

# Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance

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## Abstract

**Background:** Macular pigment (MP) is found in diurnal primate species when vision spans a range of ambient illumination and is mediated by cone and rod photoreceptors. The exact role of MP remains to be determined. In this study we investigate two new hypotheses for possible MP functions.

**Objective:** As MP absorption coincides partly with that of rhodopsin, MP may reduce rod signal effectiveness in the mesopic range, thus extend the usefulness of cone-mediated vision into the mesopic range. Forward light scatter in the eye can reduce retinal image contrast. If blue light contributes significantly to intraocular scatter, selective blue light absorption by MP could reduce the effects of scatter.

**Design:** We investigated 34 subjects from a carotenoid supplementation trial. The measurements included high mesopic contrast acuity thresholds (CATs), macular pigment optical density (MPOD), wavefront aberrations, and scattered light. The measurements were made after 6 months of daily supplementation with zeaxanthin (Z, OPTISHARP™), lutein (L), a combination of the two (C), or placebo (P), and again after a further 6 months of doubled supplementation.

**Results:** The data reveal a trend toward lower CATs in all groups supplemented, with a statistically significant improvement in the lutein group ( $p = 0.001$ ), although there was no correlation with MPOD. Light scattering in the eye and the root-mean-square wavefront aberrations show decreasing trends as a result of supplementation, but no correlation with MPOD.

**Conclusions:** The results suggest that supplementation with L or Z increases MPOD at the fovea and at 2.5°, and that supplementation can improve CATs at high mesopic levels and hence visual performance at low illumination.

**Keywords:** aberrations, contrast acuity, lutein, macular pigment, retinal carotenoids, scattered light, zeaxanthin

## Introduction

The macular pigment (MP) of the human retina consists mainly of the carotenoids lutein (L) and zeaxanthin (Z)

(Bone *et al.*, 1985; Handelman *et al.*, 1988). L and Z are not synthesised in the body, and therefore have to be obtained from the diet. The importance of nutrition in the context of the MP is illustrated by several findings. Monkeys fed a diet free of L and Z over a long period were shown to be entirely devoid of MP (Malinow *et al.*, 1980; Neuringer *et al.*, 2004). Furthermore, patients with cystic fibrosis, who have an impaired ability to absorb fat-soluble micronutrients, including carotenoids, have been shown to have decreased MP density (Schupp *et al.*, 2004). It has also been shown that diets rich in L and Z, or direct supplementation, can increase the MP density (Hammond *et al.*, 1997a; Landrum *et al.*, 1997a; Berendschot *et al.*, 2000). MP absorbs and

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attenuates the amount of blue light striking the retina, especially in the macula where xanthophylls concentration is highest (Brown and Wald, 1963; Reading and Weale, 1974; Pease *et al.*, 1987), and it is thought that selective filtering of blue light is its principal function. The precise role of the MP remains as yet undetermined. Improvement in visual performance by reduction of chromatic aberration in the eye (Reading and Weale, 1974), protection of retinal tissue from damaging short wavelength light (Kirschfeld, 1982; Schalch, 1992) and its role as antioxidant (Foote *et al.*, 1970; Snodderly, 1995) have been suggested. Evidence is often controversial, making it difficult to associate the function of the MP uniquely with any one of the benefits listed above.

In this study we put forward new hypotheses to justify the existence and the possible function of the MP. Vision in the mesopic range is mediated by both cone and rod photoreceptor signals and many studies have demonstrated interactions between these signals (Goldberg *et al.*, 1983; Frumkes *et al.*, 1985; Naarendorp and Frumkes, 1991). The fovea is densely packed with L- and M-cone receptors and yields the highest spatial resolution and the best contrast sensitivity. MP can only be found in diurnal primate species, where vision is mediated by both cone and rod receptors (Oyster, 1999). Rod-mediated vision is in general more sluggish (Barbur, 1982) and exhibits poor contrast sensitivity compared with cone-mediated vision (Puell *et al.*, 2004). Minimising the degrading effects of rod signals could be a considerable advantage. If this were the case, then the superior characteristics of cone-mediated vision may be retained throughout the mesopic range in a small central region of the visual field where rod signals are less numerous and MP density is highest. To test if the MP is likely to selectively reduce the effectiveness of unwanted rod signals we measured contrast acuity in the mesopic range in subjects with increased MP density as a result of supplementation with lutein and zeaxanthin, the two major constituents of the MP.

Forward light scatter in the eye can produce disability glare and cause a reduction of contrast in the retinal image. Recent studies have suggested that the MP may play a role in reducing the effects of 'blue haze' when viewing distant objects through the atmosphere because of preferential scattering of short wavelength light (Wooten and Hammond, 2002). In addition, if blue light contributes significantly to scatter in the eye, selective absorption by the MP is likely to reduce the effects of scattered light. Subjects with high MP density could therefore be expected to exhibit less short wavelength scatter. In order to investigate this second hypothesis we examined whether carotenoid supplementation or high levels of MP correlate with lower levels of scattered light in the eye, where the amount and the angular distribution of scattered light were measured

using a high-temperature, daylight source that is rich in blue light.

## Subjects and methods

### Subjects

The subjects investigated were already participants in the Lutein ZeaXanthin Eye Accumulation (LUXEA) trial, a double-blind placebo-controlled carotenoid supplementation trial (Kopcke *et al.*, 2005; Schalch *et al.*, 2005) involving a total of 92 healthy male subjects aged 18–40, Caucasian and with a body mass index (BMI) of 18 to 25 kg m<sup>-2</sup>. Any ocular abnormality resulted in exclusion from the study, as did a refractive error outside the range of  $\pm 3$  D sphere or  $\pm 1.5$  D cylinder.

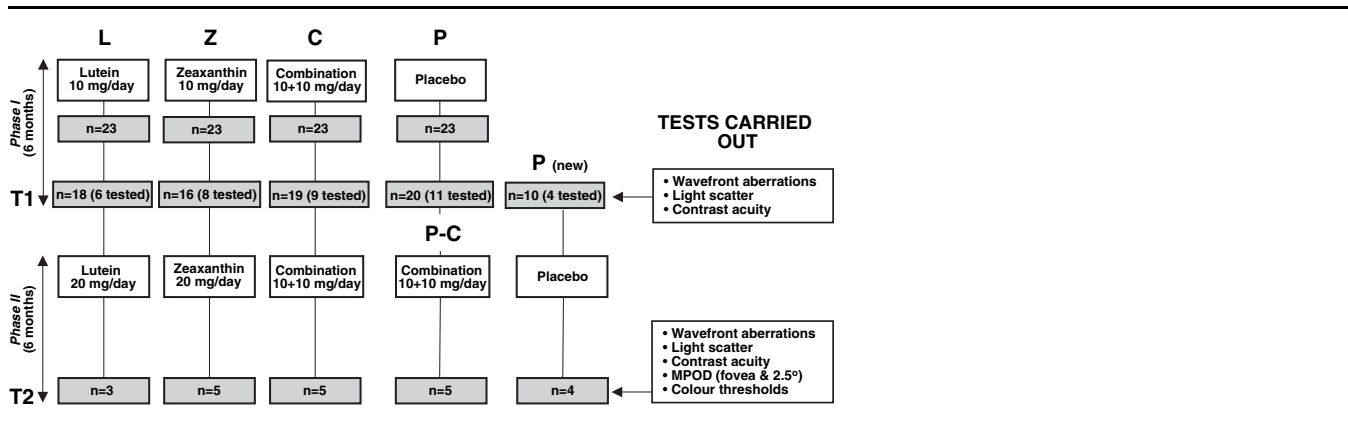
In brief, the participants in the LUXEA trial received supplementation with lutein (L; 10 mg day<sup>-1</sup>), zeaxanthin (Z; 10 mg day<sup>-1</sup>), lutein + zeaxanthin (C; 10 + 10 mg day<sup>-1</sup>) or placebo (P) for 6 months (phase I). Some of the subjects continued for a further 6 months (phase II) and received doubled supplementation, except for the C group, which continued with the same dose of supplementation as in phase I (see *Table 1*). All subjects available in the P group at the end of phase I became part of a new group, the P-C group ( $n = 5$ ), and received combined supplementation for 6 months. More subjects were recruited to form the new placebo group. This arrangement enabled us to make comparisons of the P-C group ( $n = 5$ ) after only 6 months of supplementation with the new P group ( $n = 4$ ; see *Table 1*), as well as within group comparisons of changes in contrast acuity, light scatter, aberrations and MP density as a result of the increased supplementation. Wavefront aberrations, contrast acuity and scattered light were measured at the end of phase I and phase II in a number of subject subgroups, as shown in *Table 1*, with MP density measured at the end of phase II. These investigations formed the visual performance (VIP) study.

A total of 24 subjects completed the 'VIP' study. All subjects gave informed consent before enrolment. The study followed the tenets of the Declaration of Helsinki and was approved by the research and ethical committee of City University.

### Contrast acuity assessment test

Contrast acuity was measured for a background screen luminance of approximately 1 cd m<sup>-2</sup>. This light level is normally associated with the high mesopic range where both rod and cone photoreceptor signals are effective. The short wavelength absorption of the MP is likely to selectively reduce rod signals and this in turn may lead to detectable changes in visual performance. The

**Table 1.** Schematic summary of subject groups and course of carotenoid supplementation. Measurements of *rms* wavefront aberrations, contrast acuity and scattered light were carried out at the end of phase I (time T1), and at the end of phase II (time T2), for all subject groups. Macular pigment optical density at the fovea and 2.5° in the periphery was also measured at the end of phase II for all subject groups. The various groups and subject numbers are as shown in the table. Note that at the end of phase I, group P-C was formed by enrolling all available subjects from the initial placebo group. The P-C group was then given combined carotenoid supplementation for 6 months. Ten subjects (four tested) were also recruited to form the new placebo group.



contrast acuity assessment (CAA) test was developed to detect small changes in contrast acuity thresholds following corneal refractive surgery (Barbur *et al.*, 2001). The test measures contrast acuity thresholds for a given background light adaptation level at a number of stimulus eccentricities. In this study, the Landolt ring stimulus was presented at the fovea or at an eccentricity of  $\pm 2.5^\circ$  along the horizontal meridian. The diameter of the ring was 15 min arc, a stimulus size that is considered to be functionally important, with a gap size approximately three times above the normal high-contrast acuity limit (Chisholm *et al.*, 2003). The CAA test therefore measures the contrast threshold needed to detect and discriminate correctly the orientation of the gap. The test is based on a number of interleaved staircases with variable step sizes and employs a four-alternative, forced-choice procedure. The subjects viewed the centre of a 21" Sony Trinitron monitor (Model 500 PS) driven by an ELSA Gloria XL 30 bit graphics card (ELSA, Model Gloria XL, Germany) at a frame rate of 75 Hz. Measurement of the spectral output of each phosphor gun and automatic calibration of the luminance vs applied voltage relationship for each gun provided the photometric calibration data for the display. An adjustable forehead and chin rest were used to control the head position, and the test was performed binocularly at a distance of 1.5 m.

In order to minimise the expected inter-subject variability in contrast acuity thresholds, the test was modified to control the state of light adaptation of the retina. The retinal illuminance was kept constant at 20 trolands for each subject by adjusting the screen luminance automatically to account for any differences in pupil size. Retinal illuminance is proportional to

pupil size [i.e.  $E$  (trolands) =  $L \times P_A$ , where  $L$  is the luminance of the display screen and  $P_A$  is the pupil area in square millimetres). This adjustment of screen luminance was performed during a 12 min period of adaptation to the uniform background field using a 50 Hz, infrared system for measuring the pupil size (Barbur *et al.*, 1987). In addition, during this time the subject was also trained on the task of gap orientation discrimination. Whilst the subject completed a short practise run on this test, the diameter of the pupil was measured continuously. The mean pupil diameter was computed every 2 s and used to calculate retinal illuminance. When changes were needed, the luminance of the uniform background field was ramped up or down gradually over 2 s according to the formula,  $L_{\text{screen}} (L_s; \text{cd m}^{-2}) = 20/P_A$ , where 20 represents the desired retinal illuminance in trolands. The procedure worked very well with only small adjustments needed after the first minute of adaptation. The measurements of pupil diameter taken during the last 3 min were then averaged and the mean pupil diameter stored together with the mean screen luminance needed to achieve a retinal illuminance of 20 trolands. The mean pupil diameter measured was then taken as the expected pupil diameter during the CAA test and was also used for the computation of the root-mean-square (*rms*) wavefront aberration for the eye.

#### *The scatter test*

The forward light scatter test employed is based on the flicker cancellation technique first developed by Le Grand (1937) and later extended by van den Berg and Spekrijse (1987) and Barbur *et al.* (1993). A ring

scatter source was presented on a visual display unit in conjunction with a central test target. The subjects viewed the display binocularly from a distance of 0.7 m using an adjustable chin and forehead rest. The scatter source had a mean luminance of  $50 \text{ cd m}^{-2}$  and was modulated sinusoidally at 8.6 Hz causing a burst of flicker that lasted approximately 350 ms. The luminance of a small dark disc ( $0.8^\circ$  diameter) at the centre of the annulus (i.e. the flicker nulling source) was modulated sinusoidally in counter-phase with the scatter source. The scatter source's Commission Internationale d'Eclairage (CIE) ( $x,y$ ) chromaticity was: 0.279, 0.291 (to ensure a large percentage of blue light), whilst the flicker nulling source had an ( $x,y$ ) chromaticity of: 0.522, 0.414 (to ensure a large percentage of long wavelength light). The scatter source is imaged in the periphery of the visual field, whilst the nulling source is imaged at the fovea. The presence of the MP at the fovea is therefore likely to reduce the perceived flicker caused by scattered, blue light that arrives at the centre of the ring. The light from the nulling source that cancels the flicker generated by scattered light is therefore less affected by the selective spectral absorption of the MP. An increase in MP optical density will reduce selectively the effectiveness of scattered light without affecting significantly the effectiveness of the nulling source light. This arrangement provides a useful technique for estimating glare reduction from bluish light that can be attributed directly to absorption by the MP. The subject's task was to adjust the mean luminance of the flicker-nulling source to minimise or eliminate the perception of flicker at the centre of the scatter source. The screen luminance of the flicker-nulling source needed to cancel the perception of flicker caused by scattered light is a direct measure of the amount of forward light scatter in the eye. This procedure was repeated for five different scatter ring diameters (range  $4^\circ$  to  $10^\circ$ ) so as to be able to assess the angular dependence of scattered light in the eye. An empirical function first suggested by Holladay (1926) was then fitted to the data to obtain estimates of the scatter function parameters of the eye. The amount of light scattered at an eccentricity,  $\theta$ , away from the scatter source is given by:  $L_s = kE\theta^{-n}$ , where  $L_s$  is the luminance of the screen that causes the same retinal illuminance as the scatter source,  $E$  is the illuminance level generated by the scatter source in the plane of the pupil, and  $\theta$  is the angle of the scatter source. The parameters  $k$  and  $n$  are measured for each subject and yield information on the amount and the angular distribution of light scatter, respectively. An additional parameter,  $k'$ , is also calculated by integrating the scatter function of the eye from  $2^\circ$  to infinity. This parameter is therefore proportional to the total amount of light that is scattered in the eye.

#### Wavefront aberration measurement

Measurement of wavefront aberrations (a measure of the quality of the retinal image) was carried out to eliminate subjects with very large *rms* aberrations that are likely to exhibit large contrast acuity thresholds as a result of poor retinal image quality. This procedure is likely to minimise variability and this in turn may help reveal any threshold changes that can be attributed to carotenoid supplementation. A WASCA Wavefront Analyser (Asclepion-Meditec, Fife UK) was used to measure the optical aberrations of each eye. The instrument uses the Shack-Hartmann wavefront sensing technique that was first applied to the eye by Liang *et al.* (1994). An 850 nm laser was used in conjunction with a lenslet array and a charge-coupled device (CCD) sensor to measure the distortions to the wavefront returning from the subject's eye. This gives information on the refractive errors, and also quantifies the principal aberrations of the eye. A natural pupil was used in the dark and 10 wavefront measurements were taken for each eye over a period of 10 min. The *rms* wavefront aberration is a single measure that describes the residual aberrations in the eye and was computed from the measured data for each subject for the mean pupil size measured for the lighting conditions employed in the CAA test. The *rms* values used for each subject were the third and fourth orders only. The *rms* values of 31 eyes from an additional 17 normal subjects were calculated (for the mean pupil size measured during the CAA test), and the mean and standard deviation of these values were used to derive an allowed range of  $\pm 2$  S.D. from the mean *rms* value. Subjects with an *rms* value outside this range were excluded. For the mean pupil size of  $6.97 \pm 0.90$  mm found for the CAA test, the inclusion range of *rms* values was 0.17–0.86  $\mu\text{m}$ .

In general the correlation between the aberrations of the two eyes in the same subject is high (Liang *et al.*, 1994; Liang and Williams, 1997; Porter *et al.*, 2001). As both the scatter and the CAA test were carried out binocularly, any subject with an inter-eye difference in *rms* values greater than two standard deviations (i.e. 0.345  $\mu\text{m}$ ) was excluded from the study.

#### Macular pigment optical density measurement

The optical density of the MP was estimated at the fovea and at an eccentricity of  $2.5^\circ$  in the periphery using a new implementation of the classic flicker cancellation technique (Hammond *et al.*, 1997b). The new macular assessment profile (MAP) technique employs 20 Hz sinusoidal counter-phase modulation of two spectrally broad-band beams of light, one that is maximally absorbed by the MP (peak emission wavelength/half maximum range:  $452 \pm 28$  nm), and a second beam

that extends approximately from 550 to 780 nm and is not absorbed by the MP. This was achieved by designing a notch filter with sharp band-pass spectral absorption characteristics to separate and filter out the three-phosphor outputs of a visual display. The MAP technique was implemented on a 17", high brightness visual display (EIZO Flexscan T566, Woking, UK) driven at a frame rate of 140 Hz from an ELSA Gloria XL 30 bit graphics card. In order to achieve stable display operation at the high luminance needed for this test, the background luminance was kept low and the stimulus configuration consisted of a rectangular area of  $11.57^\circ \times 5.15^\circ$  (i.e. only 11% of the total display area). The flickering test stimulus was always presented at the centre of the display and a small fixation spot surrounded by radial guides appeared at the appropriate location as needed to achieve the required stimulus eccentricity. In this part of the study the MP density was measured with respect to an eccentricity of  $6^\circ$  (i.e. we measured increments in optical density with respect to the negligible value expected at  $6^\circ$ ). The measurement was performed on the right eye at a distance of 0.7 m. The MAP test has been described briefly elsewhere (Rodriguez-Carmona *et al.*, 2004; Schalch *et al.*, 2004).

#### Data evaluation and statistics

Statistical analyses were performed with basic standard methods (descriptive statistics, scatter plots, linear regression and *t*-tests) using S-PLUS® 6.2 for Windows (PROFESSIONAL EDITION; Insightful Corporation, Seattle, WA, USA).

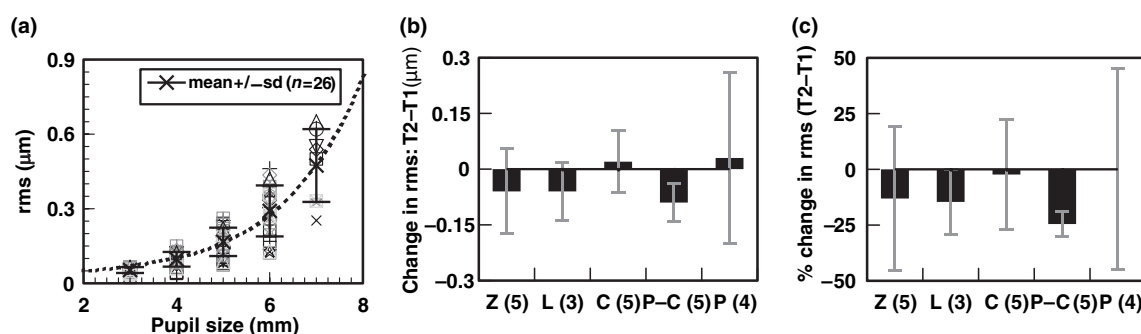
#### Results

The experimental preparations needed for the assessment of visual performance were not ready at the beginning of the LUXEA trial and consequently no

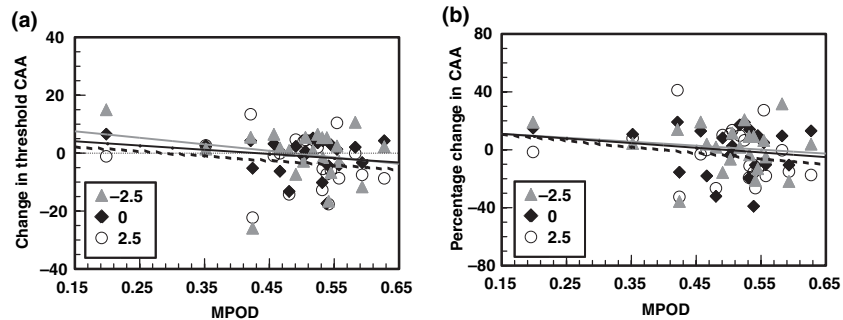
results are available for each subject before the start of supplementation. The first round of measurements was carried out after 6 months of supplementation. This period we describe as phase I (see *Table 1*) and involved the following subject numbers: L ( $n = 6$ ), Z ( $n = 8$ ), C ( $n = 9$ ) and P ( $n = 11$ ). Another group of 10 subjects were recruited and tested at the end of phase I and formed the new placebo group for the phase II of the study.

At the end of phase II, following 6 months of doubled L and Z supplementation, the same VIP measurements were carried out. A group of subjects, P-C, (drawn from the phase I placebo group) who received the combined supplementation in phase II was included in the study, and in addition a new placebo group was recruited. Some of the subjects tested in phase I were unfortunately no longer available and we were therefore unable to repeat the tests at the end of phase II with all the subjects. The subjects tested in phase II were as follows: L ( $n = 3$ ), Z ( $n = 5$ ), C ( $n = 5$ ), P-C ( $n = 5$ ), and P ( $n = 4$ ). As no data are available to describe the visual performance of the subjects before the start of supplementation, the inter-group comparison of results available for phase I and phase II can only reveal the additional effects of 6 months of increased supplementation.

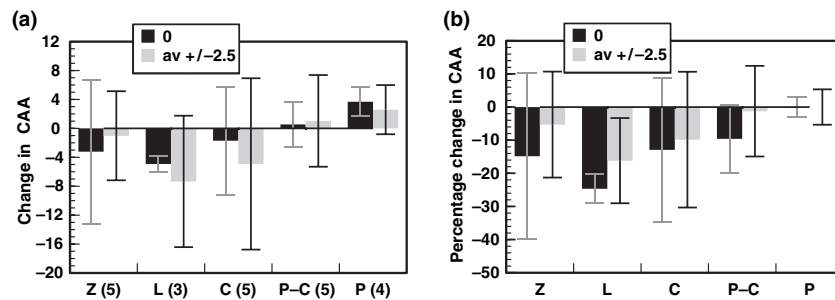
*Figure 1a* shows how *rms* wavefront aberrations vary with pupil size in the normal population. The results reveal the expected non-linear increase in *rms* aberrations with pupil size. It is of interest to note that the inter-subject variability increases quite significantly for large pupil sizes that are typical of the mesopic range. The dotted line represents the best non-linear fit based on the following equation:  $rms = 0.0283 + 0.00696 \times \exp(0.5957 \times d)$ , where the *rms* wavefront aberration is measured in micrometres and the pupil diameter, *d*, is measured in millimetres. The best fit was constrained to produce  $\sim \lambda/10$  wavefront aberration for  $< 2$  mm



**Figure 1.** Mean *rms* wavefront aberration (a parameter that relates to the quality of the retinal image) was measured in 26 subjects and is plotted as a function of pupil diameter (a). The error bars show  $\pm$ S.D. for this subject group. (b) Plots the actual changes in *rms* wavefront aberrations measured at the end of phase I and the end of phase II, following 6 months of increased supplementation (see *Table 1*). (c) Figure shows the percentage change in *rms* aberrations for each subject group at the end of phase II of the study corrected for the small changes measured in the placebo group.



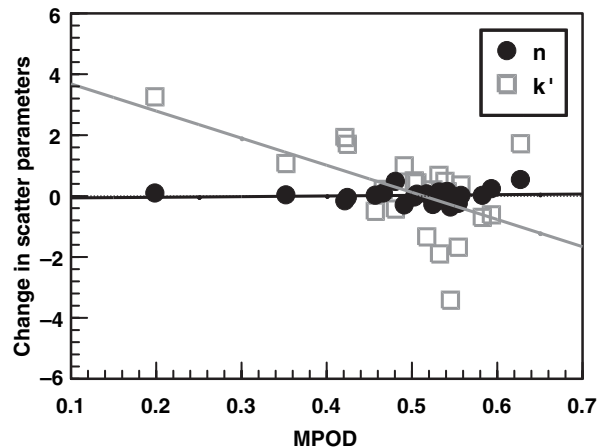
**Figure 2.** Changes in contrast acuity assessment (CAA) thresholds plotted against foveal macular pigment (MP) density for the subjects that completed phase II of the study. CAA thresholds were measured at  $-2.5^\circ$ ,  $0^\circ$  and  $+2.5^\circ$  eccentricity along the horizontal meridian. The results (a) show little or no correlation between the change in CAA thresholds and the MP density at the end of phase II [ $r^2$ : 0.050 ( $-2.5^\circ$ ), 0.044 ( $0^\circ$ ), 0.026 ( $2.5^\circ$ )]. The same data (b), but expressed as percentage changes with respect to the values measured in phase I [ $r^2$ : 0.024 ( $-2.5^\circ$ ), 0.030 ( $0^\circ$ ), 0.042 ( $2.5^\circ$ )]. The best-fit lines are also shown: grey ( $-2.5^\circ$ ), black ( $0^\circ$ ), dotted ( $2.5^\circ$ ).



**Figure 3.** Inter group comparison of changes in contrast acuity assessment (CAA) test thresholds caused by increased carotenoid supplementation during the second 6 months of the study (phase II). CAA mean thresholds were measured both at the fovea (black bars) and at eccentricities of  $\pm 2.5^\circ$  (grey bars). Actual differences are shown in (a). (b) shows the data as percentage changes between phases I and II after correction for the changes observed in the placebo group. The error bars indicate the intersubject variability ( $\pm$ S.D.).

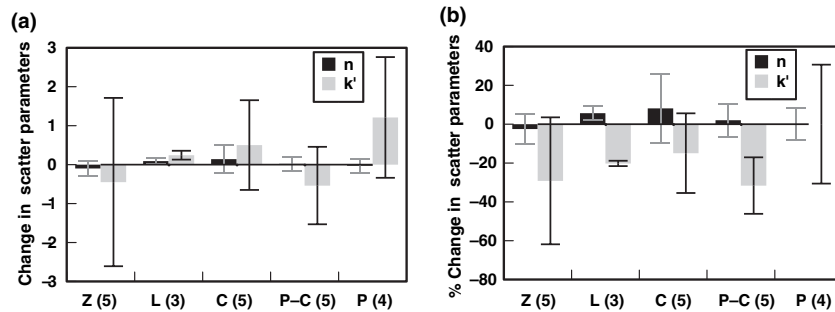
diameter pupil, a value that is not inconsistent with diffraction-limited performance. The changes in *rms* wavefront aberrations measured at the end of phase I and the end of phase II following 6 months of increased supplementation (see Table 1) is shown in Figure 1b. Figure 1c shows the same data corrected for the small changes measured in the placebo group. The results show a small trend towards lower *rms* wavefront aberrations in all treated groups. Because of the small number of subjects and the large inter-subject variability in *rms* aberrations in the mesopic range (as shown in Figure 1a), the decreases observed in the treated groups failed to reach statistical significance. These observations and particularly the decrease in higher order aberrations following supplementation were unexpected. Although lutein and zeaxanthin can be found in the human lens (Yeum *et al.*, 1995; Bates *et al.*, 1996; Landrum *et al.*, 1997b) we do not know how intake of carotenoids can affect the properties of the lens.

Figure 2a,b shows how changes in CAA thresholds correlate with MP density for the subjects that completed phase II of the study. The results show little or no correlation between MP density and the change in contrast activity thresholds.

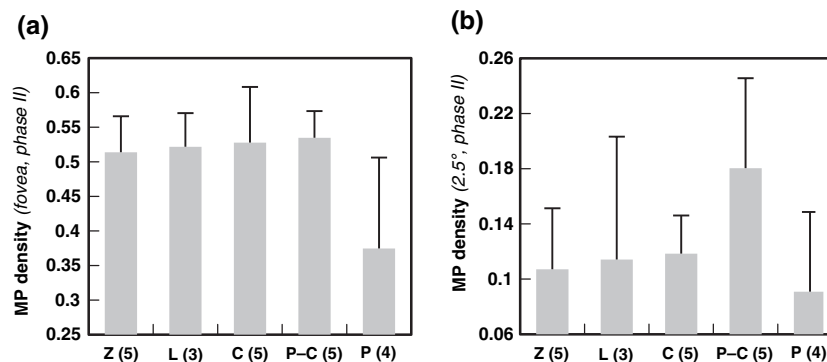


**Figure 4.** Changes in light scatter parameters in the subjects that completed phase II of the study plotted against foveal macular pigment (MP) density at the end of phase II. Parameters *n* and *K'* describe the angular distribution and the amount of scattered light, respectively. The results show little or no correlation between the change in *n* and *K'* vs MP density ( $r^2$ : 0.008 for *n*, and 0.311 for *K'*).

Inter-group comparison of changes in CAA thresholds before and after 6 months of increased carotenoid supplementation reveals a robust trend towards lower



**Figure 5.** Inter group comparison of changes in light scatter parameters that can be attributed to increased carotenoid supplementation during the second 6 months of the study (phase II). (a) shows the actual change in *n* and *k'* for each group. (b) shows the percentage change in *n* and *k'* corrected for the corresponding changes measured in the placebo group. The error bars indicate the inter-subject variability ( $\pm$ S.D.).



**Figure 6.** Macular pigment optical densities averaged for each group at the end of phase II of the supplementation study. (a) shows measurements obtained at the fovea with a test probe of  $0.4^\circ$  diameter. (b) shows estimates made at an eccentricity of  $2.5^\circ$ . The results show significantly lower macular pigment density values in the P group when compared with any of the supplemented groups. The error bars indicate the inter-subject variability ( $\pm$ S.D.) in each group.

thresholds in all supplemented groups (see *Figure 3a*). The difference in CAA thresholds referenced to the placebo group was statistically significant in the L group ( $p = 0.001$ ). The phase II CAA thresholds in the placebo group increased systematically by comparison with phase I measurements in the same subjects. Phase I measurements were taken in December and phase II tests were carried out 6 months later in July. It is highly probable that 'seasonal' differences and other unknown factors can affect CAA thresholds over a period of 6 months. If such factors exist, then the CAA changes we measure in the placebo group must apply to all the groups. Although correction for threshold changes in the placebo group does not alter the inter-group differences, the data plotted this way make the trend towards lower thresholds in the treated groups visually more obvious (see *Figure 3b*).

The same analysis for the measured light scatter parameters shows little correlation between the change in *n* or *k'* and MP density, as shown in *Figure 4*. Analysis of changes in light scatter parameters within groups shows no significant changes or trends in the angular distribution of scattered light (parameter *n*), but

a consistent trend that shows a reduction in the amount of scattered light in every one of the supplemented groups (*Figure 5b*). In spite of this consistency, the decrease in *k'* values in the supplemented group failed to reach statistical significance, because of the large inter-subject variability and the small number of subjects involved. The first phase of supplementation may again have somewhat reduced the effect one might expect to observe as a result of increased supplementation the subjects received during phase II.

Macular pigment optical densities (MPODs) measured in all subject groups, at the end of phase II of the study, show a significant increase in each of the groups that received supplementation by comparison with the placebo group. The data shown in *Figure 6* were measured both at the fovea (*Figure 6a*) and  $2.5^\circ$  in the periphery (*Figure 6b*) using the new MAP test.

## Discussion

The initial experimental findings of this study provide limited support for the hypothesis put forward in the introduction suggesting that at least one of the functions

of the MP is the slowing down of the detrimental effects of rod photoreceptor signals on cone-mediated vision in the central region of the visual field. Neither the amount of forward light scatter in the eye, nor the decreased contrast acuity thresholds in the various subject groups show good correlation with MP density. There are a number of weaknesses in this study that may account for this lack of correlation. The expected rod-cone interaction effects depend strongly on the light level of adaptation of the retina and although great effort has been put into keeping the retinal illuminance constant for all subjects during the CAA test, only one light adaptation level was investigated in this study. As the mesopic range spans almost three log units change in light level, it is conceivable that the retinal illuminance selected for this study has not been optimum to reveal the benefits of reducing the contribution of rod photoreceptor signals to cone-mediated vision. Difficulties with subject retention over a period of 12 months resulted in small subject numbers per supplementation group, particularly at the end of phase II of the study. Another important criticism concerns the measurement of MP density. The change in MP density could not be investigated and hence correlated with the various measures of visual performance as measures were only made at the end of phase II. If there had been measures at the end of phase I, it is possible that the change in MP density could correlate with changes in CAA performance or scattered light. Nevertheless, one can make intergroup comparisons that take into account the MP density at the end of phase II of the study. In this respect our investigation is not a longitudinal study but relies more on cross-sectional comparison of results within groups. The new MAP test produces spatial profiles of MP density and reveals the significantly higher densities in each of the supplemented groups at the end of phase II of the study, by comparison with the placebo group. Higher levels were measured both at the fovea and 2.5° in the periphery, as shown in *Figure 6*, but the test was not available during phase I of the study.

A separate study that examines a number of light adaptation levels in the mesopic range with increased subject numbers in each group and improved estimates of MP density is needed to adequately test the proposed hypothesis. The analysis of visual performance data within groups does not rely on the available estimates of MP density and produces intriguing results. The small numbers of subjects within each group, particularly at the end of phase II of the study, have somewhat diminished the statistical significance of the measured improvements in visual performance. In spite of this obvious limitation, significant trends have emerged both in the reduction of scattered light in the eye and in the lowering of contrast activity thresholds in all the supplemented groups. The latter is found to be statistically

significant when the reduction in contrast activity thresholds in the lutein group is compared with the placebo group (see *Figure 3b*). A reduction in the amount of scattered light that contributes to flicker detection is observed in each group that received carotenoid supplementation. Although the effects are less obvious, a downward trend in *rms* wavefront aberrations as a result of increased carotenoid supplementation in phase II of the study is evident in each group (see *Figure 1c*). It is important to remember that visual performance data were obtained at the start of phase I of the study, and that the improvements in visual performance reported here reflect only the effects of doubling the carotenoid intake in phase II of the study. Although no data are available to assess the cumulative effects of carotenoid supplementation, it is not unreasonable to expect that significant improvements as a result of phase I of the carotenoid supplementation trial have somewhat diminished the additional improvements caused by doubling the carotenoid intake during phase II of the study. This investigation suggests that supplementation with lutein and zeaxanthin can help improve human vision at low light levels and that carefully designed tests of visual performance can be used to assess and quantify such improvements.

## Conclusions

The findings that have emerged from this study suggest that supplementation with lutein or/and zeaxanthin increases MP density levels, both at the fovea and 2.5° in the periphery. Both the amount of light scatter in the eye and the *rms* wavefront aberrations show decreasing trends, as a result of increased supplementation with lutein and/or zeaxanthin. The downward trend in contrast activity thresholds is statistically significant, at least in some of the treated groups, and suggests that supplementation with lutein and/or zeaxanthin may benefit driving at night and other spatial discrimination tasks carried out under low illumination.

Although the results of this study do not support a direct link between increased MP density and changes in light scatter or contrast activity thresholds in the eye, the present findings provide convincing evidence that supplementation with lutein and/or zeaxanthin can improve significantly our visual performance when the ambient illumination is low.

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## References

- Barbur, J. L. (1982) Reaction-time determination of the latency between visual signals generated by rods and cones. *Ophthalmic Physiol. Opt.* **2**, 179–185.
- Barbur, J. L., Thomson, W. D. and Forsyth, P. M. (1987) A new system for the simultaneous measurement of pupil size and two-dimensional eye movements. *Clin. Vis. Sci.* **2**, 131–142.
- Barbur, J. L., de Cunha, D., Harlow, A. J. and Woodward, E. G. (1993) Methods for the measurement and analysis of light scattered in the human eye. In: *Non-Invasive Assessment of the Visual System (Technical Digest Series)* Optical Society of America, Washington DC **3**, pp. 170–173.
- Barbur, J. L., Chisholm, C. M., Edgar, D. F. and Harlow, A. J. (2001) Pass/fail test for assessing visual performance following corneal refractive surgery. In: *Non-Invasive Assessment of the Visual System (Technical Digest Series)* Optical Society of America, Washington DC **1**, pp. 82–85.
- Bates, C. J., Chen, S. J., Macdonald, A. and Holden, R. (1996) Quantitation of vitamin E and a carotenoid pigment in cataractous human lenses, and the effect of a dietary supplement. *Int. J. Vitam. Nutr. Res.* **66**, 316–321.
- Berendschot, T. T., Goldbohm, R. A., Klopping, W. A., van de Kraats, J., van Norel, J. and van Norren, D. (2000) Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest. Ophthalmol. Vis. Sci.* **41**, 3322–3326.
- van den Berg, T. J. T. P. and Spekreijse, H. (1987) Measurement of the straylight function of the eye in cataract and other optical media disturbances by means of a direct compensation method. *Invest. Ophthalmol. Vis. Sci.* **28** (Suppl.), 397.
- Bone, R. A., Landrum, J. T. and Tarsis, S. L. (1985) Preliminary identification of the human macular pigment. *Vision Res.* **25**, 1531–1535.
- Brown, P. K. and Wald, G. (1963) Visual pigments in human and monkey retinas. *Nature* **200**, 37–43.
- Chisholm, C. M., Evans, A. D., Harlow, J. A. and Barbur, J. L. (2003) New test to assess pilot's vision following refractive surgery. *Aviat. Space Environ. Med.* **74**, 551–559.
- Foote, C. S., Chang, Y. C. and Denny, R. W. (1970) Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection. *J. Am. Chem. Soc.* **92**, 5216–5218.
- Frumkes, T. E., Eysteinson, T. and Arden, G. B. (1985) Tonic inhibition of red-cone pathways by dark adapted rods. *Invest. Ophthalmol. Vis. Sci.* **26**, 114.
- Goldberg, S. H., Frumkes, T. E. and Nygaard, R. W. (1983) Inhibitory influence of unstimulated rods in the human retina: evidence provided by examining cone flicker. *Science* **221**, 180–182.
- Hammond, B. R., Jr., Johnson, E. J., Russell, R. M., Krinsky, N. I., Yeum, K. J., Edwards, R. B. and Snodderly, D. M. (1997a) Dietary modification of human macular pigment density. *Invest. Ophthalmol. Vis. Sci.* **38**, 1795–1801.
- Hammond, B. R., Jr., Wooten, B. R. and Snodderly, D. M. (1997b) Individual variations in the spatial profile of human macular pigment. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* **14**, 1187–1196.
- Handelman, G. J., Dratz, E. A., Reay, C. C. and van Kuijk, J. G. (1988) Carotenoids in the human macula and whole retina. *Invest. Ophthalmol. Vis. Sci.* **29**, 850–855.
- Holladay, L. L. (1926) The fundamentals of glare and visibility. *J. Opt. Soc. Am.* **12**, 271–319.
- Kirschfeld, K. (1982) Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photo-receptor cells. *Proc. R. Soc. Lond. B Biol. Sci.* **216**, 71–85.
- Kopcke, W., Schalch, W., & LUXEA-Study Group (2005) Changes in macular pigment optical density following repeated dosing with lutein, zeaxanthin, or their combination in healthy volunteers – results of the LUXEA-study. *Invest. Ophthalmol. Vis. Sci.* **46**, E-abstract 1768.
- Landrum, J. T., Bone, R. A., Joa, H., Kilburn, M. D., Moore, L. L. and Sprague, K. E. (1997a) A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp. Eye Res.* **65**, 57–62.
- Landrum, J. T., Bone, R. A. and Kenyon, E. (1997b) A preliminary study of the stereochemistry of human lens zeaxanthin. *Invest. Ophthalmol. Vis. Sci.* **38** (Suppl.), S1026.
- Le Grand, Y. (1937) Recherces sur la diffusion de la lumiere dans l'oeil humain. *Opt. J. Rev. Opt.* **16**, 241–266.
- Liang, J. and Williams, D. R. (1997) Aberrations and retinal image quality of the normal human eye. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* **14**, 2873–2883.
- Liang, J., Grimm, B., Goelz, S. and Bille, J. F. (1994) Objective measurement of wave aberrations of the human eye with the use of a Hartmann-Shack wave-front sensor. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* **11**, 1949–1957.
- Malinow, M. R., Feeney-Burns, L., Peterson, L. H., Klein, M. L. and Neuringer, M. (1980) Diet-related macular anomalies in monkeys. *Invest. Ophthalmol. Vis. Sci.* **19**, 857–863.
- Naarendorp, F. and Frumkes, T. (1991) The influence of short-term adaptation of human rods and cones on cone-mediated grating visibility. *J. Physiol.* **432**, 521–541.
- Neuringer, M., Sandstrom, M. M., Johnson, E. J. and Snodderly, D. M. (2004) Nutritional manipulation of primate retinas, I: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. *Invest. Ophthalmol. Vis. Sci.* **45**, 3234–3243.
- Oyster, C. (1999) *The Human Eye: Structure and Function*. Sinauer Associates Ltd., Massachusetts, Sunderland.
- Pease, P. L., Adams, A. J. and Nuccio, E. (1987) Optical density of human macular pigment. *Vision Res.* **27**, 705–710.
- Porter, J., Guirao, A., Cox, I. G. and Williams, D. R. (2001) Monochromatic aberrations of the human eye in a large population. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* **18**, 1793–1803.
- Puell, M. C., Palomo, C., Sanchez-Ramos, C. and Villena, C. (2004) Normal values for photopic and mesopic letter contrast sensitivity. *J. Refract. Surg.* **20**, 484–488.
- Reading, V. M. and Weale, R. A. (1974) Macular pigment and chromatic aberration. *J. Opt. Soc. Am.* **64**, 231–234.
- Rodriguez-Carmona, M., Barbur, J. L., Harlow, J. A., Schalch, W. and Kopcke, W. (2004) Chromatic sensitivity changes in relation to macular pigment optical density (MPOD) in human vision. *Invest. Ophthalmol. Vis. Sci.* **45**, E-abstract 3438.

- Schalch, W. (1992) Carotenoids in the retina – a review of their possible role in preventing or limiting damage caused by light and oxygen. *Free Radic. Aging* **62**, 280–298.
- Schalch, W., Rodriguez-Carmona, M., Harlow, J. A., Barbur, J. L. and Koepcke, W. (2004) Macular pigment optical density (MPOD) measurements using visual displays – a new method and first results. *Invest. Ophthalmol. Vis. Sci.* **45**, E-abstract 1296.
- Schalch, W., Barker, F. M. and LUXEA-Study Group (2005) Ocular and general safety of supplementation with zeaxanthin and lutein; plasma exposure levels of carotenoids and 3'-dehydro-lutein – results of the LUXEA-study. *Invest. Ophthalmol. Vis. Sci.* **46**, E-abstract 1765.
- Schupp, C., Olano-Martin, E., Gerth, C., Morrissey, B. M., Cross, C. E. and Werner, J. S. (2004) Lutein, zeaxanthin, macular pigment, and visual function in adult cystic fibrosis patients. *Am. J. Clin. Nutr.* **79**, 1045–1052.
- Snodderly, D. M. (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am. J. Clin. Nutr.* **62** (Suppl.), 1448S–1461S.
- Wooten, B. R. and Hammond, B. R. (2002) Macular pigment: influences on visual acuity and visibility. *Prog. Retin. Eye Res.* **21**, 225–240.
- Yeum, K. J., Taylor, A., Tang, G. and Russell, R. M. (1995) Measurement of carotenoids, retinoids, and tocopherols in human lenses. *Invest. Ophthalmol. Vis. Sci.* **36**, 2756–2761.