

The effects of supplementation with lutein and/or zeaxanthin on human macular pigment density and colour vision

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Abstract

Background: Both yellow-blue (YB) discrimination thresholds and macular pigment optical density (MPOD) measurements in the eye exhibit large variability in the normal population. Although it is well established that selective absorption of blue light by the macular pigment (MP) can significantly affect trichromatic colour matches, the extent to which the MP affects colour discrimination (CD) sensitivity remains controversial.

Objective: In this study, we assess whether the variability in YB thresholds is attributable to differences in MPOD, both at the fovea and in the paracentral visual field. We also investigated whether higher levels of MP offer any advantage in other visual functions such as red-green (RG) CD sensitivity.

Design: CD thresholds and spatial MPOD profiles were measured in 24 normal trichromats supplemented with zeaxanthin (OPTISHARP™) and/or lutein. Novel stimulus conditions that isolate YB and RG chromatic mechanisms were employed and MPOD profiles were measured up to an eccentricity of 8°.

Results: The data reveal an increase in MPOD in the supplemented subjects that was almost uniform within a centre region around the fovea subtending $\pm 4^\circ$. RG sensitivity was high in all subjects with thresholds well within the normal range. Unexpectedly, YB thresholds were also normal and showed no correlation with MPOD. A model for threshold CD based on appropriate combinations of cone contrast signals was developed to explain the experimental findings.

Conclusions: YB thresholds remain unaffected by supplementation with lutein and/or zeaxanthin rather, at increased MPOD levels, RG vision tends to be improved. The model accounts for the absence of correlation between MPOD and YB thresholds and predicts a marginal improvement in RG discrimination when MPOD is high.

Keywords: chromatic sensitivity, colour channels, cone-contrast, lutein, macular pigment, retinal carotenoids, zeaxanthin

Introduction

The macular pigment (MP) is composed predominantly of lutein (L) and zeaxanthin (Z) (Bone *et al.*, 1985), and

is of dietary origin (Sommerburg *et al.*, 1998). These carotenoids can be found in various body tissues; however, their concentration is highest in the macula (Landrum *et al.*, 1999) in the photoreceptor axons (Snodderly *et al.*, 1984a,b; Sommerburg *et al.*, 1999). The MP has band-pass spectral absorption characteristics with peak absorption at ~ 460 nm (Bone *et al.*, 1992), thus acting as a pre-receptor filter that selectively absorbs blue light. The spatial distribution of the MP peaks at the fovea and decreases rapidly with eccentricity (Hammond *et al.*, 1997a,b). Large,

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individual variations in macular pigment optical density (MPOD) have been reported with differences as large as 1 log unit (Ruddock, 1963). A number of putative roles have been proposed to justify the functional advantages of high MP density levels in the eye (Kirschfeld, 1982; Schalch, 1992; Khachik *et al.*, 1997; Davies and Morland, 2004). These include its antioxidant role that may reduce the risk of developing degenerative diseases of the macula such as age-related macular degeneration (ARMD) (Snodderly, 1995). The unwanted effects of chromatic aberrations (Reading and Weale, 1974) and 'blue haze' when viewing distant objects (Wooten and Hammond, 2002) can be reduced through selective absorption of blue light, and the corresponding reduction in the effectiveness of rod signals can, in principle, extend the superior characteristics of cone-mediated vision further into the mesopic range (Kvansakul *et al.*, 2004).

Two aspects of colour vision, colour matching and chromatic threshold sensitivity, have often been investigated in relation to the MP in the eye. Colour matching involves comparison of two suprathreshold coloured fields, a broadband test field that is matched in perceived colour and brightness with an appropriate mixture of three primary lights of different spectral composition, one of which is absorbed by the MP. The measurement of chromatic threshold sensitivity, on the other hand, does not require any judgement of stimulus colour and involves simple threshold detection measurements under stimulus conditions that isolate the use of colour signals (MacAdam, 1942; Barbur *et al.*, 1994). Colour matching provides a sensitive measure of differences in the colour appearance of two adjacent fields that reveals variations in the spectral responsivity of cone photopigments as well as differences in their optical densities. These factors contribute to the variability of colour matches, both in dichromats (Alpern and Pugh, 1977; Alpern, 1979) and in normal trichromats (Neitz and Jacobs, 1986). In addition, the spectrally selective absorption of light in various structures that precede the photoreceptor pigments in the eye and, in particular, the absorption of blue light by the MP and the human crystalline lens, also affect colour matches (Ruddock, 1965, 1972; van Norren and Vos, 1974; Pokorny *et al.*, 1987). The smallest difference in colour detected when a colour-defined test stimulus is presented against a uniform background is a measure of chromatic sensitivity (MacAdam, 1942). This task is functionally important and is fundamentally different from colour matching. It is generally accepted that colour threshold discrimination measurements involve only two chromatic mechanisms, a red-green (RG) mechanism that compares changes in the outputs of long (L)- and middle (M)-wavelength sensitive cones, and a yellow-blue (YB) mechanism that compares changes in the

output of short-wavelength (S) cones against the signals generated in L and M cones. Although existing findings demonstrate beyond doubt that selective absorption of blue light by the MP and the lens affects colour matching experiments, the extent to which this also affects RG and YB thresholds for detection of colour-defined stimuli is less well understood and under discussion (Moreland and Dain, 1995; Wolffsohn *et al.*, 2000). Improved techniques for the measurement of RG and YB chromatic sensitivity and MPOD make it possible to examine in greater detail the effects of supplementation with carotenoids on MPOD and chromatic sensitivity.

The purpose of this study was to assess how the spatial distribution of the MP changes as a result of supplementation with L and/or Z and to establish whether increased MPOD values reduce YB chromatic sensitivity. As supplementation with carotenoids has been shown to improve achromatic vision (Kvansakul *et al.*, 2004) we wanted to establish whether RG chromatic sensitivity may also benefit from increased amounts of MP in the eye.

Subjects and methods

Macular pigment measurements

A new heterochromatic flicker photometry technique was implemented on a CRT visual display and provides a rapid and convenient macular assessment profile (MAP) test. The MAP test is based on the use of an optical notch filter to separate the outputs of the three phosphors of the display into two components, one that is absorbed maximally by the MP and is derived only from the blue gun (i.e. the test beam) and the other that is based on a combination of red and green phosphor luminances and consists largely of long-wavelength light that is not absorbed by the MP (i.e. the reference beam). The luminance of the reference beam is 20 cd m⁻² and its modulation depth is fixed at 20%. The MAP test makes full use of the advantages of visual displays to produce stimuli of varying size at a number of randomised locations, to generate counter-phased sinusoidal modulation of the two stimulus beams (Schalch *et al.*, 2004). The frame rate of the display was 140 Hz and the stimulus modulation frequency was 20 Hz. The high temporal modulation frequency employed ensures that at the threshold one isolates the activity of luminance flicker detection mechanisms that rely only on the combined L and M cone signals. The stimulus was presented as a short burst of flicker of approximately 0.5 s duration and the subject's task was to report the presence or the absence of perceived flicker. A modified staircase procedure with variable step sizes was then used to measure the mean luminance of the test beam

needed to cancel the perception of flicker generated by the reference beam. The MAP test can be used to measure MPOD along any meridian at a number of specified locations from -8° to $+8^\circ$ eccentricity of the visual field (*Figure 1*). The test stimulus changes from a disc of 0.36° diameter, when presented at the fovea, to a sector annulus when presented at one of five discrete locations on either side of fixation across the horizontal meridian: $\pm 8^\circ$, $\pm 6^\circ$, $\pm 4^\circ$, $\pm 2.5^\circ$, $\pm 1.25^\circ$ and 0° . The width of the test annulus also increases systematically with eccentricity to facilitate the detection and the nulling of luminance flicker. Five, randomly interleaved, repeat measurements were taken at each spatial location investigated and these values are referenced with respect to 8° eccentricity on the assumption that the MP density at 8° is negligible. The test was performed at a viewing distance of 0.7 m and the stimulus was presented only to the right eye. Similar measurements made with the left eye confirmed previous findings (Handelman *et al.*, 1988; Robson *et al.*, 2003), which show good correlation in MPOD values between the two eyes. The MAP test was validated recently against MP measurements using a modified Moreland anomaloscope (Moreland and Kerr, 1979). As the latter instrument employs a narrow, short-wavelength beam with a peak output at 460 nm, the technique measures the peak optical density of the MP. The MAP test, on the other hand, employs a broader, short-wavelength beam and this slightly underestimates the peak MPOD. A simple photometric model that predicts how the mean optical density of the MP, measured with the MAP test, relates to its peak density (i.e. the optical density at 460 nm) was developed. Following correction for peak optical density, the results obtained on the two instruments were found to be in close agreement.

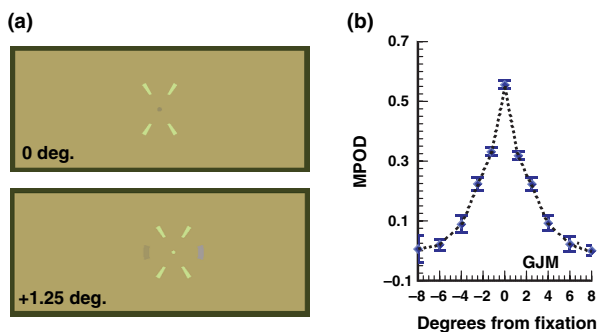


Figure 1. (a) Screen dumps showing the appearance of the stimulus employed to measure the optical density of the macular pigment (MP) for two locations centred 0° and 1.25° from fixation. At the centre of the fovea, the stimulus was a small disc and subtended 0.36° . For 1.25° eccentricity the stimulus was a sector annulus and was presented simultaneously on both sides of the fovea, an arrangement that facilitates steady fixation. (b) Example of macular pigment optical density profile obtained by measuring MP optical density at a number of retinal locations up to $\pm 8^\circ$.

Measurement of chromatic sensitivity

Chromatic detection thresholds were measured using the Colour Assessment and Diagnosis (CAD) test for a number of discrete directions in colour space in order to describe the sensitivity of both the RG and the YB chromatic channels (Rodriguez-Carmona *et al.*, 2005). The method employs dynamic random luminance contrast noise to isolate the use of colour signals by masking the detection of any residual luminance contrast (LC) components in the 'isoluminant', colour-defined test stimulus (Barbur *et al.*, 1994). The results are plotted in the CIE (Commission Internationale d'Eclairage) 1931 colour system (*Figure 2a*). The visual stimulus employed (see *Figure 2b*) is a square made up of 5×5 checks, defined by colour contrast (according to the CIE 1931 standard normal observer). The coloured stimulus is buried in a larger array of LC defined checks (15×15). During the test, the colour-defined test patch moves diagonally in one of four possible directions over a background of checks that vary randomly in luminance, every 53–80 ms, within a range specified as a percentage of the background luminance (e.g. $\pm 45\%$). The colour signal strength needed for threshold detection of the moving test stimulus for each hue direction investigated is measured using a four-alternative-forced-choice procedure (Barbur, 2004). The subject's task is to indicate or guess the direction of movement of the colour-defined stimulus by pressing one of four corresponding buttons. The mean RG and YB thresholds are computed by averaging the chromatic signal strengths measured in each of the eight, closely packed, hue directions that describe the orientation of each chromatic channel (see coloured symbols in *Figure 2a*). Examples of RG and BY thresholds measured at the fovea and 3° in the periphery for one subject are shown in *Figure 2c*. The data reveal the significantly higher thresholds for the YB channel, both at the fovea and 3° in the periphery.

Subjects

The subjects examined in this study were recruited initially for a larger carotenoid supplementation trial (Kopcke *et al.*, 2005; Schalch *et al.*, 2005), which began in 2002 and involved a total of 92 participants. The participants received supplementation consisting of L, Z, a combination of the two carotenoids or placebo for 12 months. The subjects were male, Caucasian and aged in the range of 22–39 years. The tenets of the Declaration of Helsinki were observed, and the study had the approval of the research and ethical committee of City University. All subjects gave informed consent before and during participation. A thorough optometric examination was carried out on each subject and no clinically

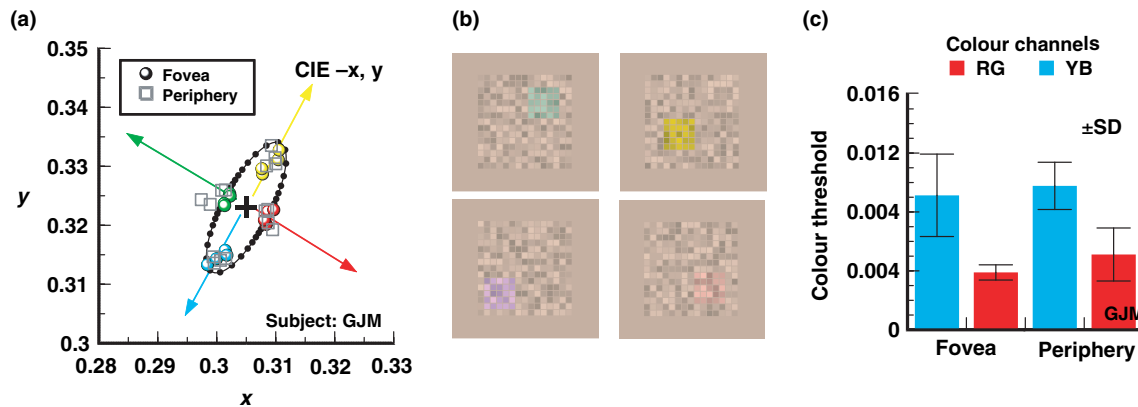


Figure 2. Colour detection thresholds for both yellow-blue (YB) and red-green (RG) discrimination measured with the Colour Assessment and Diagnosis (CAD) test. Typical results for one normal trichromat (a). The cross at the centre plots the (x, y)-chromaticity of the uniform background field and the coloured symbols show the thresholds measured in the yellow, green, blue and red directions. Measurements were taken at the fovea (solid discs) and 3° in the periphery (outline squares). The dotted ellipse plots the mean thresholds for the 'standard' CAD observer computed by averaging results for 125 subjects with normal colour vision (Rodriguez-Carmona *et al.*, 2005). (b) Screen dumps with the appearance of the visual stimulus employed. (c) Typical results for one subject at the fovea and in the periphery. The error bars show ±S.D.

detectable signs of ocular disease or other abnormalities were found.

A number of optometric and ophthalmologic tests were performed at the end of phase I (time *T1*) and phase II (time *T2*) on each subject group, as shown in *Table 1*. These tests included the measurement of wavefront aberrations, contrast acuity, scattered light and MPOD. The additional measurements needed for this study were made at the end of phase II (time *T2*) and 4 months after supplementation stopped, at the end of phase III (time *T3*), and involved measurement of MPOD profiles and YB and RG chromatic discrimin-

ation thresholds. At time *T3*, chromatic thresholds were measured at the fovea and at an eccentricity of 5°. At the same time additional measurements were made at the fovea and at an eccentricity of 3° using a smaller stimulus, as described in the caption to *Figure 2*. This was done to provide better spatial localisation of the stimulus. Two stimulus arrays placed along the horizontal meridian on each side of fixation were employed to help maintain steady fixation. Although these arrays were identical in terms of dynamic LC noise, only one of these arrays contained the colour-defined moving stimulus. The MAP test for assessment of the spatial

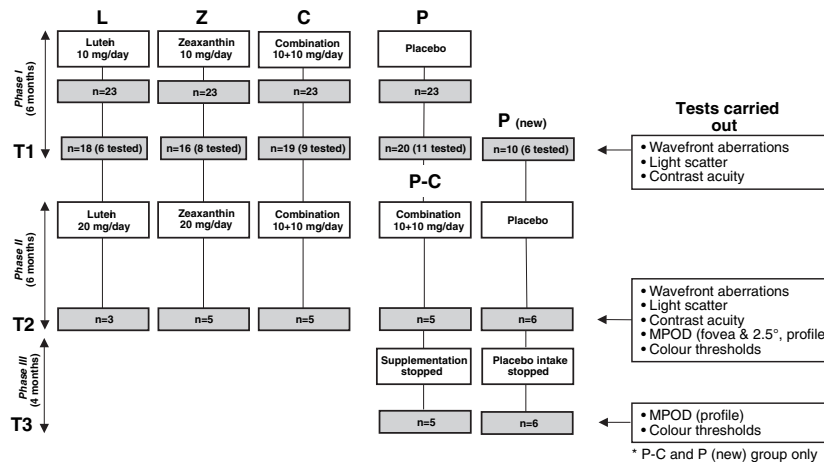


Table 1. Schematic diagram showing the various subject groups, the time course of supplementation with carotenoids and the various tests of visual performance carried out at key stages during the trial. Measurements of rms wavefront aberrations, contrast acuity, scattered light and MP density at the fovea and 2.5° in the periphery were carried out at the end of phase I (time *T1*), at the beginning of phase II (for the new placebo group) and at the end of phase II (time *T2*) for all subject groups. Colour thresholds and MP density profiles from -8° to +8° were measured at the end of phase II and phase III for the P-C and P groups only (times *T2* and *T3*, respectively). The various groups are as indicated in the table together with the amount of daily intake of carotenoids during supplementation. At the end of phase I, the P-C group was formed from enrolling all available subjects from the initial placebo group. The P-C group was then given combined L and Z supplementation for 6 months and 10 further subjects were recruited to form the new placebo group.

profile of MP was only available during phase II of the trial.

Of the 92 subjects recruited initially, only 24 subjects were available and willing to participate in the last two phases of the study (i.e. phases II and III).

The study involved the following subject numbers:

- L (10 mg of L day⁻¹ for 6 months followed by 20 mg of L day⁻¹ for another 6 months, *n* = 3)
- Z (10 mg of Z day⁻¹ for 6 months followed by 20 mg of Z day⁻¹ for another 6 months, *n* = 5)
- C (10 mg of L + 10 mg of Z day⁻¹ for 12 months, *n* = 5)
- P-C (placebo for 6 months followed by 10 mg of L + 10 mg of Z day⁻¹ for 6 months, *n* = 5)
- P (placebo for 10 months, *n* = 6)

For the second 6 months, during phase II of the study, the dose was doubled for the L and Z groups. The subjects in the P group at the end of phase I became part of a new group, the P-C group, and received combined supplementation for 6 months. More subjects were recruited and formed the new placebo group. This made it possible to compare the P-C group after only 6 months of supplementation with the new P group.

This arrangement enabled us to make group comparisons of changes in MP density and chromatic thresholds as a result of supplementation at the end of phase II (*T2*), and 4 months after the supplementation was stopped (at the end of phase III, time *T3*).

Data analysis and statistics

Analysis of variance (ANOVA) was used to compare mean estimates between groups of subjects. Scatter plots and regression analysis were used to examine the relation between MPOD and chromatic sensitivity thresholds. All analyses were performed with S-PLUS® 6.2 for Windows (Professional Edition; Insightful Corporation, Seattle, WA, USA).

Results

The first set of measurements taken in this study was carried out after 12 months of supplementation (at time *T2*), with doubled supplementation for the second 6 months of phase II (see *Table 1*).

Figure 3 shows MPODs measured in all subject groups, at the end of phase II of the study. For each group that received supplementation, the results show a significant increase in MP density (*p* < 0.05 for the C and P-C groups and *p* < 0.07 for the L and Z groups) by comparison with the placebo group. There was no statistically significant difference between the P-C group (after 6 months of supplementation) and any of the other groups (that received supplementation for 12 months). This result confirms previous findings showing that a diet rich in L and Z can cause increased levels of MP, at least in some individuals (Hammond *et al.*, 1997a; Landrum *et al.*, 1997; Berendschot *et al.*, 2000), and that increased MPOD levels could still be detected for at least 6 months after the diet was stopped (Hammond *et al.*, 1997a). The data shown in *Figure 3* were measured both at the fovea (a) and 2.5° in the periphery (b) using the new MAP test (see *Figure 1*). Panel (c) plots the actual increments in MPOD for each of the supplemented groups with respect to the placebo group, both for the fovea and for the periphery.

In order to evaluate the effect supplementation might have on the spatial distribution of the MP, measurements were made at a number of discrete eccentricities in the range +8° to -8°. These additional measurements were carried out only in the P-C and the P subject groups at time *T2* and at time *T3*, after 4 months without supplementation. *Figure 4a* reveals the clear differences in the spatial distribution of MP (measured at *T2*) for the supplemented and the placebo groups. The increase in MPOD values is significant (*p* < 0.025) in the P-C group when compared to placebo. The

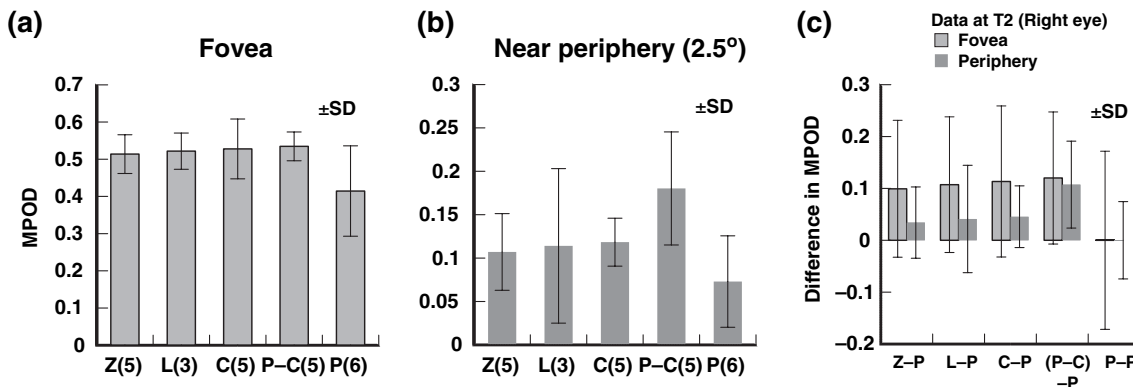


Figure 3. Estimates of mean macular pigment optical density (MPOD) for each group of subjects at the end of phase II of the supplementation study (see time *T2* in *Table 1*). Measurements were made at the fovea (a) and 2.5° in the periphery (b). The error bars indicate intersubject variability in each group. (c) The actual differences in mean MPOD values for each supplemented group with respect to the placebo group.

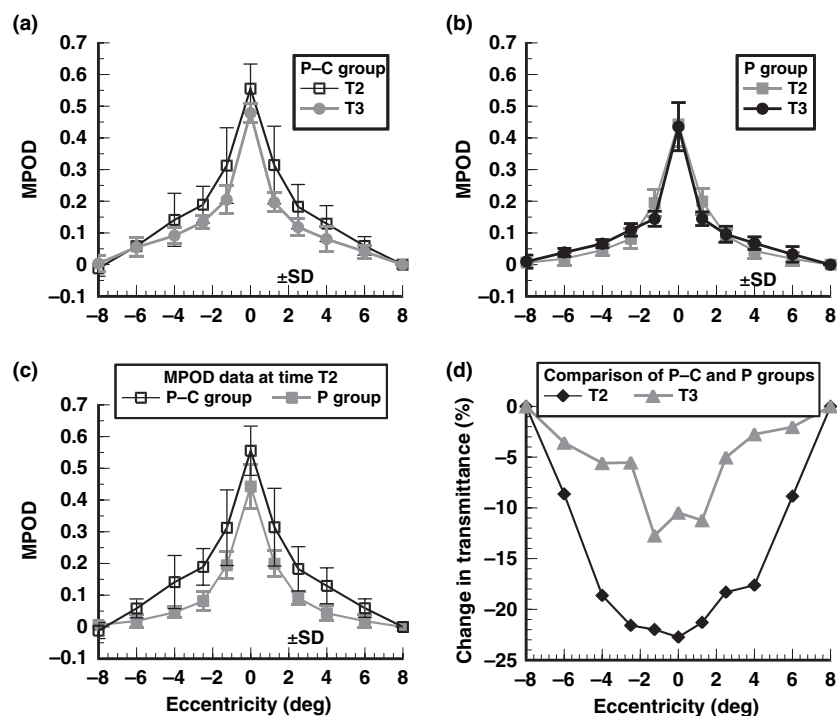


Figure 4. Comparison of MPOD spatial profiles for the P-C and the P groups measured along the horizontal meridian from $+8^\circ$ to -8° at different times during the study. Panel (a) shows data measured in the P-C group at the end of phase II (time T2) and after 4 months with no supplementation (time T3). Similar data for the P group are shown in panel (b). The error bars indicate the intersubject variability (\pm S.D.). Panel (c) shows MP profiles for the P-C and P groups measured at the end of phase II of the study (time T2). Panel (d) shows the percentage change in the transmittance of blue light (10^{-OD}) when the MPOD values in the P-C group are compared with the corresponding measurements in the P group. The diamonds show the difference between the P-C and P groups at time T2 and the triangles show the percentage difference between the same groups, but at time T3.

measured MPODs were converted to percentage transmittance values in order to express the differences between the P-C and the P groups as a percentage change in transmitted light. These comparisons computed for time T2 (after 6 months of supplementation) and at time T3 (4 months after supplementation was stopped) are shown in *Figure 4d*. The results show clearly that the percentage reduction in transmitted blue light (10^{-OD}) as a result of supplementation extends almost uniformly in the paracentral region around the fovea over a visual angle of $\pm 4^\circ$. The percentage reduction in the transmission of blue light is significantly less at time T3 after 4 months with no supplementation. *Figure 4b,c* shows the MPOD profiles for the P-C and the P groups, respectively, at both T2 and T3. Comparison of the spatial MPOD profiles for the P-C group reveals a significant decrease in MP density after 4 months with no supplementation. In contrast, the same measurements show no significant changes in the P group.

In order to establish the possible effects of MP density on chromatic discrimination sensitivity we evaluated the correlation between the measured RG and YB colour thresholds and the peak density of the MP for all subject groups. The data examined were measured at time T2

and are shown in *Figure 5*. YB thresholds are plotted in the top two panels labelled (a) and (b). Panels (c) and (d) show similar data describing RG thresholds. (a) and (c) show results measured at the fovea and the corresponding data measured 2.5° in the periphery are plotted in (b) and (d). The 15×15 check array employed to measure colour detection thresholds (see *Figure 2*) subtended a visual angle of 2.82° at the fovea and 4.23° in the periphery. The coloured stimulus consisted of 5×5 checks and subtended a visual angle of 0.94° and 1.41° , respectively. The larger stimulus size selected for the measurement of colour thresholds at 5° eccentricity compensates for the known reduction in chromatic sensitivity with eccentricity (Boynton *et al.*, 1964; Abramov *et al.*, 1991). The results show little or no correlations between the level of MP in the eye and the corresponding RG or YB colour discrimination thresholds.

Similar results were found 4 months after supplementation was stopped (at time T3) when additional measurements were made in the P-C and P groups. The additional colour discrimination thresholds measured at time T3 employed a smaller stimulus size, of 2° , that moved through a visual angle of $\pm 1^\circ$, centred both at the fovea and at an eccentricity of 3° . The spatial

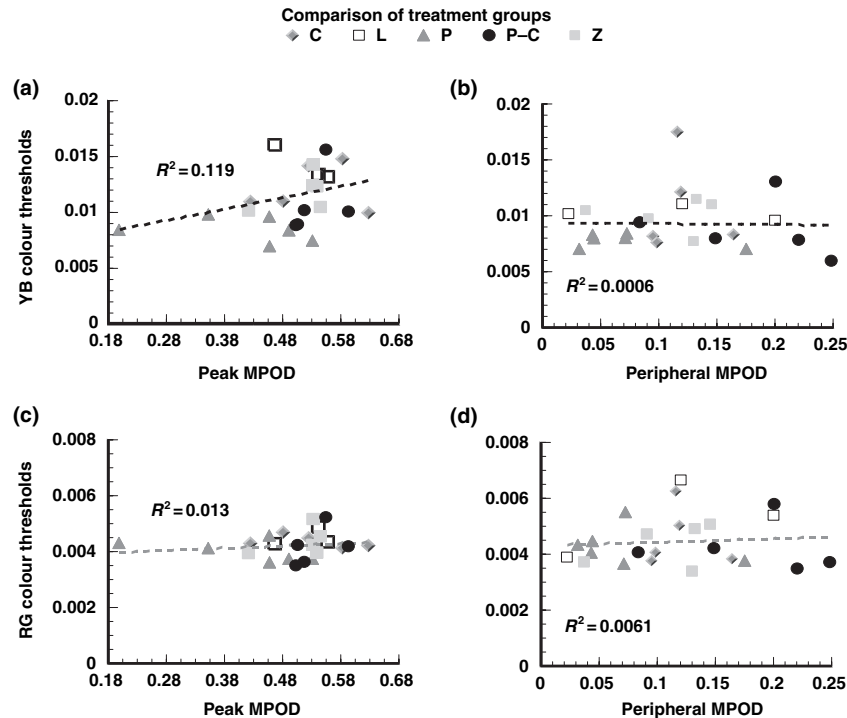


Figure 5. Chromatic detection thresholds measured at the fovea (a, c) and 5° in the periphery (b, d) plotted against the available macular pigment optical density values at the end of phase II. YB thresholds are shown in panels (a) and (b) and RG thresholds are shown in panels (c) and (d).

localisation of the coloured stimulus was improved in order to reveal the possible effects on YB colour thresholds caused by the greater absorption of blue light by the MP at the fovea. Full spatial profiles of MP density were also available for each subject at time T3. This made it possible to obtain mean MP estimates by averaging measurements taken at 0° and ±1.25° for the fovea, and at ±2.5° and ±4° for the periphery. The new RG and YB colour thresholds measured under rigorous conditions and with smaller coloured stimuli that provide better spatial localisation are shown in *Figure 6*. The results illustrate convincingly the complete lack of correlation between the level of MP in the eye and the corresponding RG or YB colour discrimination thresholds.

Plasma levels of L and Z were also measured at monthly intervals throughout the LUXEA trial as shown in *Figure 7*. During the first 2 months of supplementation plasma levels increased significantly and stayed at the higher level whilst the supplementation continued, only to decrease rapidly within 4 months after supplementation was stopped.

Discussion

This investigation exploits the advantages of two new techniques, one developed to measure the spatial distribution of the MP (Rodriguez-Carmona *et al.*, 2004; Schalch *et al.*, 2004) and the other to assess RG and YB

chromatic sensitivity with stimulus conditions that isolate the use of colour signals (Barbur, 2004). The MAP test measures MP density with respect to an eccentricity of 8°, a value considerably larger than other flicker cancellation tests that reference MP changes with respect to 5° or 6° eccentricity. Although the differences between the 5° and 8° eccentricities may be negligible in subjects with normal levels of MP density, the larger 8° reference is important for use in carotenoid supplementation studies when the accumulation of MP extends to more distant regions around the macula. The use of sinusoidal flicker and a small luminance modulation depth of 20% makes it easier for the subject to adjust the mean luminance of the test beam to cancel the perceived flicker in the reference beam and this tends to improve the overall accuracy of the measurement. The findings from this study confirm other earlier reports suggesting increased levels of MP in the eye as a result of supplementation with carotenoids (Sommerburg *et al.*, 1998). In addition, this study reveals the extent to which MP accumulates in more distant areas around the macula causing an almost uniform reduction in the amount of blue light reaching the retina within the centre ±4°. Previous studies suggest that the sparing of the very central area of the macula in a variety of macular degenerations (sometimes described as bull’s eye retinopathies) is often attributed to the presence of high levels of MP in the spared region (Weiter *et al.*, 1988). The mechanism that contributes to this outcome

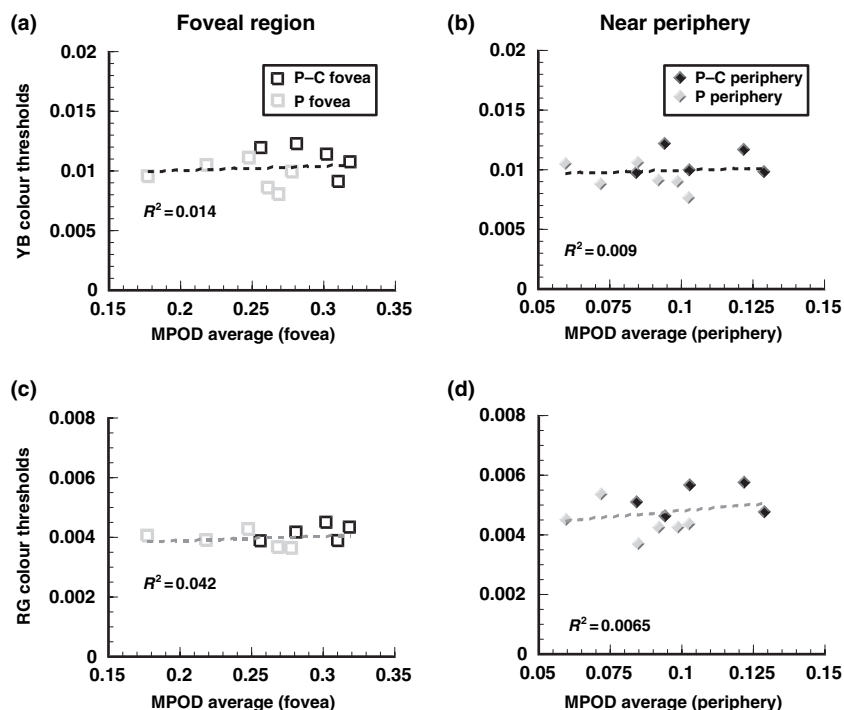


Figure 6. Chromatic detection thresholds plotted against macular pigment optical density (MPOD) values measured at the end of phase III of the study when full spatial profiles of MP density were available for the subjects investigated. The MPOD values plotted at the fovea (a, c) were obtained by averaging the measurements taken at eccentricities of -1.25° , 0° and 1.25° . For the peripheral location, a measure of mean MP density was obtained by averaging measurements made at -4° , -2.5° , 2.5° and 4° (b, d) for each subject in the P-C and P groups.

is probably the absorption of higher energy, short wavelength photons and the more effective quenching of reactive oxygen intermediates that arise from the photo-transduction process. The significant increase in MP density at and around the macula region following supplementation with L and Z implies that a larger region of the retina may receive greater protection against ARMD. The implicit claim of this hypothesis rests on obtaining further evidence to validate previous reports suggesting that the size of the spared retina in bull's eye retinopathies relates to the amount and spatial extent of the MP in the eye (Weiter *et al.*, 1988).

The principal aim of this study was to investigate the extent to which the increased variability in YB colour detection thresholds in normal trichromats reflects the large differences in MP density reported in the normal population (Ruddock, 1963). The CAD test offers unique advantages to quantify both YB and RG chromatic sensitivity both at the fovea and in the periphery where MP density is significantly reduced. The results show that although both MPOD levels and YB colour thresholds show large intersubject variability, no significant correlation exists between these two variables, suggesting that the increased variability should be

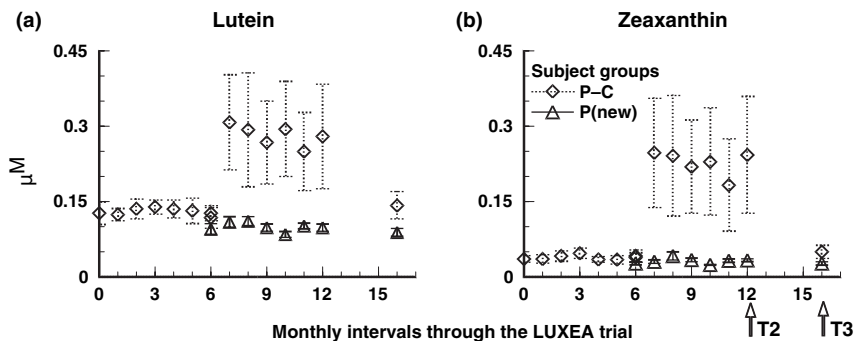


Figure 7. Plasma levels of lutein and zeaxanthin (measured in $\mu\text{M/L}$) at monthly intervals throughout the LUXEA trial for the PC group (diamonds) and the new placebo group (triangles). Time T2 indicates the end of supplementation and T3 4 months after supplementation was stopped. The full time course of supplementation is shown in Table 1.

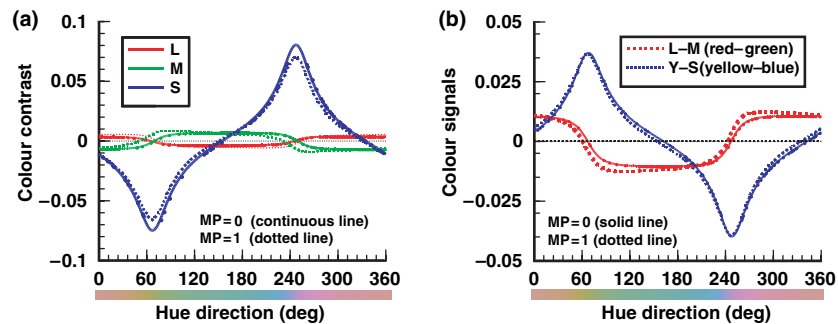


Figure 8. Analysis of cone contrasts and the corresponding colour signals generated for the mean colour detection thresholds shown as a black contour in *Figure 2a*. The solid lines plot the cone contrast signals generated in each class of cone photoreceptor for each direction in the CIE- (x, y) diagram (a), and the corresponding red-green and yellow-blue colour signals generated in the absence of MP (b). The attenuation of blue light by the MP can be simulated by filtering appropriately the light reaching the photoreceptors. The dotted lines plot the cone contrast signals (a) and the corresponding colour signals (b) when the light from the display is filtered by the MP. The results shown correspond to an MP filter with a peak optical density of 1 log unit (a value larger than that found in any subject investigated in this study).

attributed to other independent mechanisms. This finding was surprising given the well-established effect that absorption of blue light by the MP can have on trichromatic colour matches (Neitz and Jacobs, 1986). In order to explain these findings we have examined how cone photoreceptor contrasts change at threshold and how the MP affects these curves. *Figure 8a* shows the cone contrasts computed for the mean threshold discrimination ellipse (the black line shown in *Figure 2a*). The cone contrasts are calculated by evaluating the signal generated by the uniform background and the corresponding test stimulus, as a function of its hue direction in the CIE (x, y) chromaticity diagram. This computation employs the known spectral responsivity functions of cone photoreceptors (Smith and Pokorny, 1972) and knowledge of the spectral radiance data of the test and background fields. No other assumptions are involved. For example, the M cone contrast signal is given by $C_M = (M_{\text{test}} - M_{\text{bkg}}) / M_{\text{bkg}}$, where test and bkg stand for test and background field, respectively.

The dotted lines show the expected cone contrast changes when the spectral composition of the light from the display is modified by a filter corresponding to the MP spectral absorption template for a peak optical density of 1 log unit, a value significantly larger than those measured experimentally in this study. This computation is very informative in that it demonstrates clearly the independent functioning of the two chromatic channels at threshold (MacLeod and Boynton, 1979; Guth *et al.*, 1980), with the RG channel mediating colour discrimination over most of the range. The RG colour channel is formed by differencing of L and M cone signals and the YB channel is driven mostly by the S-cone signal, which is subtracted from the sum of M and L cone signals. The simple model of colour vision predicts little or no change in YB colour discrimination when an MP filter with a peak density of 1 log unit is

placed in front of the eye (*Figure 8b*). Interestingly, the RG colour signal is actually increased for some hue directions, mostly as a result of reducing selectively the signals generated by the uniform background in M and L cone photoreceptors. This improvement in RG chromatic sensitivity is probably small and the hue directions that are affected most were not investigated experimentally. Although the experimental findings and the predictions of the threshold colour discrimination model show convincingly that YB colour thresholds are not affected even by large amounts of MP in the eye, one could reasonably expect that at relatively low light levels when the signal-to-noise ratio in S cones becomes small, YB colour discrimination thresholds may no longer be independent of MP in the eye.

Conclusions

The spatial MPOD profiles measured after 6 months of supplementation with a combination of L (10 mg day^{-1}) and Z (10 mg day^{-1}) show a significant increase in MPOD, even at 6° eccentricity, making it essential to reference such measurements with respect to a larger eccentricity of 8° (*Figure 4a*). Failure to do so underestimates uniformly the increase in MPOD as a result of supplementation.

Supplementation with a combination of L (10 mg day^{-1}) and Z (10 mg day^{-1}) over a period of 6 months increases MP distribution over $\pm 8^\circ$ around the macula, causing an almost uniform reduction in the percentage of transmitted blue light over the centre $\pm 4^\circ$ (*Figure 4d*). Although a significant reduction in MP is observed in the supplemented group 4 months after supplementation was stopped, MPOD profiles in this group continue to remain higher than those in the placebo group. These findings are consistent with the measured increase in plasma levels of L and Z during

supplementation and the significant reduction observed 4 months after supplementation was stopped (see *Figure 7*).

For the stimulus conditions employed in this study YB chromatic detection thresholds do not differ significantly within the centre $\pm 5^\circ$ and do not correlate with the measured MPOD values, either at the fovea or in the periphery. All subjects showed high RG chromatic sensitivity (well within the normal range), but no correlation with MPOD values was found.

The cone contrast model developed to predict the effects of MP on colour detection thresholds accounts well for the observed experimental findings and may also account for the relatively constant short-wavelength sensitivity across the central retina (Stringham *et al.*, 2005). Hue cancellation techniques (Jameson and Hurvich, 1955) have also been employed to measure the spectral sensitivities of colour opponent mechanisms at a number of discrete locations in the visual field (Hibino, 1992). The author's findings suggest that the spectral sensitivity of the YB channel remains relatively constant with eccentricity in spite of large variation in MP optical density in the two subjects investigated. Although consistent with our findings, Hibino's experiments are more difficult to interpret because the test stimuli were scaled according to a single cortical magnification factor. The use of cone contrast signals as described in our model also explains why the differential distribution of S-cones with eccentricity has little or no effect on YB chromatic sensitivity. This may not, however, be the case at low light levels when preferential absorption of blue light by the MP in the eye could reduce the signal-to-noise ratio in S cones and hence YB chromatic sensitivity.

In addition to predicting no changes in YB colour detection thresholds, even for peak MPOD values of 1 log unit, the model unexpectedly predicts a marginal improvement on RG colour thresholds with increased MP density. In conclusion, the findings of this study suggest that at photopic levels of light adaptation increasing the density of the MP can only improve human chromatic discrimination sensitivity.

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