Contrast Sensitivity and Lateral Inhibition Are Enhanced With Macular Carotenoid Supplementation

James M. Stringham,[1] Kevin J. O'Brien,[2] and Nicole T. Stringham[1]

[1]Nutritional Neuroscience Laboratory, University of Georgia, Athens, Georgia, United States
[2]Vision Sciences Laboratory, University of Georgia, Athens, Georgia, United States

Correspondence: James M. Stringham, Department of Psychology, University of Georgia, Athens, GA 30602, USA;
psychjim@uga.edu,
jim.stringham@gmail.com.

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Purpose. Once deposited in the retina, the so-called macular carotenoids lutein (L), zeaxanthin (Z), and mesozeaxanthin (MZ) have been shown to enhance visual performance. The purpose of our study was to investigate whether increasing macular pigment optical density (MPOD) could enhance lateral inhibitory processes, and thereby improve contrast sensitivity (CS).

Methods. A total of 59 young (18–25 years), healthy individuals participated in this 1-year, double-masked, placebo-controlled study. MPOD was assessed via heterochromatic flicker photometry. Lateral inhibition sensitivity (LIS) was determined with a computer-based, user-adjustable Hermann grid. CS (at 8 cycles/degree) was determined with a two-alternative, forced-choice procedure. Subjects received either the placebo ($n = 10$), 12 mg total macular carotenoids ($n = 24$), or 24 mg total macular carotenoids ($n = 25$).

Results. MPOD, LIS, and CS increased significantly in treatment groups between baseline and 6 months, and between 6 and 12 months ($P < 0.05$ for all) versus placebo. The relationships between changes in MPOD and both LIS and CS were significant at 6 and 12 months ($P < 0.05$ for both). Changes in CS and LIS over the 12-month study period were found to be significantly related ($r = 0.41$; $P = 0.0014$).

Conclusions. Increases in MPOD led to enhanced lateral inhibitory processes, which correspond to improved CS. Because optical filtering has the same net effect on dark versus light bars, it cannot explain these improvements. Improvement in CS with increases in MPOD therefore appears to involve enhancement of the fundamental physiological systems that give rise to edge detection.

Keywords: lutein, zeaxanthin, macular pigment, contrast sensitivity, lateral inhibition

Given that it confers the ability to make objects visually distinguishable,[1] contrast sensitivity (CS) is perhaps the most important aspect of visual function. There are several factors that affect CS, including age,[2,3] ocular health,[4] fatigue,[5] and neurological disease, such as Alzheimer's.[6]

Recently, the role of diet in visual performance has become evident. Of specific relevance to the present investigation, increasing the concentration in the macular retina of the dietary carotenoids lutein (L), zeaxanthin (Z), and mesozeaxanthin (MZ) has been shown to significantly improve CS at certain spatial frequencies.[7–10] The concentration of the macular carotenoids (quantified by macular pigment optical density [MPOD]) ranges among individuals from 0 to well over 1.0 log unit (e.g., Ref. 11) and is related primarily to dietary consumption of foods that contain these carotenoids, such as spinach, kale, and orange peppers.[12] Because macular pigment (MP) lies anterior to the photoreceptors[13] and is yellow in color, it serves as a short-wavelength filter for the central retina.[14] In terms of luminance-based CS, the filtering properties of MP could not account for improvements with higher MPOD, due to the equal effect of absorption of both light and dark bars in a contrast grating: the net effect would simply cancel, and CS would remain the same regardless of MPOD. The experimental evidence noted above clearly indicates otherwise, however.

The neurophysiological basis for CS is a phenomenon known as lateral inhibition,[15] in which groups of photoreceptors are wired together in such a way as to produce a "center-surround" arrangement: light differentially affects the center versus surround regions of the receptive field and, ultimately, the perceived difference between the two yields the visual system's ability to detect edges (e.g., Ref. 16). The minimum difference in luminance detectable between center versus surround regions of the receptive field determines threshold CS, and an enhancement of the process of lateral inhibition, in which the signal-to-noise ratio is increased, would presumably improve CS. A plausible neurophysiological mechanism for an increased CS effect seen with higher MP involves optimization (via antioxidant activity) of nitric oxide levels, which has been found to enhance sensitivity of center-surround units.[17]

The purpose of this study was 2-fold: to evaluate whether augmenting MPOD affects lateral inhibition sensitivity (LIS) by lowering the contrast threshold for the perception of illusory shadows in the Hermann grid, and to determine whether changes in LIS are related to subjects' CS thresholds.

Methods

Subjects

This study was reviewed and approved by the University of Georgia Institutional Review Board. Informed consent was obtained for each subject, and the study adhered to the tenets

of the Declaration of Helsinki. Fifty-nine subjects participated in this 12-month, double-masked, randomized, placebo-controlled supplementation trial. Subjects were generally healthy, college-aged (18–25, mean = 21.5 years; 32 female) nonsmokers with a body mass index less than 27. Subjects were instructed to maintain their current diet; those that were planning on changing their diet (for whatever reason) were excluded from consideration for the trial. For those subjects enrolled in the trial, stability of diet was evaluated via questionnaire. In consideration of MP testing, all subjects had uncorrected or contact lens–corrected visual acuity of 20/20 or better in the test (right) eye, and had no current or previous history of ocular pathology. Subjects were recruited from the population of students at the University of Georgia in Athens, Georgia.

Measures of MPOD, thresholds of perceptual lateral inhibition, and CS thresholds were taken at baseline, and 6 months and 12 months.

Macular Carotenoid Supplementation

Subjects were randomly assigned to one of three groups: group 1 (placebo; $n = 10$), group 2 ($n = 24$; 12 mg/d total carotenoids), or group 3 ($n = 25$; 24 mg/d total carotenoids). Pills were brown-colored, soft gelatin capsules, with L, Z, and MZ suspended in safflower oil. Independent analysis of 100 pills in each dose category indicated that the 12-mg group supplement contained 10.86 mg L/1.33 mg Z/0.94 mg MZ, the 24-mg group supplement contained 22.33 mg L/2.70 mg Z/2 mg MZ, and placebos contained no L or Z, but only safflower oil. All reported values were within ±5% variability. Subjects were instructed to ingest one pill with a meal (preferably lunch or dinner) every day. Compliance was ensured with weekly phone calls and pill counts.

Measurement of MPOD

MPOD was assessed with a noninvasive, perceptual task called heterochromatic flicker photometry (HFP). A densitometer (Macular Metrics Corp., Rehoboth, MA, USA) described by Wooten et al.[18] was used for this purpose. The densitometer, detailed measurement procedures, and the principle of HFP have been fully described in earlier publications (e.g., Ref. 19). Briefly, subjects are presented with two superimposed lights that are temporally alternated in square-wave counterphase. This creates the perception of a flickering disc of light for the subject. The peak (550 nm) of the spectral composition of one of the lights is chosen to bypass the absorption of MP, and the other (460 nm) is strongly absorbed by MP. The subject's task is to adjust the relative radiance of the two lights until a percept of no flicker, due to perceived isoluminance, is achieved. All other factors being equal, a subject who requires more short-wave (i.e., 460 nm) relative to middle-wave (i.e., 550 nm) light to achieve null flicker has higher MPOD. This task is performed for the locations of interest within the fovea, which presumably contain MP, and for a reference location in the parafovea that does not (about 7° eccentricity). To obtain a measure of MPOD at a given test locus, the logarithmic ratio of short- to middle-wave radiance (for null flicker) at the reference location is subtracted from the corresponding logarithmic ratio found at the test locus. Although we obtained values for retinal locations across the MPOD spatial distribution, the standard 30′ retinal locus proved to account for the most variance in our other outcome measures, and therefore was used for all analyses presented in this article. The total time spent on MPOD measurement was 15 minutes per session.

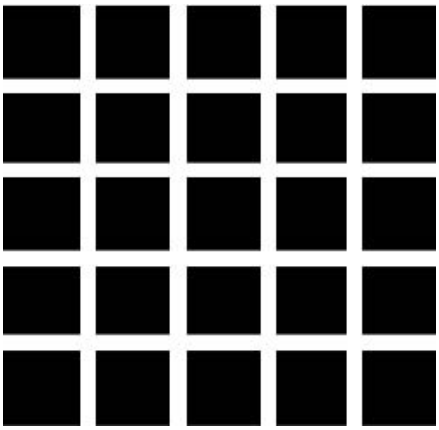

Figure 1. The Hermann grid. Illusory shadows may be seen at the junctions of the dark squares.

Measurement of Perceptual Lateral Inhibition

The stimulus seen in Figure 1 is the well-known Hermann grid.[20] The appearance of illusory shadows in the junctions of the grid is the result of lateral inhibitory interactions among retinal and central neurons that serve center versus surround portions of a receptive field.[21] When the squares are relatively dark and contrast is high (as in Fig. 1), the shadows are (usually) easily seen. Lightening the squares, however, reduces contrast and hence the effect, and at some point of square lightness, the illusory shadows will no longer be apparent. This threshold is indicative of the minimum amount of stimulus contrast required to produce a visually perceivable difference between excitation and inhibition in visual receptive fields. In our experiment, a contrast-adjustable Hermann grid, presented on an LCD display, was used to determine the threshold square lightness at which the illusory shadows were just detectable. The dark squares could be made lighter by sliding the computer mouse forward, or darker by sliding it backward. Subjects viewed the grid from a distance of 30 inches, which made the visual angle of square spacing 0.3°. This spacing was chosen based on the peak of the perceptual lateral inhibition function, per Davies and Morland.[21] The computer monitor brightness of the white background was 100 cd/m^2, and exhibited very little variation across the screen ($F = 0.42$; $P = 0.978$). The subject's task was to adjust the lightness of the dark squares to the point at which the illusory shadows were just barely detectable. Five such thresholds were determined, and the starting point of a given trial was randomized to avoid the potential for bias based on a previous setting. Subjects spent roughly 10 minutes performing Hermann grid testing.

CS Testing

CS testing was conducted on the same computer/monitor as described above. A subject's threshold for detection of a Gabor patch's orientation (tilted right or left 45° from vertical) was determined for a single stimulus, an 8 cycle/degree target subtending 2° of visual angle. A two-alternative, forced-choice staircase procedure was implemented to determine a subject's contrast threshold. If there was no response, it was recorded as incorrect. Contrast was specified as Michelson contrast:

$$\frac{L_{max} - L_{min}}{L_{max} + L_{min}},$$

where L_{max} and L_{min} represent the maximum and minimum luminance in a grating, respectively. Twenty-five stimulus presentations were used to determine a threshold, and trials

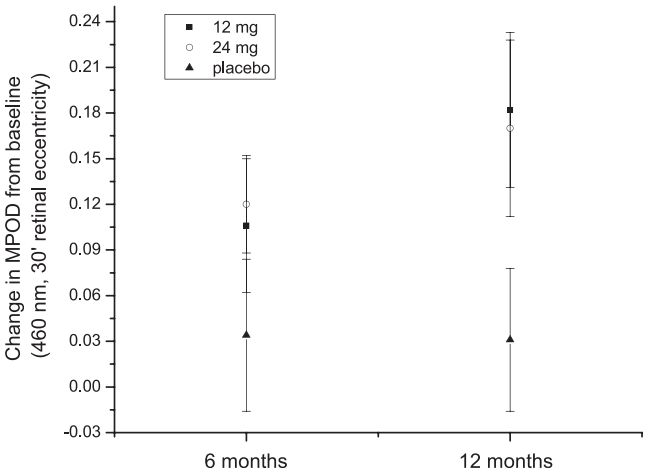

FIGURE 2. Change in MPOD at both 6- and 12-month time points, compared with baseline measure, for placebo, and 12- and 24-mg supplement groups. Symbols denote mean values, *error bars* are ± 1 SD.

always started with the Gabor set to maximum contrast (90% Michelson contrast). On correct responses, the contrast of the Gabor was decreased 27% of its previous value. Incorrect responses resulted in an increase of 21% of the previous Gabor's contrast value. Based on the results of an ideal observer model, these values most accurately predicted actual contrast thresholds for a trial consisting of 25 stimulus presentations, averaging the last three reversals. The subjects typically produced five or more reversals; actual thresholds were determined by computing the average of the last three reversals. Two thresholds were determined at each visit; the average of the two thresholds was taken as the true threshold, and used for statistical analysis. A 1-minute rest period was allowed between each trial. Total time spent on CS testing was roughly 10 minutes.

Statistical Analysis, Masking Procedure

The statistical and graphing program OriginPro 9.3 (OriginLab Corporation, Northampton, MA, USA) was used to conduct repeated-measures ANOVA, Pearson product-moment correla-

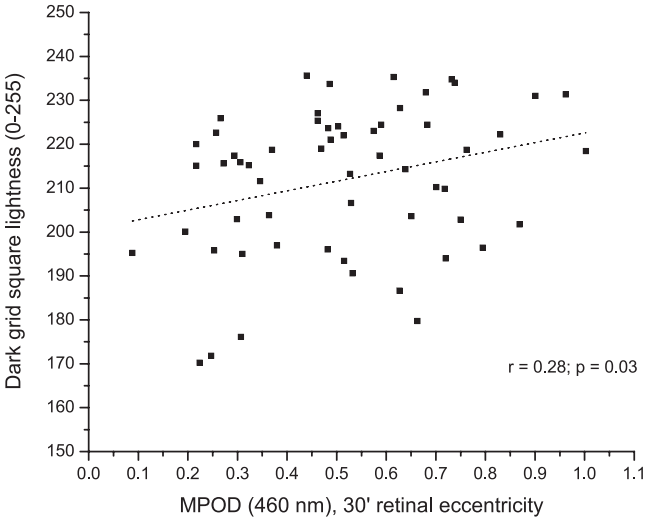

FIGURE 3. Dark grid square lightness settings in the Hermann grid task as a function of MPOD, at baseline.

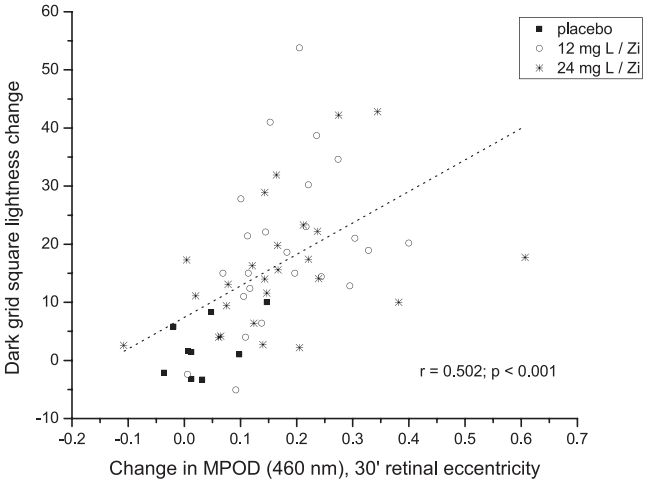

FIGURE 4. Change in lightness settings in the Hermann grid task as a function of change in MPOD, over the entire 12-month study period. Study groups with doses of L/Z isomers noted in legend.

tions, and to generate figures for the article. Assuming a placebo group of $n = 10$, an a priori power calculation was made using a 20% change in either CS or LIS in treatment groups, coupled with a standard deviation of 20%, and $\alpha = 0.05$. This calculation indicated that both 12- and 24-mg L/Z groups required 25 subjects to detect effects (if present). We assumed an attrition rate of roughly 20%, and therefore enrolled 75 subjects. As noted above, 59 completed the trial.

The randomization sequence was generated by the study coordinator, who performed random allocation to the three study groups. The study investigator (JMS) received a box of supplements labeled only with the participant identification number. On completion of the study, the randomization sequence was revealed, and data analysis ensued.

RESULTS

Main Effects

Repeated-measures ANOVAs determined that MPOD, LIS, and CS increased significantly in both treatment groups between baseline and 6 months, and between 6 and 12 months ($P < 0.05$ for all) versus placebo (which did not change appreciably in any of these respects throughout the study). MPOD increased significantly in both 12-mg ($P = 0.029$) and 24-mg ($P = 0.009$) groups versus placebo; groups did not differ from each other at any time point during the study (see Fig. 2).

Correlations

At baseline, subjects' MPOD ranged from 0.088 to 1.03 at 30′ retinal eccentricity, and was significantly correlated to subjects' LIS values ($r = 0.28$; $P = 0.03$; see Fig. 3). MPOD was nearly significantly correlated to CS ($r = 0.24$; $P = 0.08$) at baseline. Over the total 12-month study period, changes in MPOD were significantly correlated to changes in LIS ($r = 0.502$; $P < 0.001$; see Fig. 4) and CS ($r = 0.432$; $P < 0.001$; see Fig. 5). Additionally, changes in LIS and CS over 12 months were found to be significantly related ($r = 0.41$; $P = 0.0014$; see Fig. 6).

DISCUSSION

The findings from this study indicate that augmentation of MPOD leads to increases in CS and LIS, and suggest that CS is

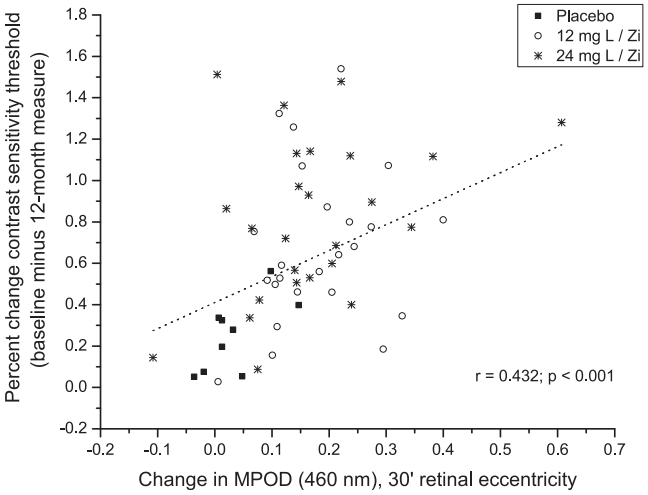

FIGURE 5. Change in CS as a function of change in MPOD, over the entire 12-month study period. Study groups with doses of L/Z isomers noted in legend.

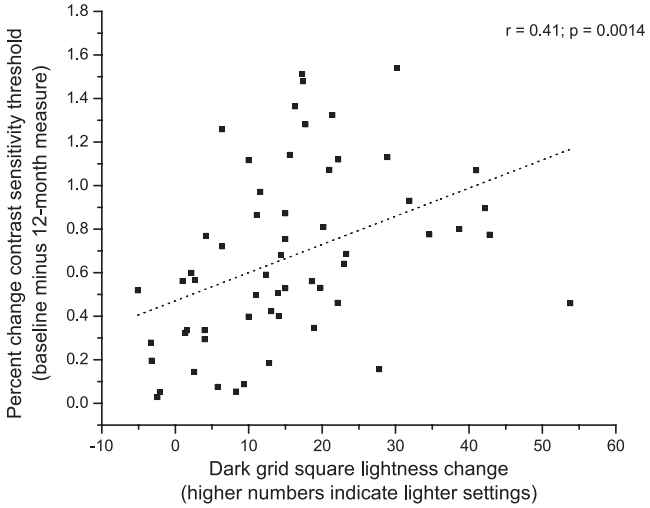

FIGURE 6. Change in CS as a function of the change in lightness settings in the Hermann grid task, over the entire 12-month study period.

improved via enhancement of LIS. The significant changes in MPOD, CS, and LIS versus placebo are supportive of this idea, and the significant relationship between the changes in CS and LIS over the entire 12-month study period further bolsters the argument. Although MP acts as an optical filter and this fact can explain many of its effects on vision, light filtration cannot account for an increase in CS because the percent absorption of the light versus dark bars of the grating is equivalent. Moreover, an overall reduction in luminance would serve to *decrease* signal-to-noise ratio, which runs counter to our results. The mechanism responsible for this effect must therefore involve something other than modification of the retinal image by MPOD. The most plausible possibility is enhancement of the neurophysiology of the retina, where increased MPOD, via antioxidant action, would serve to increase the metabolic efficiency of the visual cycle (see e.g., Ref. 10). To a first approximation, this would lead to faster photopigment regeneration, which would manifest as faster dark adaptation with higher MPOD. This relationship was found recently in a cross-sectional study of healthy young adults,[22] and Patryas et al.[23] produced data that trended in this same direction for a sample of relatively older subjects.

Enhancing the visual cycle via increasing antioxidant capacity, however, would also have effects on the post–receptoral circuitry serving the aforementioned center-surround receptive fields. In fact, maintaining an optimal "redox homeostasis" (in which the balance between oxidation and antioxidant capacity is optimal for health and function) has been suggested as a mechanism for increasing the efficiency of several neural processes.[24] In terms of retinal physiology (as noted in the Introduction), the soluble gas neurotransmitter nitric oxide (NO) has been shown to amplify the difference between outputs of the center versus surround of a receptive field.[17] This would increase the signal-to-noise ratio in the output of center-surround receptive fields, would lead to increased sensitivity in the Hermann grid task (as was found in our study), and would ultimately lead to greater CS (also found in our study). The function of NO is strongly affected by the oxidative state of the tissue in which it is active[24]; at advantageous levels, NO promotes efficient neural function. But if oxidative stress is too high, NO will itself generate potentially neurotoxic radical species, such as N_2O_3 and peroxynitrite (ONOO–).[25] Sufficient antioxidant capacity therefore appears crucial to neural health and function. Following this line of reasoning, the increase in MPOD seen in our study may have brought subjects closer to a point of optimal oxidative equilibrium in the retina, and may explain not only the findings of enhanced LIS and CS presented in this article but also the significant improvements in CS with increased MPOD reported in previous investigations.[7–10]

Although the effects characterized in this article were related to increases in MPOD, it is noteworthy that there was no statistically significant difference between 12- and 24-mg supplement groups for any of the outcome measures. Generally, higher doses of L, Z, and/or MZ are associated with higher retinal response rates in terms of MPOD.[26] It is possible that, despite random assignment to groups, response was simply more robust in subjects within the 12- versus 24-mg groups. Indeed, there were seven subjects in the 12-mg group who responded very strongly to the supplement, increasing by at least 0.25 OD over the 12-month study period, and there were no "nonresponders" (OD increase <0.05) in this group. By contrast, only five of the subjects in the 24-mg group increased by 0.25 OD or more, and there were three nonresponders in this group. There are several potential sources of variability in response among subjects, including the efficiency of mechanisms involved in transport[27] and binding,[28] and/or perhaps demand for these carotenoids for more immediate uses, such as the reduction of systemic inflammation or oxidation.[29] Because of the many health and performance benefits derived from increased systemic and local concentrations of these carotenoids, determining the factors that contribute to absorption, transport, binding, and deposition has become one of the most important scientific questions for this area. Ultimately, our results appeared to depend on the change in MPOD that supplementation produced, regardless of dose level or subject response kinetics.

In terms of application to "real-world" vision, the results of our study elucidate some important points. First (and most generally), these results illustrate that improvements in very specific aspects of nutrition can confer significant neurophysiological and visual performance improvements, even in healthy, young subjects. Because CS is fundamental to visual performance, improvements in this dimension should manifest as appreciable improvements in daily visual (and related) tasks. Perhaps the clarity of distant objects would be relatively better, such that a sign may be read at increased distance while driving. An improvement in recognition would, in turn, yield

additional time for decision making, or result in faster reaction time. In a driving scenario, this would presumably improve driving performance and safety. In support of this line of reasoning, a recent simulation of visibility of objects at a distance viewed through atmospheric haze has been shown to be better as a function of MPOD.[30] With respect to relatively long-term effects on vision, improved CS may improve the visual resolution of text (e.g., presented on a computer monitor/tablet, or on a printed page). Although this improvement may be very slight, over a time span of several hours, the cumulative effect could result in less visual strain (e.g., squinting), which may result in reduced visual fatigue and perhaps lower incidence of headache. We plan to evaluate these hypotheses in a future study.

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