The Impact of Macular Pigment Augmentation on Visual Performance Using Different Carotenoid Formulations

James Loughman,[1,2] *John M. Nolan,*[3,4] *Alan N. Howard,*[5] *Eithne Connolly,*[3,4] *Katie Meagher,*[3] *and Stephen Beatty*[3,4]

Purpose. To investigate changes in macular pigment optical density (MPOD) and visual performance following supplementation with different macular carotenoid formulations.

Methods. Thirty-six subjects (19 male, 17 female; mean ± SD, age 51 ± 13 years) were recruited into this single-masked placebo-controlled study, and were randomly assigned to one of the following three intervention (supplementation) groups: (1) group 1 (20 mg lutein [L] and 2 mg zeaxanthin [Z]); (2) group 2 (10 mg L, 2 mg Z, and 10 mg *meso*-zeaxanthin [MZ]); and group 3 (placebo). Outcomes measures included visual performance and MPOD response. Data were collected at baseline, at 3 months, and at 6 months.

Results. At 3 and 6 months, a statistically significant increase in MPOD was found at all eccentricities (other than the most peripheral 3° location) in group 2 ($P < 0.05$ for all), whereas no significant increase in MPOD was demonstrable at any eccentricity for subjects in groups 1 and 3. Statistically significant improvements in visual performance measures including visual acuity and contrast sensitivity with and without glare were observed for group 2 only. Only mesopic contrast sensitivity at one spatial frequency improved significantly by 6 months ($P < 0.05$) for group 1. No improvements in any parameters of visual performance were observed for subjects supplemented with placebo ($P > 0.05$ for all).

Conclusions. These results suggest that supplementation with all three macular carotenoids potentially offered advantages over preparations lacking MZ, both in terms of MPOD response and visual performance enhancement. (*Invest Ophthalmol Vis Sci.* 2012;53:7871–7880) DOI:10.1167/iovs.12-10690

From the [1]Optometry Department, Dublin Institute of Technology, Dublin, Ireland; the [2]African Vision Research Institute, Faculty of Health Sciences, University of KwaZulu Natal, Durban, South Africa; the [3]Macular Pigment Research Group, Department of Chemical and Life Sciences, Waterford Institute of Technology, Waterford, Ireland; the [4]Institute of Vision Research, Whitfield Clinic, Waterford, Ireland; and the [5]Howard Foundation, Downing College, University of Cambridge, Cambridge, United Kingdom.

Supported by the Howard Foundation, Cambridge, CB22 5LA, United Kingdom.

Submitted for publication August 2, 2012; revised October 15 and October 27, 2012; accepted November 1, 2012.

Disclosure: **J. Loughman**, Howard Foundation (C); **J.M. Nolan**, Nutrasight Consultancy Limited (I), Howard Foundation (C), Bausch & Lomb (C); **A.N. Howard**, Howard Foundation (I); **E. Connolly**, None; **K. Meagher**, None; **S. Beatty**, Nutrasight Consultancy Limited (I), Howard Foundation (C), Bausch & Lomb (C)

Corresponding author: James Loughman, Optometry Department, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland; james.loughman@dit.ie.

The macula mediates central vision, provides sharpest visual acuity (VA) and facilitates best color discrimination. *Meso*-zeaxanthin (MZ), lutein (L) and zeaxanthin (Z) are uniquely concentrated in the inner and central layers of the primate macula[1] where they are collectively known as macular pigment (MP).

MP absorbs short-wavelength (blue) light[1] and possesses antioxidant properties.[2] While MP is hypothesized to protect against age-related macular degeneration (AMD), the primary evolutionary advantage of such exquisite and biologically selective accumulation at the fovea and central macula likely rests on MP's capacity to optimize and enhance vision. The properties of MP have prompted the articulation of various theories to explain the influence of MP on vision, and include the optical,[3] glare,[4] and visibility hypotheses.[5] Traditional views of the possible optical advantages that MP confers on visual acuity, contrast sensitivity, and visual comfort have been supplemented with further observations, such as (1) the dichroic properties of MP (which may facilitate the reduction of discomfort and disability glare through preferential absorption of plane polarized light),[6] and (2) the possible beneficial effects of MP on root-mean-square wavefront aberrations (in particular, higher order aberrations).[7]

Although the effects of optical filtration are often assumed to be the sole mediator of visual performance benefits attributable to MP,[8] there is another, and important, mechanism whereby MP may affect visual performance and experience. The antioxidant properties of the MP carotenoids may attenuate the deleterious effects of free radical damage on the physiologic functionality of photoreceptors and their axons, and thereby refine and preserve visual function. Such a pathway may also, perhaps, explain improvements in functional vision observed in cases of ocular disease following MP augmentation.[9,10] Importantly, recent evidence suggests that, in terms of antioxidant properties, MZ appears to be the most potent of the macular carotenoids.[11]

No study investigating the effect of MP on visual performance has emphasized or evaluated the potential role of MZ, in combination with the other carotenoids, L and Z. Aside from its antioxidant potency, MZ is also somewhat intriguing from a visual performance perspective. Firstly, MZ, which is less available than L or Z in a typical diet, is produced by the body in sufficient quantities to become the dominant carotenoid at the central macula (where visual performance is maximal)[12]; secondly, the absorbance spectrum of MZ extends the range of prereceptoral blue light filtration capacity and its' orientation (compared to L) in the membrane of Henle's fibers, likely conferring beneficial polarization properties[13]; thirdly, it has been shown that, in older subjects and smokers (known risk factors for AMD), MP occasionally displays an atypical central dip profile[14,15] but can be uniquely rebuilt with supplements containing MZ[14]; and finally, it has been suggested that some individuals may lack the capacity to convert retinal L to MZ, possibly attenuating visual performance in a way that may go

unreported by the individual who is unaware of his/her deficiency in this respect (somewhat akin to color vision defects).

For the aforementioned reasons, we designed a study to investigate the impact of supplementation with two different preparations (one containing L and Z, and the other containing L, Z, and MZ) on macular pigment optical density (MPOD) and visual performance.

Methods

Subjects and Study Design

Thirty-six subjects (19 male, 17 female) were recruited into this single-masked, randomized, placebo-controlled trial. All subjects signed an informed consent document, and all subjects were treated in accordance with the Declaration of Helsinki. Ethics approval was granted by the Research Ethics Committees at Dublin Institute of Technology, Dublin, Ireland, and Waterford Institute of Technology, Waterford, Ireland. Inclusion criteria for participation in this study were as follows: age 18 to 70 years; refractive error less than six dioptres spherical equivalent; no known presence of ocular or systemic pathology; best corrected visual acuity (BCVA) of LogMAR 0.5 (20/60) or better in the study eye (actual acuity range 20/25 to 20/10); not taking L and/or Z and/or MZ dietary supplements in the 12-month period prior to study recruitment.

Twelve subjects were randomly assigned to each of the three supplementation groups as follows: group 1 was supplemented with a product containing 20 mg L and 2 mg Z (Ultra Lutein, Natures Plus, Melville, NY) (tests at two laboratory facilities indicated that the product we used contained approximately 2 mg Z, compared to the 0.86 mg stated on the product literature); group 2 was supplemented with a product containing 10 mg MZ and 10 mg L and 2 mg Z (Macushield, Macuvision Europe Ltd, Solihull, UK); and group 3 was supplemented with placebo (G & G Food Supplies Ltd, West Sussex, UK). All subjects were instructed to take one capsule per day with a meal for the 6-month study duration. The importance of compliance with the supplementation protocol was emphasized to subjects at the baseline visit and in the subject information leaflet. Regular reminder text messages and phone calls were made, and subjects were requested to return their supplement packs at the 3- and 6-month visits to facilitate tablet count checks on compliance.

Iris color, lifestyle information, and demographic information were recorded at baseline, while body mass index measurements and blood serum samples were taken at each study visit. For each subject, the full range of visual performance and MP measurements was conducted on three separate visits: at baseline, 3 months, and 6 months. All subjects were naïve to the method of measurement of MP and visual performance (other than BCVA) employed in the study.

MPOD Measurement

A spatial profile of MPOD was generated across 0.25°, 0.5°, 1°, 1.75°, and 3° of retinal eccentricity with respect to a 7° reference location, using the Macular Densitometer, which employs a heterochromatic flicker photometry (HFP) technique. Subjects were shown an explanatory video of the technique, and afforded a practice session prior to test commencement. HFP flicker frequencies were optimized following determination of individual critical flicker fusion (CFF) frequency measurements in a customization process that optimizes MP measurements, and which has been described in detail elsewhere.[16] The MPOD measurement comprised the average of six readings (computed as the radiance value at which the subject reported null flicker) at each retinal eccentricity, and was deemed reliable and acceptable only when the standard deviation of null flicker responses was below 0.1.

Visual Performance Assessment

Best corrected visual acuity was measured at baseline with a computer-generated logMAR test chart (Test Chart 2000 Pro; Thompson Software Solutions, Hatfield, UK) at a viewing distance of 4 m, using the Sloan LogMAR ETDRS letterset. VA was measured using single-letter scoring, and recorded as the average of three measurements facilitated by the software letter randomization feature. The eye with better VA was chosen as the study eye; however, when both eyes had the same corrected acuity, the right eye was chosen as the study eye.

Contrast sensitivity was measured using a functional acuity contrast test (Optec6500 Vision Tester; Stereo Optical Co., Inc., Chicago, IL), which incorporates sine wave gratings presented as Gabor patches at spatial frequencies of 1.5, 3, 6, 12, and 18 cycles per degree (cpd) to produce a contrast sensitivity function. Testing was performed under mesopic (3 candelas per square meter [cd/m^2]) and photopic (85 cd/m^2) conditions. Contrast sensitivity was also assessed under glare conditions using the same test but in the presence of an inbuilt circumferential LED glare source (42 lux for mesopic and 84 lux for photopic glare testing). The LED glare source rendered a daylight-simulating color temperature of 6500°K and a spectral emission profile with a single large peak at 453 nm (close to peak MP spectral absorbance). These tests have been described in more detail elsewhere.[17,18] The subjects' tasks, and the nature of the test, were explained in detail prior to test commencement. Subject performance was monitored closely by a trained examiner during the test, and the subject was reinstructed if necessary.

Photostress recovery time (PRT) of the short wavelength sensitive (SWS) visual system was assessed using a macular automated photostress test, an adaptation of the Humphrey visual field analyzer (Model 745i; Carl Zeiss Meditec, Inc., Dublin, CA) for the assessment of foveal incremental light threshold.[19,20] To isolate SWS cones, mid- and long-wavelength sensitive cones were desensitized using a 3-minute sustained exposure to a 100 cd/m^2, 570 nm bleaching background. A Goldmann V, 440nm stimulus, presented for 200 milliseconds, was used to test the sensitivity of the SWS system before and after photostress. Following the 3-minute adaptation and practice session (during which subject performance was assessed for reliability and understanding), subjects were directed to fixate centrally between four circumferential light stimuli and to respond to the detection of a *blue* stimulus at that location using the response button provided. Foveal sensitivity was determined as the average of three consecutive measurements recorded in decibels, with each decibel representing a 0.1 log unit sensitivity variation. Following baseline foveal sensitivity calculation, the subject was exposed to a short-wavelength dominated photostress stimulus, which consisted of a 5-s exposure to a 300-W lamp viewed at 1 m through a low-pass glass dichroic filter, thus creating a temporary foveal blue after-image to mask fixation and reduce foveal sensitivity. Immediately postphotostress, a continuous and timed cycle of foveal sensitivity measurements was conducted and recorded. The reduction in foveal sensitivity from baseline (i.e., the magnitude of the reduction in foveal sensitivity caused by the photostress stimulus, calculated as baseline sensitivity [decibel] minus immediate post photostress sensitivity [decibel]), along with the recovery characteristics of the SWS system sensitivity, was recorded. Pupil diameter was again recorded for background light conditions and in the presence of the photostress light source. Iris color was also graded using a standardized iris classification scheme as defined by Seddon et al.[21]

Ocular straylight was measured using an Oculus C-Quant (OCULUS, Optikgeräte GmbH, Wetzlar, Germany), an instrument designed to quantify the effect of light scatter on vision. The assessment of ocular straylight was investigated because of MP's optical properties and consequential potential capacity to attenuate scatter of short-wavelength (blue) light following supplementation and concomitant augmentation of MPA. Central bipartite 14° test field was viewed monocularly through the instrument eyepiece. Subjects were instructed to respond, using the appropriate response button, to indicate the

Table 1. Baseline Demographic, MP, and VA Data for Each Intervention Group

Variable	Group 1: 20 mg L; 2 mg Z; Mean ± SD (range)	Group 2: 10 mg MZ; 10 mg L; 2 mg Z; Mean ± SD (range)	Group 3: Placebo; Mean ± SD (range)	*P* Value
N	12	12	12	
Age (y)	56 ± 8 (30–66)	51 ± 13 (23–70)	46 ± 20 (21–68)	0.3
BMI	27 ± 3	25 ± 3	26 ± 5	0.31
BCVA	−0.14 ± 0.1	−0.18 ± 0.12	−0.16 ± 0.12	0.72
MPOD 0.25	0.32 ± 0.13	0.37 ± 0.13	0.35 ± 0.18	0.69
MPOD 0.5	0.25 ± 0.14	0.27 ± 0.12	0.28 ± 0.16	0.88
MPOD 1.0	0.15 ± 0.14	0.20 ± 0.07	0.16 ± 0.11	0.46
MPOD 1.75	0.07 ± 0.10	0.10 ± 0.07	0.04 ± 0.04	0.16
MPOD 3	0.07 ± 0.08	0.08 ± 0.07	0.04 ± 0.05	0.26

BMI, body mass index.

position of the most strongly flickering right or left test hemifield. Subjects were allowed a defined practice session, during which reliable understanding of the task was assessed by the trained examiner. Test results were deemed acceptable only when the standard deviation of measured straylight value (ESD) was $\leq$ 0.08, and the reliability coefficient (Q) was $\geq$ 1. Absolute straylight values were recorded in logarithmic form (log[s]).

Statistical Analysis

A statistical software package (PASW Statistics 18.0; SPSS, Inc., Chicago, IL) was used for analysis. All quantitative variables investigated exhibited a typical normal distribution. Means ± SDs are presented in the text and tables. Statistical comparisons of the three supplementation groups at baseline were conducted using one way ANOVA, while paired samples Student's *t*-tests and repeated measures ANOVA (RM ANOVA) (using a general linear model approach) were used to analyze visual performance and MPOD measures in each supplementation group for change across study visits as appropriate. Where relevant, the Greenhouse-Geisser correction for violation of sphericity was used. A 5% level of significance was used throughout the analysis, except for the intergroup analysis, where a more stringent 1% significance level was used to reflect the multiple tests conducted.

With 10 to 11 subjects per treatment group, this exploratory study had adequate statistical power to detect only large within-group or between-group differences. To illustrate, on standard assumptions (5% level of significance, two-tailed tests) and with 11 subjects in a treatment group, the power was 0.85 for detecting a pre-post difference of one standard deviation using a paired samples Student's *t*-test, but power of only 0.32 for detecting a difference of half that magnitude.

Results

Following randomization, one-way ANOVA revealed no significant differences between groups, at baseline, in terms of demographic, MP, visual performance parameters, or other variables, as illustrated for select parameters in Table 1 ($P >$ 0.05 for all).

Of the 36 subjects recruited, 32 completed the trial, with one dropout from each of the intervention groups and two dropouts from the placebo group. All further analysis is confined to those subjects with a complete dataset (group 1, n = 11; group 2, n = 11; group 3, n = 10).

Serum Analysis

One-way ANOVA revealed no significant difference between study groups for baseline serum concentrations of L (P = 0.496), Z (P = 0.977), or total carotenoids (P = 0.595). RM ANOVA revealed a statistically significant change in serum L, Z, MZ, and total carotenoid concentrations across study visits in group 2 only. Serum L concentrations did not change significantly in group 1 and group 3 across the three study visits or, indeed, between any two study visits (paired Student's *t*-test; $P > 0.05$ for all).

Serum carotenoid concentrations (μmol/L) split by group intervention at baseline, 3 months and 6 months, along with significance of change (*P*) values, are presented in Table 2.

MPOD Response

Table 3 presents MP data for each group and for each eccentricity measured, at baseline and following 3- and 6-months' supplementation with macular carotenoids or placebo. The data show a statistically significant increase in MPOD at 3 months, but only for group 2. The increase was significant at all retinal eccentricities tested, other than at 3°. There was no significant change in MPOD between 3 and 6 months for any group. RM ANOVA reveals a statistically significant increase in MPOD at follow-up visits in group 2 only, across all retinal eccentricities other than at 3°.

The MPOD response at 0.25° of retinal eccentricity among supplementation groups across the three study visits is presented in Figure 1.

Visual Performance Response

There was no significant change in BCVA at 3 months for any of the study Groups ($P <$ 0.05 for all). At 6 months, paired Student's *t*-test analysis revealed a statistically significant improvement in BCVA compared to baseline for group 2 (P = 0.008). RM ANOVA confirms a significant change in BCVA from baseline at subsequent study visits for group 2 (P = 0.034).

Mesopic and photopic contrast sensitivity improved from baseline values across a range of spatial frequencies at 3 months and, in particular, at 6 months. At 3 months, statistically significant improvements were noted at 1.5 cpd (P = 0.008) for mesopic conditions, and at 3 cpd (P = 0.024) and 12 cpd (P = 0.025) for photopic conditions for group 2. At 6 months, statistically significant improvements in contrast sensitivity were noted across a substantially broader set of spatial frequencies, most notably under mesopic conditions, for group 2 (see Table 4). Mesopic contrast sensitivity at 6 cpd improved significantly for group 1 at 6 months ($P <$ 0.05). RM ANOVA confirms the improvements from baseline contrast sensitivity values to be statistically significant at subsequent study visits for at least three of the five spatial frequencies tested under mesopic and photopic conditions. A detailed summary of contrast sensitivity results is provided in Table 4.

Table 2. Serum L, Z, MZ, and Total Macular Carotenoid Concentrations and Response (Absolute Values) for Each Intervention Group

	Baseline (μmol/L)	3 mo (μmol/L)	6 mo (μmol/L)	*P* Value*
L				
Group 1	0.26 ± 0.12	0.91 ± 0.99	0.54 ± 0.48	0.14
Group 2	0.27 ± 0.05	1.26 ± 0.66	0.94 ± 0.46	0.00
Group 3	0.21 ± 0.05	0.23 ± 0.07	0.22 ± 0.06	0.39
Z				
Group 1	0.06 ± 0.03	0.12 ± 0.05	0.07 ± 0.03	0.04
Group 2	0.06 ± 0.02	0.12 ± 0.05	0.09 ± 0.03	0.02
Group 3	0.06 ± 0.03	0.06 ± 0.03	0.06 ± 0.04	0.77
MZ				
Group 1	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.01	0.14
Group 2	0.00 ± 0.00	0.10 ± 0.05	0.09 ± 0.06	0.00
Group 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-
TC†				
Group 1	0.33 ± 0.14	1.03 ± 1.03	0.61 ± 0.50	0.13
Group 2	0.35 ± 0.07	1.56 ± 0.73	1.20 ± 0.49	0.00
Group 3	0.26 ± 0.07	0.29 ± 0.10	0.28 ± 0.09	0.52

* Significance (*P*) values represent repeated measures (Greenhouse-Geisser) significance for the change in serum concentrations across the three study visits.

† TC, total macular carotenoid, combined L, Z, MZ response; group 1 ($n = 12$): high L group (20 mg L/day, 1 mg Z/day); group 2 ($n = 12$): combination group (10 mg L/day, 2 mg Z/day, 10 mg MZ/day); group 3 ($n = 12$): placebo.

The change in mesopic contrast sensitivity function, from baseline to exit visit, across supplementation groups is presented in Figure 2.

Mesopic and photopic contrast sensitivity under glare conditions improved from baseline across a range of spatial frequencies at 3 months and at 6 months. At 3 months, statistically significant improvements were noted at 12 cpd ($P = 0.048$) for mesopic conditions, and at 1.5 cpd ($P = 0.023$) and 3 cpd ($P = 0.033$) for photopic conditions for group 2. At 6 months, statistically significant improvements were noted across a substantially broader set of spatial frequencies for group 2. RM ANOVA revealed no statistically significant change, at any spatial frequency, in glare-affected mesopic or photopic conditions within groups 1 and 3. The statistically significant improvements in group 2, under glare-affected mesopic and photopic conditions, for all spatial frequencies tested (other than 18 cpd) were robust to RM ANOVA. A detailed summary of glare-affected contrasts sensitivity results are provided in Table 5.

The change in glare-affected photopic contrast sensitivity function, from baseline to exit visit, across supplementation groups is presented in Figure 3.

Photostress recovery time did not improve significantly for any of the groups during the study period ($P > 0.05$ for all).

Table 3. MPOD Response and Significance at Each Retinal Eccentricity across Study Visits

Group Intervention	Baseline	3 mo	Student's *t*-test	6 mo	Student's *t*-test	RM ANOVA
	MPOD0.25	MPOD0.25	*P**	MPOD0.25	*P*†	*P*‡
Group 1	0.32 ± 0.12	0.38 ± 0.15	0.08	0.41 ± 0.14	0.44	0.09
Group 2	0.37 ± 0.13	0.49 ± 0.14	0.00	0.50 ± 0.20	0.01	0.00
Group 3	0.35 ± 0.20	0.38 ± 0.20	0.71	0.37 ± 0.18	0.64	0.81
	MPOD0.50	MPOD0.50	*P*	MPOD0.50	*P*	*P*
Group 1	0.27 ± 0.13	0.32 ± 0.22	0.46	0.30 ± 0.14	0.46	0.09
Group 2	0.28 ± 0.12	0.38 ± 0.16	0.01	0.37 ± 0.21	0.04	0.01
Group 3	0.28 ± 0.17	0.31 ± 0.16	0.40	0.28 ± 0.16	0.97	0.57
	MPOD1.0	MPOD1.0	*P*	MPOD1.0	*P*	*P*
Group 1	0.16 ± 0.14	0.18 ± 0.12	0.46	0.15 ± 0.14	0.77	0.53
Group 2	0.21 ± 0.08	0.28 ± 0.10	0.03	0.27 ± 0.14	0.09	0.04
Group 3	0.16 ± 0.12	0.14 ± 0.11	0.95	0.13 ± 0.10	0.40	0.99
	MPOD1.75	MPOD1.75	*P*	MPOD1.75	*P*	*P*
Group 1	0.08 ± 0.10	0.08 ± 0.10	0.86	0.07 ± 0.10	0.87	0.93
Group 2	0.11 ± 0.07	0.19 ± 0.05	0.00	0.18 ± 0.10	0.04	0.03
Group 3	0.03 ± 0.03	0.03 ± 0.05	0.77	0.03 ± 0.05	0.73	0.82
	MPOD3.0	MPOD3.0	*P*	MPOD3.0	*P*	*P*
Group 1	0.05 ± 0.02	0.07 ± 0.06	0.59	0.03 ± 0.03	0.19	0.67
Group 2	0.09 ± 0.07	0.11 ± 0.11	0.28	0.10 ± 0.07	0.71	0.92
Group 3	0.02 ± 0.03	0.02 ± 0.03	0.81	0.02 ± 0.05	0.68	0.48

Group 1 (20 mg L, 2 mg Z), $n = 11$; group 2 (10 mg MZ, 10 mg L, 2 mg Z), $n = 11$; group 3 (placebo), $n = 10$.

* Difference between baseline and 3 months (paired samples Student's *t*-test).

† Difference between baseline and 6 months (paired samples Student's *t*-test).

‡ RM ANOVA across all visits.

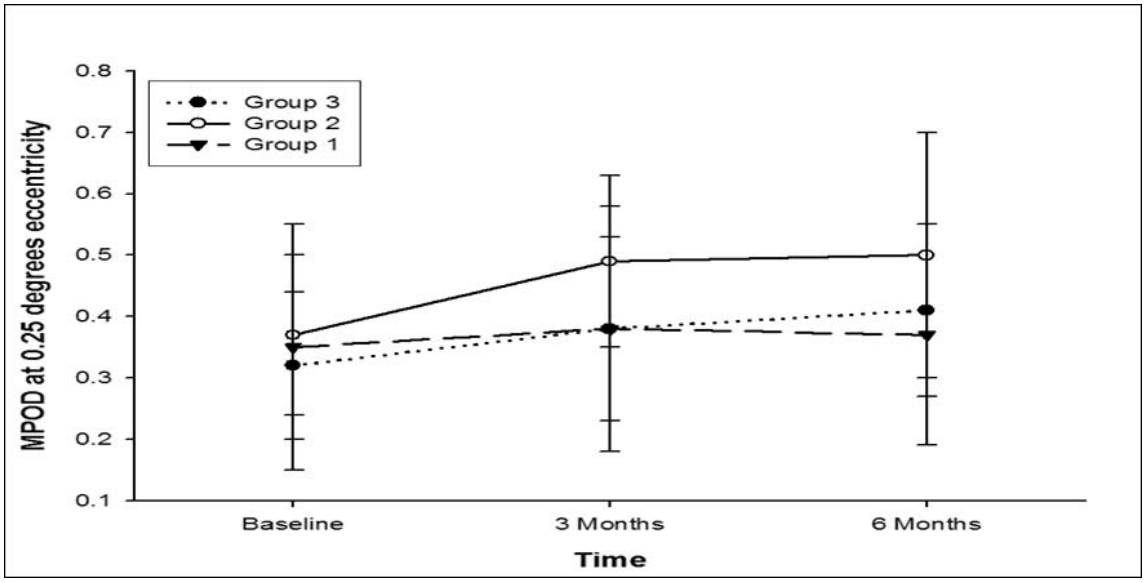

Figure 1. MPOD response at 0.25° of retinal eccentricity among study groups across study visits.

Paired Student's *t*-test analysis revealed, however, that the improvement in PRT for group 2 (PRT 37 seconds [or 21%] shorter on average at 6 months compared to baseline) approached, but did not reach, statistical significance ($t = 2.067$, $P = 0.06$), while no such improvements were observed for groups 1 or 3. Ocular straylight measures did not change significantly for any group ($P > 0.05$ for all). No association was observed between iris color and MPOD or any visual performance measures ($P > 0.05$ for all).

Intergroup Analysis

At baseline, one way ANOVA revealed no statistically significant differences between serum carotenoid levels, MPOD levels or visual performance measures, including BCVA, mesopic and photopic contrast sensitivity (with and without glare) ($P > 0.05$ for all 29 statistical tests). Following 6 months' supplementation, however, statistically significant differences between groups were demonstrated across a range of measures. Serum carotenoid differences were observed between groups for L and MZ ($P < 0.01$, for both), but not Z. MP level differences were observed at 1, 1.75, and 3 degrees of retinal eccentricity ($P < 0.01$ for each), while contrast sensitivity differences were noted across five of the 20 test condition combinations, although these were not significant at the 1% level ($P = 0.02$–0.04). Post-hoc analysis (Tukey Test) isolated the difference to group 2, which revealed a statistically significant bias towards higher MP and serum carotenoid levels in group 2.

Discussion

We report no significant change in MPOD at any eccentricity, at 3 or at 6 months, in subjects supplemented with a preparation that does not contain MZ or in subjects given placebo. In contrast, subjects supplemented with all three macular carotenoids exhibited a significant increase in MPOD at four of the five eccentricities tested, at 3 months and at 6 months. These findings are neither counterintuitive nor inconsistent with the serum response noted herein, or with the findings of previous reports or with emerging data.[22–24] For example, the double-masked, randomized, placebo-controlled COMPASS trial,[17,18] which used a supplement formulation containing 12 mg L and 1 mg Z (but not MZ), reported a modest, albeit statistically significant, rise in MPOD, but only following 12 months' supplementation. Although the macular response at 6 months in subjects supplemented with L and Z (but not MZ) in the current study was better when compared to the same time point in COMPASS (most likely due to the higher overall carotenoid content and oil-based formulation used in the current study), the observed change in MPOD at 6 months was not statistically significant in either investigation. This finding is also consistent with previously published studies reporting delayed and modest retinal response to macular carotenoid supplements that do not contain MZ.[24–27] In contrast, however, recent reports have consistently demonstrated that supplementation with preparations containing MZ results in a rapid response in central MPOD,[22,23] even in subjects with atypical spatial profiles at baseline characterized by the lack of a typical central peak.[23]

The spatial profile of MP is a composite of the respective contributions of its three constituent carotenoids: L, Z, and MZ. Z and MZ are the predominant carotenoids in the foveal region, whereas L predominates in the parafoveal region.[28] The concentration of MZ peaks centrally, with a MZ:Z ratio of 0.82 in the central retina (within 3 mm of the fovea) and 0.25 in the peripheral retina (11–21 mm from the fovea).[12] These observations are probably attributable to the fact that retinal MZ is primarily generated from isomerization of retinal L (but not Z),[29] thus accounting for relatively lower levels of L and higher relative levels of MZ in the central macula, and vice versa in the peripheral macula, and would also explain why MZ accounts for approximately one-third of total MP, in spite of its absence (or very low concentrations) in a typical diet.[30] Consequently, and by definition, the typical central peak of MP is dependent upon, and determined by, this process of isomerization of retinal L and any factors that might influence it, and not solely on dietary intake of carotenoids.

It is likely, therefore, that the variability in the rapidity and magnitude of the observed retinal response to different preparations of macular carotenoids is attributable to the presence or absence of MZ in the formulation used. It is reasonable to hypothesize that the macular response to a preparation containing L (but not MZ) will be delayed at the epicenter (because of the need to convert the supplemental L

Table 4. Contrast Sensitivity Change and Significance Levels at each Spatial Frequency Tested under Mesopic and Photopic Conditions

Group Intervention	Contrast Sensitivity at Baseline	Contrast Sensitivity at 6 mo	Student's *t*-test	RM ANOVA
	Photopic at 1.5 cpd	Photopic at 1.5 cpd	*P**	*P*†
Group 1	44 ± 26	53 ± 20	0.05	0.12
Group 2	49 ± 30	68 ± 28	0.07	0.12
Group 3	52 ± 22	62 ± 29	0.41	0.28
	Photopic at 3.0 cpd	Photopic at 3.0 cpd		
Group 1	85 ± 37	85 ± 29	0.96	0.68
Group 2	73 ± 25	100 ± 28	0.00	0.00
Group 3	95 ± 36	94 ± 46	0.84	0.81
	Photopic at 6.0 cpd	Photopic at 6.0 cpd		
Group 1	99 ± 27	100 ± 28	0.71	0.43
Group 2	95 ± 36	114 ± 45	0.23	0.26
Group 3	103 ± 54	116 ± 64	0.83	0.88
	Photopic at 12.0 cpd	Photopic at 12.0 cpd		
Group 1	30 ± 10	39 ± 17	0.18	0.26
Group 2	32 ± 13	50 ± 30	0.01	0.01
Group 3	57 ± 43	62 ± 42	0.64	0.92
	Photopic at 18.0 cpd	Photopic at 18.0 cpd		
Group 1	8 ± 5	12 ± 9	0.17	0.38
Group 2	12 ± 6	23 ± 17	0.06	0.04
Group 3	20 ± 17	17 ± 14	0.53	0.73
	Mesopic at 1.5 cpd	Mesopic at 1.5 cpd		
Group 1	57 ± 30	63 ± 23	0.62	0.83
Group 2	52 ± 18	76 ± 24	0.00	0.00
Group 3	65 ± 27	75 ± 24	0.20	0.24
	Mesopic at 3.0 cpd	Mesopic at 3.0 cpd		
Group 1	78 ± 45	74 ± 35	0.79	0.91
Group 2	58 ± 17	88 ± 38	0.00	0.00
Group 3	68 ± 39	96 ± 44	0.10	0.11
	Mesopic at 6.0 cpd	Mesopic at 6.0 cpd		
Group 1	41 ± 13	53 ± 21	0.06	0.00
Group 2	50 ± 19	77 ± 49	0.14	0.06
Group 3	53 ± 46	63 ± 43	0.58	0.82
	Mesopic at 12.0 cpd	Mesopic at 12.0 cpd		
Group 1	7 ± 4	9 ± 6	0.20	0.16
Group 2	10 ± 6	33 ± 30	0.04	0.01
Group 3	13 ± 14	21 ± 25	0.40	0.50
	Mesopic at 18.0 cpd	Mesopic at 18.0 cpd		
Group 1	2 ± 0	2 ± 0	NS	0.17
Group 2	2 ± 9	11 ± 14	0.04	0.02
Group 3	4 ± 5	5 ± 3	0.59	0.28

RM ANOVA, Repeated measures ANOVA across all study visits; NS, nonsignificant (statistic not computed, SE of difference = 0).
Group 1 (20 mg L; 2 mg Z): $n = 11$; Group 2 (10 mg MZ; 10 mg L; 2 mg Z): $n = 11$; Group 3 (placebo): $n = 10$.
* Difference between baseline and 6 months (paired samples Student's *t*-test).
† RM ANOVA across all visits.

that has been captured by the retina) and attenuated in the peripheral macula (because some of the captured L at this location will be isomerized to MZ and, therefore, depleted). Such a biologically plausible rationale is consistent with our observed rapid and significant response, in terms of central MPOD, in subjects supplemented with all three macular carotenoids. The corollary to this hypothesis, therefore, would include negation of the need for conversion of (captured) retinal L to MZ in a subject supplemented with MZ, thus allowing stabilization and accumulation of L at the site of this carotenoid's natural dominance (i.e., the peripheral macula), and such a corollary is consistent with the saturation of central MP in subjects supplemented with all three macular carotenoids (reflected in the observation that the central peak of MP did not increase beyond 3 months). In other words, it is possible that inclusion of MZ in a preparation of supplemental macular carotenoids may not only result in a more rapid and greater central retinal response, but may facilitate MP augmentation in the peripheral macula. This is consistent with the findings of the current and recent reports.[22–24]

The contribution of MP to visual performance and subjective visual experience has been the subject of intense investigation in the recent past, informing the emerging and evidence-based consensus that MP is indeed important in this respect.[31] Associations identified from cross-sectional studies include: visual discomfort attributable to glare, which has been shown to be wavelength-dependent and more severe for short wavelength visible light than for medium or long wavelength visible light,[32] is inversely related to MPOD[33]; the intensity of short wavelength light required to elicit photophobia is positively related to MPOD[34]; and PRT has an inverse relationship with MPOD.[9] Further, these findings have been replicated under free-viewing conditions.[9] However, the published cross-sectional data are less consistent with respect to the relationship between MP and psychophysical outcome measures that are not designed to test function under conditions of glare. Nevertheless, a positive association

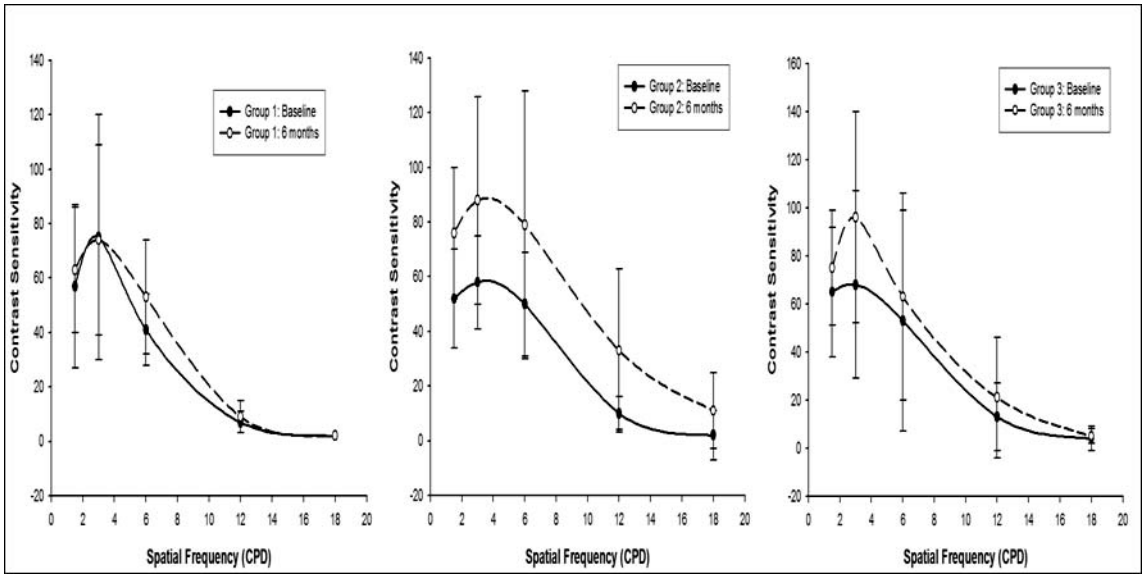

Figure 2. Change in mesopic contrast sensitivity function across supplementation groups from baseline to exit visits.

between MPOD and corrected distance VA and contrast sensitivity, under mesopic and under photopic conditions, has been demonstrated in one cross-sectional investigation,[17] although these findings were not replicated in another study.[35]

Interestingly, temporal aspects of vision, such as CFF and the temporal modulation transfer function (TMTF) at the fovea also appear to be associated with MP.[36,37] The limits of the TMTF are determined by postreceptoral neural processing constraints, so any relationship between MP and CFF, for example, should not be influenced by optical filtration at a prereceptoral level.[38,39] Further, recent findings suggestive of cognitive benefits following L supplementation,[40] taken together with the positive and significant correlation between MP and cerebral concentrations of its constituent carotenoids,[41] indicate that that the role of macular carotenoids extends beyond its passive and absorptive properties and suggests that these compounds may influence postreceptoral neural processing efficiency.

Interventional (supplementation) studies have focused their attention on the impact of MP augmentation on visual function in, and/or the natural history of, retinal disease (such as AMD and hereditary retinal degenerations).[10,42] Interestingly, MP augmentation has been shown to enhance visual function in subjects with such conditions,[9,10,42] but meaningful comment on the impact of such augmentation on the natural course of these and other pathologies would require periods of follow up that far exceed those of the studies concerned. There is a paucity of data, however, on the impact of MP augmentation in normal subjects with no ocular pathology in terms of visual performance and in terms of the potential for prevention of ARMD (or, indeed, on the age-related decline in macular function in the absence of pathology). For example, one eloquent study has demonstrated that higher MP is associated with the preservation of visual sensitivity into old age.[43] Given the unprecedented ageing of our society, and given that the vast majority of subjects respond (in terms of MPOD) to supplementation with macular carotenoids (thus indicating that these individuals have less-than-saturation levels of these carotenoids in their central retina),[44] the impact of such augmentation on visual performance (in the presence or absence of macular disease), and on potential for disease prevention, warrants study.

The current study demonstrates a novel and important effect of MP augmentation on visual performance among healthy subjects without ocular disease. Across a broad range of testing modalities and conditions, visual performance improved significantly among subjects who exhibited a significant rise in MPOD. Specifically, improvements in contrast sensitivity (across virtually all spatial frequencies, under daytime and nighttime conditions, with and without glare conditions), and improvements in VA, were demonstrated in subjects supplemented with all three macular carotenoids, but no such observations were seen in the placebo control subjects or in subjects supplemented with L and Z (but not MZ). It is likely that these improvements in contrast sensitivity, both with and without glare, are clinically meaningful. The improvements may be of value, for example, to patients who fail to meet contrast sensitivity requirements to fulfill eligibility criteria for driving where measures of contrast sensitivity are a mandatory component of such testing (in the European Union, e.g.), and may represent the difference between eligibility and noneligibility to drive. The data support the view that MP influences visual performance through its optical filtration effects,[3–6] as the glare test protocol included an LED glare source that exhibited a short-wavelength peak emission profile matching the known spectral absorbance of MP. The observed improvements in acuity and contrast sensitivity, however, are less consistent with a solely optical explanation. The stimuli used, however, do contain a relatively small short wavelength component. It is possible, therefore, that MP augmentation results in optical image enhancement through a reduction of the deleterious effects of chromatic aberration and light scatter, and thereby improves VA and contrast sensitivity for such spectrally broadband stimuli. It is also possible that the macular carotenoids, which are intracellular compounds,[1] also play a neurobiologic role, thereby contributing to, and/or facilitating, optimal neurophysiologic performance, and, hence, visual function (the limits of spatial vision represent the combined influence of optical and neural efficiency limits). Observations on the relationship between MP and temporal visual function,[36,37] high concentrations of L and Z in the primary visual cortex,[45] and the influence of L supplementation on cognitive function,[40] do suggest a neurophysiologic role for these compounds, the so-called *neural efficiency hypothesis*.[36,37]

Table 5. Glare-Affected Contrast Sensitivity Change and Significance Levels at each Spatial Frequency Tested under Mesopic and Photopic Conditions

Group Intervention	Contrast Sensitivity, under Glare, at Baseline	Contrast Sensitivity, under Glare, at 6 mo	Student's *t*-test	RM ANOVA
	Photopic at 1.5 cpd	Photopic at 1.5 cpd	*P**	*P*†
Group 1	56 ± 27	67 ± 20	0.06	0.12
Group 2	50 ± 22	67 ± 22	0.06	0.03
Group 3	60 ± 25	74 ± 29	0.13	0.24
	Photopic at 3.0 cpd	Photopic at 3.0 cpd		
Group 1	84 ± 26	95 ± 31	0.18	0.28
Group 2	86 ± 24	121 ± 34	0.003	0.002
Group 3	96 ± 30	97 ± 44	0.96	0.92
	Photopic at 6.0 cpd	Photopic at 6.0 cpd		
Group 1	114 ± 43	96 ± 37	0.18	0.26
Group 2	91 ± 39	130 ± 40	0.03	0.04
Group 3	105 ± 51	112 ± 58	0.64	0.80
	Photopic at 12.0 cpd	Photopic at 12.0 cpd		
Group 1	34 ± 13	32 ± 14	0.79	0.13
Group 2	42 ± 20	70 ± 25	0.004	0.006
Group 3	29 ± 21	62 ± 48	0.06	0.13
	Photopic at 18.0 cpd	Photopic at 18.0 cpd		
Group 1	17 ± 11	23 ± 12	0.35	0.08
Group 2	33 ± 13	65 ± 20	0.17	0.23
Group 3	33 ± 15	46 ± 22	0.41	0.75
	Mesopic at 1.5 cpd	Mesopic at 1.5 cpd		
Group 1	23 ± 8	45 ± 35	0.08	0.05
Group 2	39 ± 26	58 ± 29	0.08	0.04
Group 3	32 ± 24	38 ± 23	0.76	0.25
	Mesopic at 3.0 cpd	Mesopic at 3.0 cpd		
Group 1	36 ± 10	61 ± 43	0.07	0.06
Group 2	40 ± 14	74 ± 40	0.009	0.02
Group 3	54 ± 39	59 ± 46	0.82	0.93
	Mesopic at 6 cpd	Mesopic at 6 cpd		
Group 1	64 ± 41	90 ± 53	0.15	0.17
Group 2	50 ± 19	77 ± 49	0.07	0.049
Group 3	53 ± 46	64 ± 43	0.66	0.71
	Mesopic at 12 cpd	Mesopic at 12 cpd		
Group 1	5 ± 2	10 ± 17	0.30	0.35
Group 2	5 ± 2	12 ± 8	0.01	0.01
Group 3	7 ± 5	10 ± 7	0.24	0.15
	Mesopic at 18 cpd	Mesopic at 18 cpd		
Group 1	2 ± 0	2 ± 0	0.34	0.44
Group 2	2 ± 1	11 ± 13	0.16	0.21
Group 3	4 ± 5	5 ± 3	0.14	0.22

Group 1 (20 mg L, 2 mg Z), $n = 11$; group 2 (10 mg MZ, 10 mg L, 2 mg Z), $n = 11$; group 3 (placebo), $n = 10$.
* Difference between baseline and 6 months (paired samples Student's *t*-test).
† RM ANOVA across all visits.

Indeed, the current study lends support to such a view, reflected in the observed lag between maximally augmented MPOD (at 3 months) and demonstrable improvements in visual performance (at 6 months, at least for most outcome measures), suggesting that the observed improvements in visual performance were not solely attributable to the increase in MPOD-mediated optical filtration.

To our knowledge, there has been one double masked, randomized controlled trial designed to investigate the impact of supplemental macular carotenoids (12 mg L; 1 mg Z) on visual performance in normal subjects (MZ was not included in the study formulation).[18] In that study (COMPASS), benefits, in terms of contrast sensitivity, BCVA, or glare disability, were not demonstrable in the intervention or placebo groups. It should be stated, however, that the COMPASS intervention group did exhibit a rise in MPOD although the rise was modest (an average increase of 0.10 optical density units in the intervention group after 12 months) and did not achieve statistical significance until 12-months' supplementation. Consistent with the findings of COMPASS, it remains possible that prolonged high-dose L supplementation could augment MPOD over a 12-month time course, but there is no evidence to support the view that such augmentation would be associated with the enhancements in visual performance observed here for formulations containing MZ. Therefore, for subjects supplemented with a preparation lacking MZ, the consistency of our findings with those of COMPASS in terms of the absence of a visual performance benefit, prompts further questions. For example, and again, it remains unclear whether the enhanced visual performance following supplementation with all three macular carotenoids, observed in the current study, is attributable solely to the ability of such a preparation to augment MP across its spatial profile, or whether some other as yet unidentified property of MZ is playing a role.

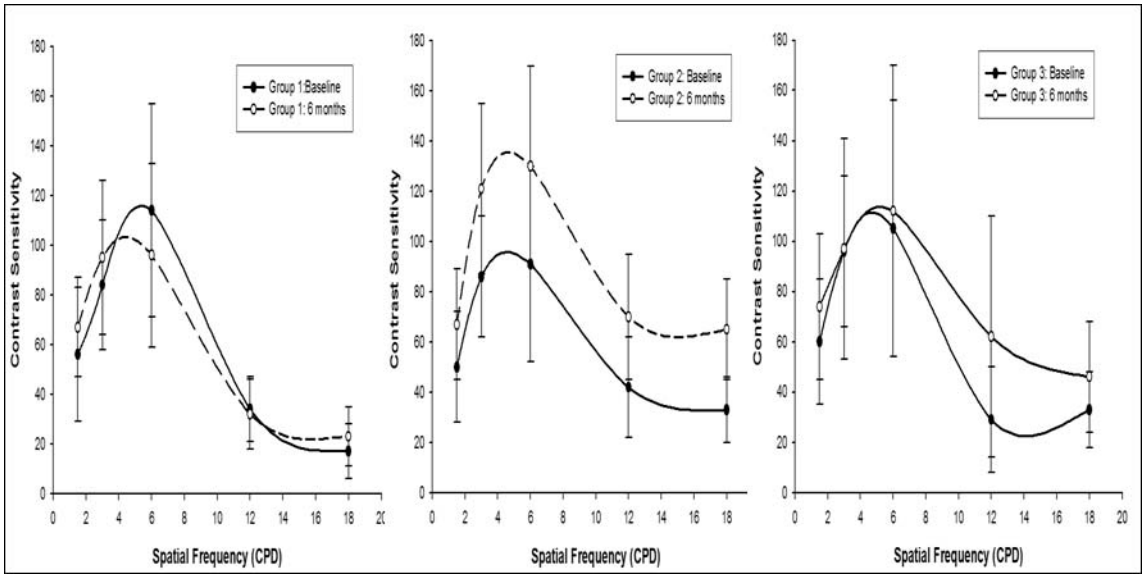

Figure 3. Change in photopic contrast sensitivity function, under glare-disability conditions, across supplementation groups from baseline to exit visits.

Of note, the safety of macular carotenoids has been examined in both animal and human trials, with no adverse effects reported.[22,46] In the animal trial,[46] the results confirmed that the No-Observed-Adverse-Effect-Level was in excess of 200 mg/kg/day, which is far greater than doses used in dietary supplements, which are typically less than 0.5 mg/kg/day. Also, in the human trial,[22] clinical pathology analysis after supplemental MZ (in combination with L and Z) was not suggestive of any associated toxicity. It is likely, therefore, that macular carotenoid supplementation, at the combined dosage level employed here (22 mg total), is safe for long-term consumption for the purposes of vision enhancement or disease prevention, although further research is required in this regard.

The decision to employ a single masked experimental design merits discussion. The relevant capsules were provided in a blank white container to the subjects, and contained no possible identifiers as to the likely contents of the particular capsules. Although the appearance of three capsule types used for the active and placebo formulations were similar, the subtle and visible differences were potentially discernible to the investigators, thereby rendering complete and meaningful masking impossible in this respect. Of note, however, the member of the research team charged with collection, recording, and archiving all data relating to any outcome measure was excluded from the processes of subject recruitment and randomization, and from data analysis, thereby minimizing the potential for bias arising from the nondouble-masked nature of the study design.

In conclusion, this investigation has demonstrated a rapid and sustained rise in MPOD following supplementation with all three macular carotenoids, and this was not observed in placebo-controlled subjects or in subjects supplemented with a preparation lacking MZ. Further, supplementation with all three macular carotenoids resulted in significant improvements in contrast sensitivity (under photopic and mesopic conditions, with and without glare) and in corrected distance VA, whereas no such changes were seen in placebo controls or in subjects supplemented with a preparation lacking MZ. The variable response to supplementation observed was such that, by the end of the study period, serum carotenoid, macular pigment, and visual performance levels were statistically higher for those subjects supplemented with all three macular carotenoids when compared to the other two groups (whereas no such intergroup differences existed at baseline). These findings have potentially important implications for people engaged in activities where optimization of visual function is important, and warrant further study.

References

1. Snodderly DM, Auran JD, Delori FC. The macular pigment 2. Spatial-distribution in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:674–685.
2. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci*. 1997;38:1802–1811.
3. Reading VM, Weale RA. Macular pigment and chromatic aberration. *J Opt Soc Am*. 1974;64:231–234.
4. Stringham JM, Hammond BR Jr. The glare hypothesis of macular pigment function. *Optom Vis Sci*. 2007;84:859–864.
5. Wooten BR, Hammond BR. Macular pigment: influences on visual acuity and visibility. *Prog Ret Eye Res*. 2002;21:225–240.
6. Hemenger RP. Dichroism of the macular pigment and Haidinger's brushes. *J Opt Soc Am*. 1982;72:734–737.
7. Kvansakul J, Rodriguez-Carmona M, Edgar DF. Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. *Ophthal Physiol Opt*. 2006;26:362–371.
8. Stringham JM, Garcia PV, Smith PA, McLin LN, Foutch BK. Macular pigment and visual performance in glare: benefits for photostress recovery, disability glare, and visual discomfort. *Invest Ophthalmol Vis Sci*. 2011;52:7406–7415.
9. Weigert G, Kaya S, Pemp B, et al. Effects of lutein supplementation on macular pigment optical density and visual acuity in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:8174–8178.
10. Richer S, Stiles W, Statkute L, et al. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry*. 2004;75;216–230.

11. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch. Biochem. Biophys*. 2010;504:56–60.
12. Bone RA, Landrum JT, Friedes LM, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp Eye Res*. 1997;64:211–218.
13. Okulski W, Sujak A, Gruszecki WI. Dipalmitoylphosphatidylcholine membranes modified with zeaxanthin: numeric study of membrane organisation. *Biochim Biophys Acta*. 2000;1509: 216–228.
14. Connolly E, Beatty S, Thurnham DI, et al. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study. *Curr Eye Res*. 2010;35;335–351.
15. Kirby ML, Beatty S, Loane E, et al. A central dip in the macular pigment spatial profile is associated with age and smoking. *Invest Ophthalmol Vis Sci*. 2010;51:6722–6728.
16. Stringham JM, Hammond BR, Nolan JM, et al. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res*. 2008;87:445–453.
17. Loughman J, Beatty S, Akkalli M, et al. The relationship between macular pigment and visual performance. *Vis Res*. 2010;50:1249–1256.
18. Nolan JM, Loughman J, Akkali MC, et al. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. *Vis Res*. 2011;51:459–469.
19. Dhalla MS, Fantin A, Blinder KJ, Bakal JA. The macular automated photostress test. *Am J Ophthalmol*. 2007;143;596–600.
20. Dhalla MS, Fantin A. Macular photostress testing: sensitivity and recovery with an automated perimeter. *Retina*. 2005;25: 189–192.
21. Seddon JM, Sahagian CR, Glynn RJ, et al. Evaluation of an iris classification system. *Invest Ophthalmol Vis Sci*. 1990;31: 1592–1598.
22. Connolly EE, Beatty S, Loughman J, Howard AN, Louw MS, Nolan JM. Supplementation with all three macular carotenoids: response, stability, and safety. *Invest Ophthalmol Vis Sci*. 2011;52:9207–9217.
23. Nolan JM, Akkali MC, Loughman J, Howard AN, Beatty S. Macular carotenoid supplementation in subjects with a "central dip or plateau" in their macular pigment spatial profile. *Exp Eye Res*. 2012;101:9–15.
24. Liew M, Gilbert CE, Spector T, et al. The heritability of macular pigment: a twin study. *Invest Ophthalmol Vis Sci*. 2005;46: 4430–4436.
25. Bone RA, Landrum JT, Guerra LH, Ruiz CA. Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. *J Nutr*. 2003;133:992–998.
26. Trieschmann M, Beatty S, Nolan JM, et al. Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res*. 2007;84:718–728.
27. Johnson EJ, Hammond BR, Kyung-Jin Y, et al. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr*. 2000;71;1555–1562.
28. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest Ophthalmol Vis Sci*. 1988;29:843–849.
29. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas, III: effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Invest Ophthalmol Vis Sci*. 2005;46:692–702.
30. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci*. 1993;34:2033–2040.
31. Loughman J, Davison P, Akkalli M, Nolan J, Beatty S. Visual performance and macular pigment. *J Optom*. 2010;3:73–89.
32. Stringham JM, Fuld K, Wenzel AJ. Action spectrum for photophobia. *J Opt Soc Am*. 2003;20:1852–1858.
33. Stringham JM, Fuld K, Wenzel AJ. Spatial properties of photophobia. *Invest Ophthalmol Vis Sci*. 2004;45:3838–3848.
34. Wenzel AJ, Fuld K, Stringham JM, Curran-Celantano J. Macular pigment optical density and photophobia light threshold. *Vis Res*. 2006;46:4615–4622.
35. Engles M, Wooten B, Hammond BR. Macular pigment: a test of the acuity hypothesis. *Invest Ophthalmol Vis Sci*. 2007;48: 2922–2931.
36. Hammond BR, Wooten BR. CFF thresholds: relation to macular pigment optical density. *Ophthalmic Physiol Opt*. 2005;25: 315–319.
37. Renzi LM, Hammond BR. The relationship between macular carotenoids, lutein and zeaxanthin, and temporal vision. *Ophthalmic Physiol Opt*. 2010;30:351–357.
38. Ratliff F, Hartline HK, Miller WH. Spatial and temporal aspects of retinal inhibitory interaction. *J Opt Soc Am*. 1963;53:110–120.
39. Powell RR. Flicker fusion as a typological index of nervous system reactivity. *Percept Mot Skills*. 1983;57:701–702.
40. Johnson EJ, McDonald K, Calderella SM, Chung HY, Troen AM, Snodderly DM. Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. *Nutr Neurosci*. 2008;11:75–83.
41. Vishwanathan R, Neuringer M, Snodderly DM, Schalch W, Johnson EJ. Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates [published online ahead of print July 9, 2012]. *Nutr Neurosci*. doi:10.1179/1476830512Y.
42. Dagnelie G, Zorge IS, McDonald TM. Lutein improves visual function in some patients with retinal degeneration: a pilot study via the Internet. *Optometry*. 2000;71:147–164.
43. Hammond BR, Wooten B, Snodderly DM. Preservation of visual sensitivity of older subjects: association with macular pigment density. *Invest Ophthalmol Vis Sci*. 1998;39:397–406.
44. Sabour Pickett S, Nolan JM, Loughman J, Beatty S. A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration. *Mol Nutr Food Res*. 2012;56:270–286.
45. Craft NE, Haitema TB, Garnett KM, Fitch KA, Dorey CK. Carotenoid, tocopherol, and retinol concentrations in elderly human brain. *J Nutr Health Aging*. 2004;8:156–162.
46. Chang CJG. Thirteen week oral (gavage) toxicity of mesozeaxanthin in Han Wister rats with a 4-week recovery. *Study no*. 1567-04370. Gaithersburg, MD: Gene Logic Laboratories, Inc., 2006.