantly lower than those found in maternal peripheral blood (Yeum et al., 1998). These samples were obtained at the time of delivery and may not represent the situation during gestation. It is tempting to deduce that the transfer of macular carotenoids across the placenta is low or absent. It has also been shown that levels of lutein are two to three times higher than β-carotene in human breast milk, whilst their blood levels are nearly the same (Jewell et al., 2001). Firm evidence about the presence of MP in the retinae of premature infants (17–22 weeks gestation) was provided by Bone et al. (1988), where retinae were dissected and carotenoid levels were measured by HPLC. The delicate nature of the tissues did not allow dissection of the macula specifically, and whole retinae were analysed. Both L and Z were detected in such retinae, with abundance comparable to that of adults on the basis of mass per unit area. However, in the prenatal retinae, no yellow spot was seen in the macula region. In the postnatal infants, a yellow spot was visible after ~ 6 months of age.

In neonates and infants up to the age of 2 years, both L and Z were detected in a disc of tissue of diameter 4.7 mm taken from the macula. Bone et al. (1988) found in infant retinas below the age of 2 years that lutein was the major pigment, whereas in older subjects zeaxanthin was predominant. The mean ratio of L:Z in the infants was 1.44 ± 0.16 for those less that 2 years of age and fell to 0.77 ± 0.20 for those greater than 2 years. The retinal distribution of MP in the neonatal and infant macula was not studied. From this study it is apparent that MP is present in the retina before birth; however, unanswered questions about the accumulation and concentration of MP in the macula itself remain. As a final point, the causes of death in these unfortunate infants were not given in the study and it remains a possibility that the retinae analysed were not representative of the true situation in vivo.

Handelman et al. (1988) measured MP in some young retinae using HPLC and again found that L and Z were present in the eyes of a 1-week-old neonate and also a 2-month old. Levels were similar to those found in some young adults, but older adults had higher levels in whole retinal specimens. No maculae from infants were studied.

8.5. MPOD in different populations

MP density is known to vary widely between different individuals and there are many studies in the literature from different centres across the world. In this section, we review the findings from different centres, to investigate whether there may be systematic differences in MP density in different populations across the world. It could be hypothesized that differences in race, diet, sunlight exposure and other environmental factors might give different populations different average MP densities.

The majority of the work to date on MP density has been performed in the United States, the United Kingdom and the Netherlands. To our knowledge, there are no studies of MP density in people living in Eastern Europe, Scandanavia, France, Germany, Italy, Greece, Turkey, the Middle East, India, most other Asian countries, South and Central America or Africa.

In Section 7 above, we described a variety of different methods that are available for measuring MPOD and noted that there can be systematic differences in MPOD measured in the same individuals using different techniques. (e.g. photographic techniques tend to underestimate MPOD in comparison to psychophysical methods). At present, there is no agreed 'Gold Standard' method for measuring the in vivo correlate of MP density. Review of the work performed to date in different centres around the world shows that a wide range of the available techniques have been used. There is also methodological variation within the same general measurement technique. For example, many studies have employed HFP to measure MPOD, but the studies

have used different target sizes, stimulus wavelengths, background stimulus conditions and locations for the eccentric target position. All of these stimulus features are likely to have an impact on estimates of MPOD and if comparisons are to be made between studies with differing stimulus features then (at the very least) assumptions have to be made concerning the underlying spatial and spectral properties of macular pigmentation. It is very difficult, therefore, to evaluate differences between the means of samples drawn from populations in different geographical regions unless identical techniques have been used.

We tabulate the results of studies performed on different populations across the world and also make note of the numbers of subjects assessed. We also draw the reader's attention to the methods used in each study and note that they frequently differ and that stimulus attributes in studies that share the same methodology also differ (Table 3). There are a few studies that have evaluated MPOD in different geographical regions using comparable methods and we review these below.

The earliest study to assess population differences was conducted by Bone and Sparrock (1971). HFP was used to measure MPOD in 49 subjects and the analysis directed towards comparison of MPOD across different racial groups, age, colour of iris and also hair. The spectral absorption characteristics were obtained for the MP from 400 to 580 nm in 10 nm steps. In this study, there was no systematic difference in peak MP density at 460 nm in the different racial groups, or with age or iris colour. There was no information reported about whether these subjects lived in the same geographical area or were recruited from different locations. The only significant association was a high MPOD in subjects with red hair.

Some of the studies conducted in the US by Hammond and co-workers have used the same technique, which does allow for valid comparison of data obtained in the different geographical locations within the US. A large study was performed by Ciulla et al. (2001), using HFP to assess MPOD in a group of 280 healthy adults from the Indianapolis area in the Midwestern US. Overall, the mean MPOD was 0.21 (SD 0.13) log units. Using the same technique and stimulus attributes, Hammond and Caruso-Avery (2000) measured MPOD in 217 men and women from Pheonix, Arizona. The mean MPOD in this group was 0.22 (SD 0.13) log units and thus it appears that the means of samples taken from mid-western and South-western populations are no different. The mean MPOD seems low in both studies compared to other evaluations of MPOD using HFP and is likely to be a result of the extrafoveal flicker measurement was made at 4° eccentricity.

In summary, we find very little data to date that allow insight into whether any systematic variation exists in

Table 3
Mean MPOD obtained for samples of 30 or more normal subjects

Author and year	Location	Method	Number of subjects	Subgroup	MPOD (log units)	SD
Davies and Morland (this article)	UK	Colour Matching	30		0.56	
Bone and Sparrock (1971)	Jamaica	HFP	49		0.49–0.69	
Beatty et al. (2001)	UK	HFP	46	OD	0.29	0.16
				OS	0.30	0.16
Davies and Morland (2002)	UK	Colour matching	34		0.32	0.24
Mellerio et al. (2002)	UK	HFP	124		0.41	0.16
Broekmans et al. (2002)	Netherlands	Spectral reflectance	376		0.33	0.15
Berendschot et al. (2002)	Netherlands	Spectral reflectance	289		0.33	0.16
Hammond et al. (1996c)	Boston, USA	HFP	30		0.34	0.15
Hammond et al. (1996a)	Boston and New Hampshire, USA	HFP	88	Males	0.38	0.22
				Females	0.24	0.16
Hammond et al. (1996b)	Boston and New Hampshire, USA	HFP	95	Blue/Grey	0.25	0.20
				Green/Hazel	0.32	0.15
				Brown/Black	0.38	0.24
Ciulla et al. (2001)	Mid West USA	HFP‡	280		0.21	0.13
Hammond and Caruso-Avery (2000)	South West USA	HFP‡	217		0.22	0.13
Chang et al. (2002)	Taiwan	Fundus reflectometry	55		0.23	0.07

Those studies marked with the symbol ‡ can be compared with each other on the basis that the technique and stimulus attributes are the same. Although other studies have employed the same technique, the stimulus attributes vary; so direct comparison of mean MPOD values is not possible. The column labelled 'Subgroup' indicates which subgroups were investigated in studies that differentiated their normal subjects into different categories.

MPOD in different populations across the world. It would require significant dedication and time to investigate this, but it is entirely possible. Would such an investigation be worthwhile? We believe that it would be; the results of a large study using one or more methods would indicate if some populations exposed to highly different environments have different MP levels. This in turn may indicate whether MP does indeed play an active physiological role in humans.

8.6. Other factors influencing MPOD in normal populations

8.6.1. Tobacco smoking

Levels of MP were first measured in smokers in comparison with non-smokers by Hammond et al. (1996c). Increased oxidative stress may be one of the causative factors in several smoking-related diseases and the study was performed to investigate the possible relationship between smoking and macular carotenoid levels. Smoking has been shown to significantly decrease levels of carotenoids in serum and also to increase the risk of neovascular age-related maculopathy (Klein et al., 1993; Paetkau et al., 1978; Snodderly, 1995; Vingerling et al., 1996).

Thirty-four smokers and 34 non-smokers were studied. There were no differences in age, weight, different skin tones, hair or iris colour between the two groups. The results showed a significantly lower MPOD in the smokers in comparison with non-smokers (mean MPOD 0.16, SD 0.12 vs. 0.34, SD 0.15; $p<0.0001$). The difference in MPOD could not be explained by differences in dietary intake of carotenoids assessed by questionnaire. Also, the reduction in MPOD in the smokers showed an inverse relationship with the number of cigarettes smoked; smoking more than 25 cigarettes per day having a pronounced effect on MPOD.

Following this study, other studies have included smoking as part of a sub-analysis. In the Southwest USA, smoking has been identified with a low MP density (Hammond and Caruso-Avery, 2000), but no significant relationship was found in a Midwest population (Ciulla et al., 2001).

As a part of the validation of different measurement techniques, Delori et al. (2001) assessed MPOD in 27 smokers compared with 102 non-smokers. The results showed a small but statistically significant reduction in MPOD measured using lipofuscin autofluorescence (mean 0.40 ± 0.15 vs. 0.49 ± 0.16 log units). No difference was noted between the two groups when MPOD was measured using fundus reflectance, however. Details of the amount of tobacco consumed were not given. Using HFP alone, Mellerio et al. (2002) also noted a smoking-related reduction in pigment density.

These studies indicate that the MPOD is likely to be reduced by tobacco smoking, most particularly in heavy consumption (>25 cigarettes per day). The implications of this finding are discussed more fully in the context of age-related maculopathy.

8.6.2. Iris colour

A study published in 1996 was specifically designed to investigate whether there is an association between iris colour and MPOD (Hammond et al., 1996b). This study was performed based on the finding that a dark iris was associated with reduced risk of AMD (Hyman et al., 1983; Weiter et al., 1985). It is known that eyes with light-coloured irides transmit significantly more light than those with dark irides (van den Berg et al., 1991) and the hypothesis was suggested that MP levels are lower in eyes with light iris colour because of increased oxidative stress due to light exposure.

Ninety-five non-smokers participated in the study and iris colour was classified into one of three groups, blue or grey, green or hazel and brown or black. There was a reasonably even distribution of gender, race and age between the groups. MPOD was measured using HFP and serum L and Z levels by HPLC. There was no significant difference between the serum levels of L and Z in the three groups, nor of dietary carotenoid intake.

There was a significant difference in MPOD between those with blue/grey and brown/black iris colour, but no difference between those with green/hazel and brown/black irides or blue/grey and green/hazel irides. There was no difference in MPOD with respect to gender.

8.6.3. Lens density

The presence of carotenoids in the crystalline lens has been documented by Yeum et al. (1995, 1999) and Bernstein et al. (2001). Hammond measured both MPOD using HFP and lens optical density using scotopic sensitivity in a total of 41 subjects (Hammond et al., 1997b). They found that there was no relationship between MPOD and lens OD for subjects aged 24–36 years. A significant relationship was found, however, for middle-aged subjects (range 48–66 years) and even more so for older subjects (age range 67–82 years).

The finding of a lower MP level in the eyes with lenses that are more optically dense was used to support the hypothesis that retinal MP levels act as markers for lenticular MP levels, and that lenticular carotenoids act to protect the lens from age-related increase in lens density.

We can suggest an alternative hypothesis for the above finding. Retinae screened from high-energy light (by the lens) do not need to accumulate MP as a protection against its effects. As the mechanisms controlling incorporation of MP into the retina are not understood, it does not seem implausible to suggest that MP levels may be controlled partly by the amount

of incident light. This hypothesis could also be employed to explain our findings in patients with diabetes (Davies and Morland, 2002) (see later).

8.6.4. Obesity

Lutein and zeaxanthin are stored in greatest proportion in body fat and obesity is known to be a risk factor for age-relatel macular degeneration (AMD) (Schaumberg et al., 2001). To investigate whether total body fat and body mass index (BMI) influence the MPOD, Hammond et al. (2002) undertook a study of 680 individuals. MPOD was measured using HFP, and data were collected on BMI and body fat percentage. There was a significant negative relationship between MPOD and both BMI and percentage body fat. It was made note of that this relationship was mainly the result of the significantly lower pigment density (21%) in subjects with a BMI greater than 29.

9. Macular pigmentation in disease

9.1. AMD

AMD is the commonest form of blindness in the developed world. The mechanisms underlying its pathogenesis are not fully elucidated and treatment options once the disease is established are limited. There is evidence that photocoagulative and, more recently photodynamic laser, treatment ('Report' 2000; Barbazetto et al., 2003) is beneficial in some subgroups and in other subgroups there is now evidence that anti-oxidants have a protective effect (ARED report no. 8, 2001). Although it is not within the scope of this article to investigate the pathogenesis of AMD in detail, here we review the data relevant to AMD and the carotenoids.

There is epidemiological evidence that people at a higher risk of AMD have lower intake and serum levels of L and Z (EDCC). Other risk factors for AMD include being female gender, smoking tobacco (Klein et al., 1993; Paetkau et al., 1978; Snodderly, 1995; Vingerling et al., 1996), having a light iris colour (Sandberg et al., 1994), and obesity (Schaumberg et al., 2001). In a search for a common thread with these risk factors, Hammond et al. have conducted a series of studies measuring the MPOD as a function of the above parameters in isolation (see Section 8 for discussion of these results). All of the studies found a relationship with low MP levels in the variables studied. This finding, coupled with the knowledge that MP can act as antioxidants, that the macula is a site with great potential for oxidative stress (see later) and in particular the hypothesized role that oxidative stress plays in the pathogenesis of AMD (Beatty et al., 2000a) may indicate that the MPs could have a role in protecting the macula from the changes that ultimately lead to AMD. Another study noted that the fovea can be preserved and the perifovea affected by degeneration (Weiter et al., 1988), suggesting that the fovea is protected by the high density of MP located there. (From the evolutionary point of view, we wonder what mechanism could have driven the accumulation of a substance that protects the animal from a disease that only occurs after the age of reproduction and rearing.)

Below we review the results of studies that have been designed to measure the MPOD in patients with or at risk of AMD. Beatty et al. (2001) used HFP to measure MPOD in a small group of 9 subjects at high risk of age-related macular degeneration and in a group of 46 healthy volunteers in the age range 21–81 years.

The eyes at risk of AMD were chosen from 9 patients who had advanced neovascular AMD in the fellow eye and yet no macular abnormality in the study eye. This prerequisite for the study was necessary as the study used HFP and thus requires the assumption of normal receptoral function and comparable spectral sensitivity foveally and extrafoveally. However, it should be pointed out that the patients in this category form an unusual group. AMD is predominantly a bilateral condition and most patients with advanced neovascular change in one eye have a degree of AMD in the fellow eye. This is borne out in the small number of 'high-risk' eyes studied. It is also known that fellow eyes with no macular abnormality have the lowest risk of progression to neovascular AMD in comparison with fellow eyes with soft drusen, focal hyperpigmentation and systemic hypertension (Bressler, 2001). The results showed that the high-risk eyes had a significantly lower MPOD in comparison with healthy subjects (0.147 ± 0.144 log units vs 0.331 ± 0.206 log units, $p = 0.015$, Wilcoxon ranked sum test) after matching for age, gender, iris colour, smoking habits and lens density.

Using Raman spectroscopy, Bernstein et al. measured MPOD in 220 eyes of 18 normals and 93 eyes of 68 patients with AMD (Bernstein and Gellermann, 2002). The results from both right and left eyes were pooled. The mean spectrometer counts were given from a subset of the control group (those greater than 60 years) and in AMD patients taking supplements containing $\geqslant 4$ mg lutein and those whose supplemental intake of lutein was zero or less than 4 mg/day. The counts were 219 ± 134 for controls, 212 ± 169 for AMD taking lutein and 148 ± 147 for the no-supplement group. This result indicates that the patients with AMD not taking any lutein supplement had a significantly lower MP level (32%) than age-similar normals or those taking lutein. A calibration figure is given in the paper for spectrometer counts as a function of MPOD (based on a standard sample). Using this graph we find that the levels of MPOD in all groups are very low—a count of 200 gives an MPOD of slightly less that 0.05 log units.

Using spectral fundus reflectance, Berendschot et al. (2002b) measured MPOD in a group of 289 eyes with no

AMD and 146 eyes with AMD (single eye per subject). All participants were $\geqslant 55$ years of age. It was made note of that none of the subjects were using lutein supplementation at the time of measurement. The MPOD was measured using a specific optical model (van de Kraats et al., 1996). The effect of drusen was ignored in the model. However, the results suggested a spectrally flat reflectance from the drusen, which would not affect the determination of MPOD. Interestingly, the results of this study showed a mean MPOD in the control group of 0.33 ± 0.15 and 0.33 ± 0.16 in eyes with any stage of AMD (analysis used a general linear model of age and AMD stage).

9.2. Diabetes

We used colour matching to assess MPOD (Ruddock, 1963) in a group of 34 patients with diabetes and 34 healthy controls (Davies and Morland, 2002). The Wright tristimulus colorimeter was used (Wright, 1939), with a test field consisting of 490 nm desaturated with 650 nm and matching fields of 460 and 530 nm, desaturated with 650 nm. The field was a bipartite square of angular dimension $1^\circ 20''$ and the extrafoveal match made at 5° of eccentricity. In this study, the mean MPOD of the normal subjects was 0.32 ± 0.24 log units and of the diabetic subjects 0.13 ± 0.20 log units ($p = 0.0015$). The MOPD showed a negative relationship with increasing grade of maculopathy. We modelled the change that would be expected in measurement of MPOD based on differential receptor dysfunction at foveal and extrafoveal locations (see above) and found that the effect on MPOD measurement was small and could not explain the results observed. The findings of this study are interesting, as it can be argued that the macula in diabetes is prone to greater oxidative stress than without and that the development of anatomical changes may be a good marker of the severity of the retinal microvascular disease.

To date, we are not aware of any other studies that have measured MPOD in patients with diabetes.

9.3. Other conditions

9.3.1. Albinism

The study of MPOD in human albinism has revealed that negligible pigment can be detected in such individuals. This has been revealed by fundus photography by Abadi and Cox (1992). In this case, an objective measurement is particularly useful, because behavioural tests applied to subjects with albinism will be prone to underestimate pigment density because of the nystagmus that these subjects frequently display. Behavioural measurements in subjects with albinism and very little nystagmus undertaken in our laboratory have confirmed the findings of Abadi and Cox (1992) with a group of eight oculocutaneous albinos having a mean density of 0.04 that was not significantly different from zero. It is interesting that individuals with very low levels of ocular melanin also have negligible levels of the MPs that are derived from dietary intake of carotenoids. It remains to be seen if the retinae of such subjects are devoid of the pigments, or whether measurements are incapable of finding the concentration of them in the macula that is typical in normal subjects. It is certain that subjects with albinism do not have a normally developed macula, so perhaps this structural abnormality prevents the appropriate accumulation of the relevant pigmentation in one area of the retina. A comparison of MP levels in patients with aniridia may be fruitful in terms of disambiguating the relative role of hypopigmentation and abnormal foveal development on MPOD.

9.3.2. Choroideraemia

MP levels and macular function were measured in a group of patients with choroideraemia (Duncan et al., 2002). This inherited disease causes progressive degeneration of photoreceptors, RPE and choroid. Thirteen patients with chorioderaemia and 40 controls took part. Lutein supplement in a dose of 20 mg/day was taken for a period of 6 months. The MP levels prior to supplement were not different in the two groups and both showed a rise in MPOD with the supplement. The patients with chorioderaemia had reduced central rod and cone function, as measured with two-colour dark adapted sensitivity. There was no change in the retinal sensitivity after the supplementation period. This study concluded that there was no short-term benefit of oral supplement in this group. The need for a longer term study on the effects of oral MP supplement was noted.

9.3.3. Retinitis pigmentosa

MPOD was measured using HFP in patients with RP and Usher's syndrome (Aleman et al., 2001). The MPOD in the patients with RP was similar to that of normals and bore the same relationship with markers of low MP in normals (i.e. lower in females, smokers and persons with light-coloured irides). Oral supplementation with lutein resulted in a rise in MPOD in only half of the patients and there was no detectable change in central visual function.

10. Dietary supplement of macular pigmentation

The fact that the MPs are entirely of dietary origin and the thought that they may play some role in protecting the macula has encouraged researchers to investigate the effect that increasing consumption of L & Z has on the MP density in the eye. Landrum et al. (1997) performed a study of lutein supplementation on

two subjects for a period of 140 days and followed them for 1 year. Lutein esters were given (derived from marigolds) at a dose of 30 mg/day. MPOD was measured using HFP 4–5 times per week and serum concentrations determined by HPLC. For both subjects there was a clear rise in MPOD for both eyes (39% for one subject and 21% for the other), which reached a plateau around 50 days after cessation of supplement. This rise in MPOD was maintained for the duration of measurement.

In another study, Hammond et al. (1997a) used dietary supplement with spinach and sweetcorn to give an intake of approximately 10 mg of lutein and 0.7 mg zeaxanthin. Thirteen persons participated in the study, of which 12 adhered to the dietary supplement for 15 weeks. Serial measurements were made using HFP and serum levels monitored using HPLC. Analysis of results indicated three different responses to the supplement of spinach and sweetcorn. The first group (8/11) had increases of both serum and retinal levels of MP. The second (2/11) had a serum, but not a retinal response, and one subject showed neither a serum nor a retinal response. The two other subjects were given sweetcorn only (i.e. zeaxanthin supplement only) and one showed a significant rise in MPOD, whilst the other did not.

Two different MP measurement techniques were used by Berendschot et al. (2000) to assess the effect of lutein supplement on eight male volunteers. A 10 mg daily dose of lutein was taken and MPOD measured using log reflectance maps obtained by a scanning laser ophthalmoscope and also using spectral reflectance. All subjects showed a linear rise in MPOD with a mean 4-week increase of 5.3%. This study is the first to use an objective technique to measure the effect of lutein supplement on MPOD.

More recently, a further supplement study reported the results of HFP measurements on 38 subjects, with different dosages of lutein and zeaxanthin. This allowed the estimation of the serum response with respect to L + Z dose and also the MPOD increase with respect to the serum response (Bone et al., 2003). Overall, the response looked linear, with two-thirds of the variance of serum levels attributable to oral dose and one-third of the variance of MPOD attributable to serum levels.

These studies clearly indicate that increasing dietary consumption of the macular carotenoids can raise both the serum and retinal levels of MP. Although the response is variable across different individuals, there exists the possibility that oral supplement of MP could be used for either visual or therapeutic purposes.

11. Putative roles for MP

The presence of two selected carotenoids in the macula is generally assumed to imply that they serve some useful function for the animal. From an evolutionary perspective, it could be argued that the MP confers survival advantage to the animal and should do so within its reproductive lifespan. Two roles for MP (not mutually exclusive) have been proposed for their function, and in this section we review the evidence for both.

11.1. Role in improving visual function

Improving visual function would seem to us to be the most logical role for MP. As the macula is specialized for high spatial resolution and for colour vision, it would seem that MP could be involved in these processes.

11.1.1. Chromatic aberration

The eye suffers from a relatively large amount of chromatic aberration. Longitudinal chromatic aberration (LCA) results from the dispersion characteristics of the ocular media. With the preferred accommodation on the wavelengths of peak sensitivity (550 nm), the SW light is focused anterior and the LW light posterior to the retina. This results in a penumbra on the retina of both blue and red light. The amount of LCA in the eye has been measured experimentally (Bedford and Wysecki, 1957) and is relatively constant across individuals (Fig. 8). The range is approximately 2.1 D from 400 to 700 nm. With the eye accommodated on 550 nm light, light of 460 nm has a defocus of approximately −1.2 D. Due to the dispersion, the LCA for longer wavelengths is less, being around +0.5 D for 650 nm light.

Transverse chromatic aberration (TCA) also affects the eye, and its effects have been studied in less detail. TCA results in LW light being deviated less than SW light. This has the effect of giving a red blur at the edge of the image. Taken together and when viewing white light, LCA and TCA would result in the presence of a purple penumbra to the image.

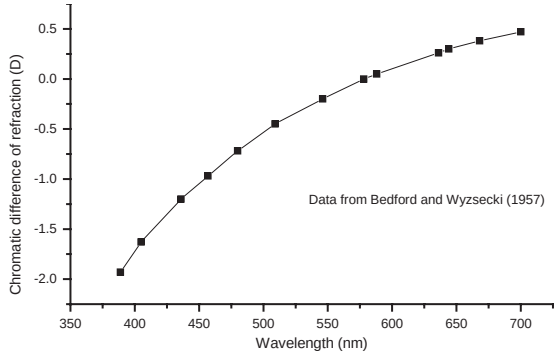

Fig. 8. Longitudinal chromatic aberration of the eye. Data from Wysecki and Stiles (1982) Table 1 (2.4.3) (originally reported by Bedford and Wysecki, 1957).

In 1866, Schultze proposed a role for MP of absorbing sufficient SW light to reduce the blur in the retinal image resulting from LCA. This was investigated by Reading and Weale (1974), who showed that a filter required to reduce radiance of the SW blur circle to a subthreshold value covered a spectral range similar to that of the MP.

This led to the hypothesis that the role of MP is to reduce SW chromatic blur and thus to enhance spatial vision. In their article in this journal, Wooten and Hammond (2002) have termed this the 'Acuity Hypothesis'. They note that there have been many trials of the use of additional filters to enhance spatial vision. When measuring high-contrast acuity, there is no proven benefit and even when considering contrast sensitivity, some studies have produced positive results and others have found no effect. Overall, all studies have shown that there are wide-ranging effects across different individuals, some experiencing visual improvement, others no change and others detriment to their vision (Kelly et al., 1984; Wolffsohn et al., 2000). Amazingly enough, no study has measured MPOD in the participants and correlated this with any improvement in visual function with the use of a supplemental filter. A hypothesis to explain the wide variation of response to supplemental filters is that those subjects with high MP levels would experience little or no visual enhancement, whilst those with lower pigment levels may experience a greater effect.

This does, however, raise the important issue of why the MP levels are so variable across individuals. If MP does indeed improve vision by removing the effect of LCA, why are MP levels not uniformly high (or controlled to lie within a narrow range), particularly as the effects of LCA are consistent across individuals?

It has recently been suggested that retinal image quality is in fact relatively independent of wavelength (McLellan et al., 2002) and that the quality of the image for shorter wavelengths is not degraded by LCA. This study used a spatially resolved refractometer (Marcos et al., 1999) to measure wavefront aberration data as a function of wavelength in three individuals. Wave aberration data were used to calculate the modulation transfer function (MTF) of the eye and this was used as the metric of image quality.

Aberrations were measured at six wavelengths (450, 490, 530, 570 and 650 nm). The area under the MTF was examined as a function of wavelength and compared with a theoretical MTF for a model eye with LCA only. The results showed a relatively flat function for all three subjects, whereas for the model eye the MTF area decreased significantly as wavelength deviated from the optimal focus of 550 nm. It should be noted, however, that the subjects' pupils were dilated with 0.5% tropicamide only. At this concentration, tropicamide will allow some pupillary dilatation but is unlikely to paralyse accommodation. It is possible that in the course of the measurements the subjects were accommodating onto the lights at the different wavelengths used and thus reduced any blur from LCA, artificially improving the MTF. An optical channel was included in the system with a background of text to give an accommodative target but this may not provide hard control of accommodative state. To allow a formal assessment of MTF as a function of wavelength in the presence of LCA, it would be best that the subjects undergo complete cycloplegia with several drops of cyclopentolate or indeed atropine. Also, the study was conducted with a pupil diameter of 6 mm and at a relatively low luminance of 100 cd/m^2. It may not be safe to generalize the results to the smaller pupil that occurs in natural light of significantly higher luminance. Perhaps firmer evidence questioning the role of MP in this context could be attained by a more comprehensive experiment with subjects under cycloplegia and measurements made over a wider range of pupil sizes.

As a final point, in an earlier study by the same group (Marcos et al., 1999), the analysis of optical quality with respect to wavelength was presented in three ways: volume under the MTF, RMS wavefront error and the Strehl ratio. Interestingly, the volume under the MTF for one subject decreased with increasing wavelength (even when truncated for spatial frequencies >100 cpd). The RMS value was relatively flat with respect to wavelength (RMS indicates the change in phase of the pupil function but does not include diffraction effects). The Strehl ratio, however, increased significantly with increasing wavelength. These different measures gave optical quality metrics that were different for the same individual and one must be careful about the interpretation.

In summary, we note that the evidence for and against this role for MP is associative only and that further study needs to be undertaken to confirm or refute the hypothesis that MP improves visual function by reducing the effect of chromatic aberration.

11.1.2. Visibility

Wooten and Hammond (2002) propose another hypothesis of how MP may improve our vision. The physics of light scatter is such that SW light is scattered more than longer wavelengths both by air molecules (Rayleigh scatter model) and by larger particles in the atmosphere (haze aerosol, treated with the Mie scatter model). Both of these lead to the blue colour of the sky and the blue haze attained by objects viewed in the distance. They show that a yellow filter can increase the visibility of a target viewed against a background by reducing the blue of the background. At the same time, the spectral energy of any target is reduced in the shorter wavelengths by scatter. Hence, the overall effect of a

yellow filter will be to reduce the luminance of the background with respect to the target, increasing its contrast.

Further calculation showed that the visibility range of an object seen at 10 km with an MPOD of 0.0 is increased to 11.9 km with MPOD of 0.5 log units. The corollary of this is that in the presence of MP, nearer objects may become visible in the presence of MP that would otherwise have remained sub-threshold. The visibility hypothesis is attractive, but the gains in visual function are rather modest.

11.2. MPs as antioxidants

The macula (and indeed the whole retina) is a very delicate tissue and as with all neural tissues does not have the ability to regenerate after damage. The retina serves a vital role for the animal as the transducer of the dominant sensory organ. Also, the retina is the most metabolically active tissue in the body and the photoreceptor layer is maintained at a high oxygen tension and contains a high concentration of polyunsaturated fatty acid (Anderson et al., 1984). This environment sets the scene for a tissue that is at risk of damage from two sources. Firstly photic, from the incident and absorbed light and secondly from internal processes, as both of these mechanisms can lead to the release of reactive oxygen species (superoxide anion, hydroxyl free radical and hydroperoxyl radicals).

Ham (Ham et al., 1978) measured the damaging effects of SW light on rhesus monkey retina. Depending on exposure duration, the power required to result in photic damage was 70–1000 times lower for 441 nm light than for infrared light of 1064 nm (durations from 1 to 1000 s).

From these findings, it is apparent that the macula is prone to irreversible damage from light of SW and it therefore seems logical to propose that during evolution protective mechanisms may have developed if such damage were likely to occur during the reproductive age of the animal. Thus, the second major hypothesized role for the MP is as an antioxidant.

The MP could have two distinct roles in acting as a protector of the macula. Firstly, given its location as a prereceptoral filter (Snodderly et al., 1984a), to protect the macula by reducing the amount of SW light reaching the photoreceptor outer segments and secondly by acting directly as a scavenger of reactive oxygen species once liberated; either from light-induced damage or from other internal mechanisms.

Considering the photic damage hypothesis, it is interesting to note that MP is not concentrated in the inferior retina. With blue-sky overhead, most of the SW light will be imaged on the inferior retina and we do not observe inferior retinal changes routinely in subjects whose retinae are exposed to such influence. This observation may go against the SW damage idea, except to point out the counter argument that the macula is a specialized area of the retina with the highest receptor density and thus may be more prone to damage derived from light exposure.

Here we examine the evidence that MP plays a protective role for the preservation of the neural elements that initiate our central vision. The mechanisms for retinal damage by reactive oxygen species have been reviewed by Beatty (Beatty et al., 2000a) in particular in relation to age-related macular degeneration and the interested reader is referred to this article.

The carotenoids as a family have clear antioxidant properties and have been shown to react with singlet oxygen, free radicals and also to prevent lipid peroxidation (Khachik et al., 1997). Also, oxidation products of lutein and zeaxanthin have been identified in the retina (Khachik et al., 1997).

The anatomical location of lutein and zeaxanthin in the photoreceptor axons and in the inner plexiform layer (Snodderly et al., 1984a) will shield photoreceptors from SW light, but MP is not well located here to act as a scavenger of free radicals and oxygen species released near the photoreceptor outer segments. In view of this, two studies have been performed to investigate whether MP is also found associated with the receptoral membranes (Rapp et al., 2000; Sommerburg et al., 1999). It was found that lutein and zeaxanthin are both present in the membranes of rod outer segments, representing somewhere between 10% (Rapp et al., 2000) and 25% (Sommerburg et al., 1999) of the total retinal amount of MP. We are not aware of any studies investigating specifically the presence of MP in cone receptor outer segments.

The effect of MP as an antioxidant in the central retina has been investigated in several studies. The earliest was that by Haegerstrom-Portnoy (1988). In this study, S- and L-cone sensitivities were measured across the central retina in two groups of normals. In the older group, there was a significant differential loss of S-cone sensitivity across the retina compared to the younger group, with increased S-cone sensitivity loss away from the fovea. No such change was noted for the L-cones. This study was interpreted as supporting the hypothesis that MP protects the retina from light-induced damage. Weiter et al. (1988) noted that there are some subjects in whom the central fovea is preserved whilst the perifoveal tissue degenerates. This again may imply a protective role of the MP.

A more detailed study has been performed more recently, where lens density, MPOD and Stiles π_1 increment (related to S-cone) thresholds were measured in a group of normals over a wide age range (Hammond et al., 1998). The MPOD was measured using HFP and the lens density using equivalent rhodopsin thresholds in the dark-adapted eye. The Stiles π_1 sensitivity was

measured at 440 and 500 nm for a 1° foveal target. This study found that there is a reduction of visual sensitivity in the $\pi - 1$ mechanism (corrected for both MPOD and lens density) with increasing age. The decline in sensitivity was correlated with MPOD, in that subjects with a low MP (0–0.39) showed a significant age-related reduction in sensitivity at 440 nm, whilst those with a high MP density (0.40–0.97 log units) had no age-related decline. Foveal sensitivity as a function of MPOD also showed a correlation for the older subjects. Those aged 60–84 years had reduced foveal sensitivity as MPOD declined, whereas the younger group (24–36 years) did not show such a relationship. It seems reasonable to conclude from this that higher levels of MP are associated with maintenance of foveal S-cone function in older persons, although this association may not be causal.

Studies of oral supplementation with the macular carotenoids are underway in patients with AMD. One study investigated the effects of antioxidant supplement on central retinal electrophysiological function in a group of patients with AMD (Falsini et al., 2003). Here, the supplement consisted of lutein (10 mg), vitamin E (20 mg) and nicotinamide (18 mg). Focal electroretinograms were used to assess central retinal response and the supplements were taken for a period of 180 days. The results showed a significant increase in the ERG amplitude in the patients with AMD taking the supplement in comparison with those not taking it. Furthermore, the values returned toward the pre-treatment levels 180 days after cessation of supplement. The control group showed a similar increase in amplitude at 180 days. Unfortunately, in this study, no measurements of serum or macular levels of MP were made, which makes it impossible to draw conclusions about the benefit the lutein supplement alone.

The Lutein Antioxidant Supplementation Trial (Richer et al., 2002) has been published in abstract form only and consisted of lutein vs. lutein/antioxidants supplements in a group of 90 elderly patients with AMD. MPOD increased on average by 0.09 log units (assessed by HFP), and there was a significant improvement in glare recovery, contrast sensitivity and both near and distance acuities in both treatment groups. We await the full publication of this and of other ongoing trials into the effect of MP supplements with great interest.

12. Outstanding issues

In this section, we present some issues that we believe require investigation. The reader is also directed to the previous section in which various potential avenues of study that should shed light on the role of the MP are also outlined.

12.1. The importance of measuring the distribution or peak MPOD

A very interesting result has recently come to light: Peak MPOD measured for a small central region of the fovea does not correlate well with the overall level of pigmentation within the macula (Robson et al., 2003). Given the increasing prominence of the hypothesis that increased macular pigmentation may help prevent AMD, it seems timely to question whether it is the overall pigmentation of the macula or the peak MPOD that plays the most significant protective role. It would be valuable if future works explicitly evaluate which of the two factors is most important in preserving visual function and whether either or both can be modulated by significantly by dietary supplement.

12.2. How should macular pigmentation be measured?

We reviewed psychophysical and objective methods for evaluating macular pigmentation. In our view, there is an increasing need to obtain as much information about macular pigmentation as possible, not least because of the issue we described above. We believe, therefore, that the objective methods, which can readily obtain estimates of peak MPOD and information on the spatial distribution of MPs, should be preferred. However, objective methods appear to provide consistently lower estimates of MPOD. This does not render these methods invalid as long as they are reliable and reproducible, which has been shown to be the case most particularly for AF. Imaging methods also offer a great advantage of not requiring the subject/patient to undergo demanding observations. This is a key issue for work that must be undertaken on elderly subjects, those with macular pathology or ocular instability. It must be noted, however, that light scatter in the ocular media, which increases with age, could reduce the accuracy of MPOD estimates with many objective measures.

Although objective techniques are the most desirable procedures to take forward, it must be recognized that the methods are currently used in the few laboratories with the necessary sophisticated equipment. This provides a reason why psychophysical procedures have been the ones that most large-scale surveys of MPOD have adopted. In our view, there is now a demand for an objective imaging technique that can be readily implemented to measure MPOD and MP spatial distribution quickly and reliably. A modification to a standard imaging ophthalmoscope would be most appropriate and would allow ophthalmologists to readily acquire data on macular pigmentation on large number of subjects. Moreover, such a modified instrument would enable the clinician to monitor the effect of dietary

supplementation on macular pigmentation and perform longitudinal studies.

12.3. Can macular pigmentation be modulated to serve a protective role?

There appears to be converging evidence that macular pigmentation can be enhanced by increased intake of L and Z. In addition to evaluating the long-term effects of L and Z, more data on how macular pigmentation may be enhanced in disease is also required to add to the encouraging preliminary results reviewed in this article.

12.4. Correlation and causality

There is evidence that suggests lower levels of macular pigmentation in groups of subjects with AMD than in groups of control subjects. From this finding, it is tempting to draw the conclusion that low levels of macular pigmentation may be a causative factor in AMD. However, it is equally likely that lower levels of macular pigmentation may result from neural loss in the macula associated with AMD (i.e. a secondary effect). In order to resolve the presence and direction of a causal link between AMD and macular pigmentation, it is necessary to perform longitudinal studies that can follow subjects without AMD for sufficient time for AMD to develop in some but not others. It may also be of value to examine the relative proportions of oxidized products of L and Z in the diseased retina.

12.5. Mechanisms of deposition of the MPs

An issue that remains truly outstanding is how L and Z are deposited in the macula and how and if oxidized products of L and Z are removed from the retina. Addressing this issue will be a difficult task, but is fundamental to understanding how macular pigments may benefit individuals in health and disease. Genetic factors that influence the population variation of MPOD remain outstanding and work in this area may yield better understanding of the fundamental processes governing L and Z deposition in the macula.

13. Conclusions

The work over the past 50 years has identified and characterized the MPs in health and in different disease states. The relationship between dietary intake, serum levels and MPOD is better understood. There has been an increase in the number of techniques for in vivo measurement of MP; each method has its own advantages and disadvantages. We also note that the multitude of methods used across the world makes comparison of different works very difficult.

Perhaps, it is now time for the scientific community interested in MP to agree on a preferred or standardized technique for its measurement in humans. The design of studies needs to move away from the gathering of associative data and should aim towards understanding the underlying mechanisms with the aim of addressing causation of disease.

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