Dietary Modification of Human Macular Pigment Density

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Purpose. The retinal carotenoids lutein (L) and zeaxanthin (Z) that form the macular pigment (MP) may help to prevent neovascular age-related macular degeneration. The purpose of this study was to determine whether MP density in the retina could be raised by increasing dietary intake of L and Z from foods.

Methods. Macular pigment was measured psychophysically for 13 subjects. Serum concentrations of L, Z, and β-carotene were measured by high-performance liquid chromatography. Eleven subjects modified their usual daily diets by adding 60 g of spinach (10.8 mg L, 0.3 mg Z, 5 mg β-carotene) and ten also added 150 g of corn (0.3 mg Z, 0.4 mg L); two other subjects were given only corn. Dietary modification lasted up to 15 weeks.

Results. For the subjects fed spinach or spinach and corn, three types of responses to dietary modification were identified: Eight "retinal responders" had increases in serum L (mean, 33%; SD, 22%) and in MP density (mean, 19%; SD, 11%); two "retinal nonresponders" showed substantial increases in serum L (mean, 31%) but not in MP density (mean, −11%); one "serum and retinal nonresponder" showed no changes in serum L, Z, or β-carotene and no change in MP density. For the two subjects given only corn, serum L changed little (+11%, −6%), but in one subject serum Z increased (70%) and MP density increased (25%).

Conclusions. Increases in MP density were obtained within 4 weeks of dietary modification for most, but not all, subjects. When MP density increased with dietary modification, it remained elevated for at least several months after resuming an unmodified diet. Augmentation of MP for both experimental and clinical investigation appears to be feasible for many persons. Invest Ophthalmol Vis Sci. 1997;38:1795–1801.

A leading cause of blindness in Western countries is age-related macular degeneration (AMD). Because there are currently no effective treatment strategies for most patients with AMD, attention has focused on efforts to prevent the damage leading to this condition.[1] Of particular interest is the possibility that nutritional counseling or intervention might reduce AMD incidence or retard AMD progression.[2]

One possible approach is to increase the dietary intake of the carotenoids lutein (L) and zeaxanthin (Z). Nutritional epidemiologic reports[1] have suggested that dietary intake of foods rich in carotenoids—especially L and Z[3]—protect against AMD. The protection may be explained by the fact that L and Z are selectively accumulated in the retina and are particularly dense in the macula.[1] In the macula, these carotenoids are referred to as macular pigment (MP). By preventing light-initiated oxidative damage to the retina and retinal pigment epithelium (RPE), MP may protect against age-related deterioration. Thus, it is possible that increasing MP density would retard age-related changes leading to AMD.

We hypothesized that MP density might be increased by enriching the diet with carotenoid-rich foods. Consistent with this idea, we recently showed that MP density is positively correlated with L and Z in the blood.[4] However, for women, the blood-retina correlation was weaker and there was no significant correlation between MP density and L and Z in the diet.[4] Hence, it was unclear how the MP of women

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would respond to dietary manipulation. Previous nutritional studies have shown that persons vary widely in the extent to which blood carotenoid concentrations change during dietary manipulation.[5,6] Consequently, we expected that we might find important individual differences in the transfer of carotenoids from the diet via the blood to the retina. A combination of such individual differences, together with the weak correlations between diet and MP of women could obscure the protective effect of dietary L and Z. In addition, such individual differences would help to explain why some epidemiologic studies[7] have failed to find a relationship between serum concentrations of L and Z and risk for AMD.

In the current study, we measured MP optical density in vivo with a noninvasive psychophysical technique that allows repeated assessment of the retina of the same person. Although most subjects responded to increases in dietary intake of L and Z with increases of MP, some did not. The challenge for the future will be to determine whether the nonresponders are at special risk for developing AMD.

MATERIALS AND METHODS

Subjects

Thirteen subjects (four men, nine women) participated in the study. The ages of the subjects ranged from 30 to 65 years. Five subjects were of Asian descent, seven were white (four men, three women), and one was Hispanic. All subjects were nonsmokers. Subjects had no history of ocular disease, alcoholism, pancreatic disease, atrophic gastritis, hyperlipidemia, insulin-requiring diabetes, and bleeding disorders and were not taking medications that are known to interfere with carotenoid absorption. Informed consent was obtained from all subjects and the tenets of the Declaration of Helsinki were followed.

Acquisition and Measurement of Nutrient Content of Food

Frozen spinach and corn were purchased in a single lot and their carotenoid content was analyzed by the modified official method of analysis of the Association of Official Analytical Chemists.[8] The analyses were conducted with the same high-performance liquid chromatography (HPLC) system that was used for serum analyses. HPLC analysis was conducted on the food after cooking as it would be prepared for consumption. The spinach (1/2 cup, 60 g) contained 10.8 mg L, 0.3 mg Z, and 5.0 mg β-carotene; the corn (1 cup, 150 g) contained 0.3 mg of Z and 0.4 mg L. For comparison, we note that a typical diet high in fruits and vegetables would be expected to contain about 2.3 mg/day of L and 0.3 mg/day of Z.[8] Therefore our subjects were expected to receive about 4 times as much L and 2 to 3 times as much Z as in a normal healthy diet.

To evaluate the actual changes in carotenoid intake associated with our dietary modification, the regular dietary intake of our subjects was estimated using the Health Habits and History Questionnaire.[9] Vitamin and mineral supplement use was also assessed.

Dietary Modification Protocol

At the start of the study, two baseline fasting blood samples were obtained on separate days. Spinach (60 g/day) and corn (150 g/day) were distributed to the subjects in one-week allotments. Ten subjects were given both corn and spinach, one subject was given only spinach, and two subjects were given only corn. Those receiving only corn were avoiding spinach because of a history of kidney stones, which can be formed from oxalate in spinach.

Subjects were asked to modify their diets by eating the spinach and/or corn with a meal and/or a fat source while otherwise maintaining their usual dietary habits. One male subject ate the modified diet for 6 weeks, and MP and serum measurements were obtained at 4 and 6 weeks. All other subjects ate the modified diets for 14 to 15 weeks. One MP and one serum measurement were obtained from each person at 4, 8, 12, and 14 to 15 weeks. An additional MP and serum measurement was obtained 1 to 6 months after dietary modification was discontinued. To minimize bias, the results of the serum analyses were unknown to the investigator measuring MP density. Similarly, the results of the MP measurements were unknown to the investigator measuring the serum.

Measurement of Macular Pigment Optical Density

Macular pigment density was measured psychophysically with a centrally fixated 1° stimulus as described in Hammond et al.[4] To assess the reliability of the MP measurements we compared MP density measured in the two baseline sessions. In general, there was good agreement between the two baseline sessions (the absolute change between the first and second session averaged 0.08 ± 0.017; $y = 0.03 + 0.83x$, $r = 0.72$) indicating that these subjects provided reliable data.

Additional measurements of MP at different retinal locations were made for three subjects to evaluate the effect of dietary modification on the MP spatial distribution. The retinal eccentricities and stimulus sizes used for these measurements are provided in the Results. For details regarding the method used to measure MP spatial profiles, see Hammond et al.[10]

Measurement of Lutein, Zeaxanthin, and β-Carotene in Serum

Serum was prepared from fasting blood samples (800g for 15 minutes at 4°C). Before analysis, serum was stored for periods not exceeding 6 months at −70°C. L and Z in serum samples were prepared for extraction as previously described[11] using 200-μl samples. Echinenone, in ethanol, was added as an internal standard. The extracted sample was analyzed for carotenoids using a reverse-phase, gradient HPLC system and method also previously described.[12]

RESULTS

When considering subjects who were supplemented with spinach and corn ($n = 10$) or spinach only ($n = 1$), a matched pairs Student's t-test indicated significant increases in MP density from baseline at 4, 12, and 14 weeks ($P < 0.05$). Further analysis, however, revealed distinct differences in the response of persons to addition of spinach and corn to their diet. When the average values for the two baseline samples were compared with the average values of all the samples taken during dietary modification, three types of responses could be identified:

> Eight persons had average increases of at least 13% in MP density (19% ± 11%, mean ± SD). These persons we refer to as "retinal responders." These persons all had increases (33% ± 22%) in serum concentrations of L (but not Z and β-carotene). The average increases in MP density and serum L during the test period for this group were statistically significant ($P < 0.05$, as determined by a matched pairs Student's t-test). One of these subjects received spinach only but since the data from this subject were indistinguishable from subjects given both corn and spinach, her data were combined with the spinach–corn group.
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> Two persons showed a slight decrease in MP density during the study period (mean, −11%), in spite of substantial increases in serum concentrations of L (mean, 31%). These persons, whose MP did not increase, we refer to as "retinal nonresponders."
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> One subject showed minimal changes in MP density and in serum concentrations of L, Z, and β-carotene (6% or less for all values) and is considered a "retinal and blood nonresponder."

Responses to Spinach and Corn: Retinal Responders

The time course of changes in MP and serum L averaged across persons for each of the response types is shown in Figure 1. Data from the retinal responders are presented in the top panel. Within one month of dietary modification, mean MP density of the retinal responders was already significantly different from baseline. Although mean MP density was not significantly different from baseline at 8 weeks, at all other

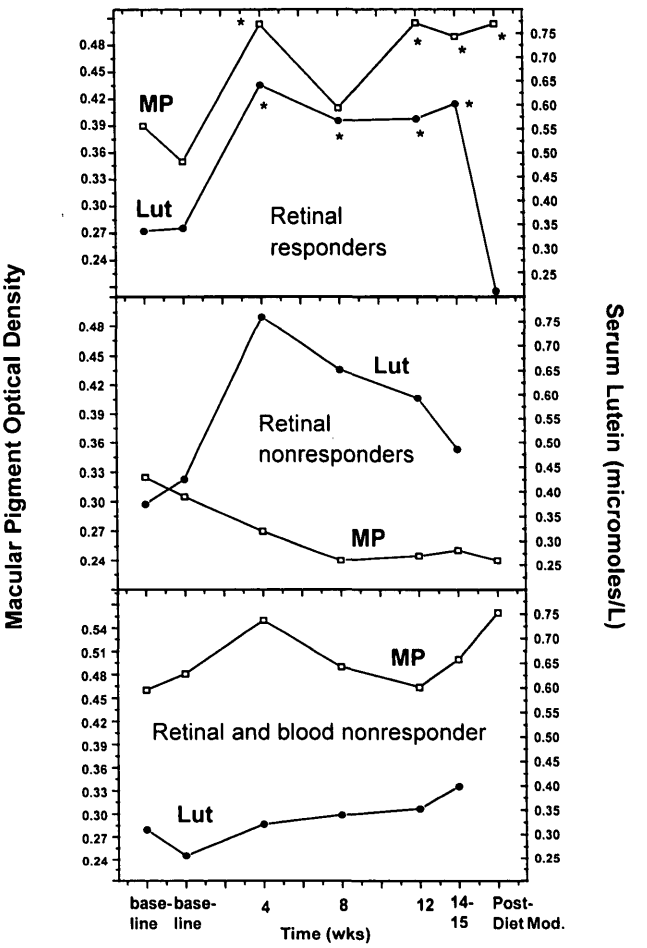

FIGURE 1. Mean macular pigment (MP) (*open squares*) and serum concentrations of lutein (L) (*filled circles*) during the study period for the three different types of responses to dietary modification. (*top panel*) Retinal responders ($n = 7$). For MP, SDs for the means of the retinal responders were 0.09 at baseline, and ranged from 0.11 to 0.14 at the other time points. For serum lutein, baseline SD was 0.16. Between 4 weeks and 12 weeks, SDs were 0.26 to 0.28, and at 14 to 15 weeks, 0.34; postdietary modification SD was 0.11. Asterisks indicate those measurements that were significantly different from baseline ($P < 0.05$) as determined by a matched pairs Student's t-test. (*middle panel*) Retinal nonresponders ($n = 2$). The averages of the two persons are plotted. The shapes of the curves for the two persons were similar, but the magnitudes were displaced by a scalar of approximately 23%. (*bottom panel*) Data for the one person whose serum L did not change and whose MP increased only 6% (classified as a retinal and blood nonresponder). Postdietary modification values were obtained at different time points for each person (range, 1 to 6 months; mean, 3 months).

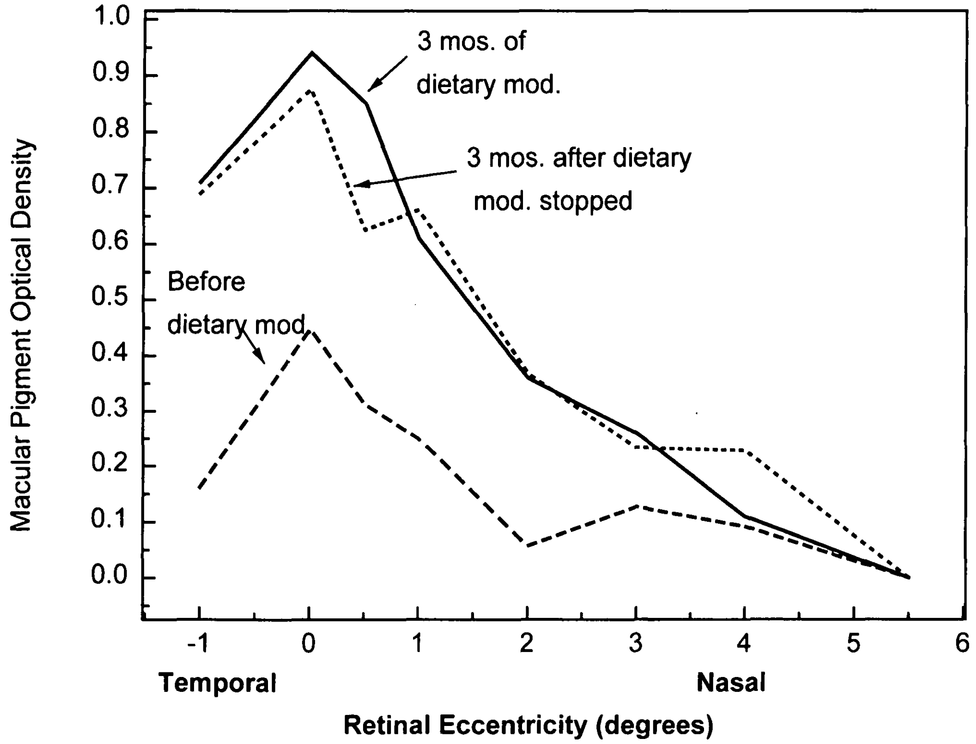

FIGURE 2. The macular pigment density distribution of a retinal responder before dietary modification (*dashed line*), after 3 months of adding spinach/corn to her diet (*solid line*), and 3 months after dietary modification was discontinued. Macular pigment density was measured at the following retinal locations using the stimulus sizes that are indicated parenthetically: 1° temporal (20′); 0° (12′); 0.5° nasal (12′); 1°, 2°, and 3° nasal (20′); 4° nasal (1°); and 5.5° nasal (1.2′).

time points (12, 14 to 15 weeks, and at the posttest time point, obtained 1 to 6 months after discontinuing the diet), it was significantly elevated ($P < 0.05$, as determined by a matched pairs Student's *t*-test). Serum L was significantly higher than baseline ($P < 0.05$) for all time points except for the postdiet measure, which did not differ from the predietary modification baseline. Thus MP remained elevated after serum L declined. Serum concentrations of Z and β-carotene (not shown) did not change significantly from baseline throughout the study period for the retinal responders.

Although MP density was only measured in the center of the retina for most subjects, we measured MP density at several retinal locations for three subjects (two retinal responders and one retinal nonresponder). For the two retinal responders, we found that MP density increased at all measured locations. No change at any location was found for the retinal nonresponder. Figure 2 shows the increase in the MP spatial distribution of one retinal responder after 3 months of dietary modification. Peak MP density of this person changed by a factor of two and was maintained at this elevated level when measured again 3 months after discontinuing the diet. Data from another retinal responder whose MP distribution was measured are shown in Figure 3. Because this subject did not complete the entire protocol (he ate the modified diet for only 6 weeks), his data are not included in Figure 1. Dietary modification for only 6 weeks, however, was sufficient to cause a substantial and enduring increase in this subject's MP distribution.

Responses to Spinach and Corn: Retinal Nonresponders

For three persons, two white men and one Asian woman, MP density did not increase significantly during dietary treatment with spinach and corn. For two of these subjects, one man and one woman, MP declined slightly in spite of the fact that serum L increased by a mean of 31%. Mean data for these persons are shown in the middle panel of Figure 1. One male subject, the retinal and blood nonresponder, (bottom panel of Fig. 1) did not have a substantial increase in either serum concentrations of L or in MP density. This subject, a coauthor of the current article, was questioned carefully and maximal compliance with the dietary protocol was confirmed.

Responses to Corn Only

Two persons, one Asian woman and one Hispanic woman, modified their diet by adding corn only for

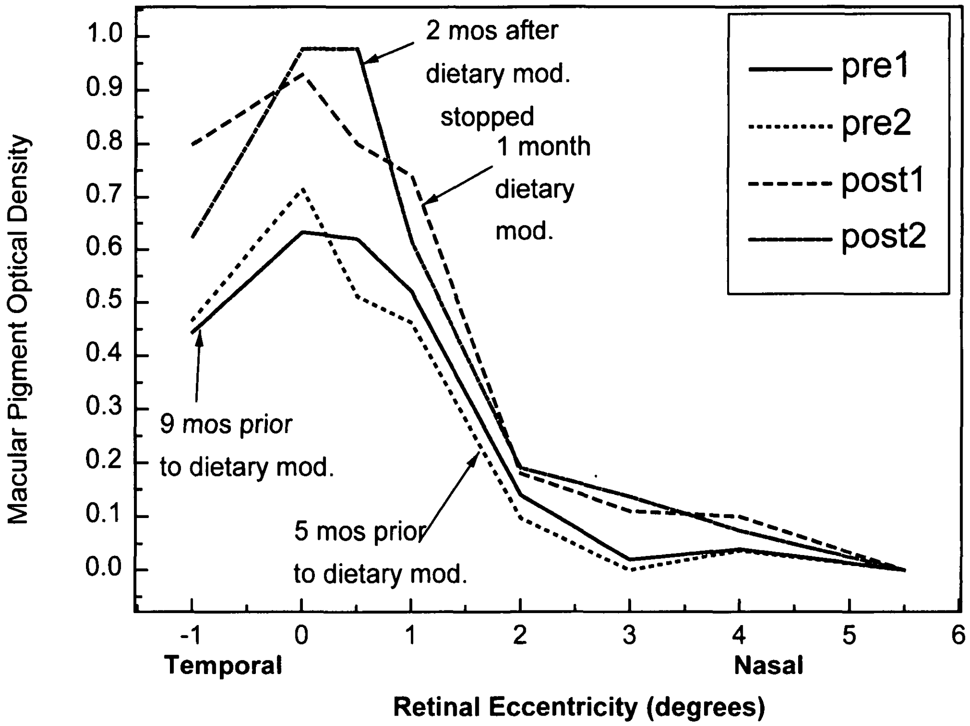

FIGURE 3. The macular pigment density distribution of a retinal responder at two time points before dietary modification (5 and 9 months) (*dashed lines*), after 1 month of dietary modification (*solid line*), and 2 months after dietary modification was discontinued (*dotted line*). Macular pigment measurements were made in the same manner as indicated for Figure 2.

14 to 15 weeks. As a percentage change, the addition of corn to the diet should have little impact on the intake of L, but approximately double the intake of Z. Consistent with this expectation, these subjects had only slight changes in serum L (+11%, −6%) with decreases in serum β-carotene (23%, 12%). However, the Asian woman had a substantial increase in serum Z (70%) and in MP density (25%), whereas the corresponding parameters changed little for the Hispanic woman (6%, 7%). Although preliminary, these data suggest that individual differences in response to specific foods may need to be considered when formulating dietary recommendations.

DISCUSSION

The major finding in the current study was the demonstration of significant increases in mean MP density in response to dietary modification with spinach and corn. For the responders, increases in MP density were obtained for all time points except the 8-week point. The temporary return to baseline at 8 weeks was displayed by all subjects, with decreases from the 4-week values ranging from 16% to 37%. Because the 8-week measurement did not occur on the same day for all subjects, we think it is unlikely that this temporary decline is caused by experimental error. Instead, interaction with other tissues that accumulate L and Z may contribute to the temporal fluctuations in MP. The concentration of L and Z in adipose tissue was measured in some of the subjects who participated in the current study. Our preliminary results indicate an inverse relationship between L and Z in the retina and L and Z in adipose tissue, both before and during dietary supplementation.

An analysis of the dietary records of the retinal responders for whom we had complete data indicated that modification of their diets increased their average intake of L and Z by a mean factor of 6.7. (The nutritional database used to assess the diets only has values for the sum of L and Z.) This increase in dietary intake was sufficient to raise mean serum concentrations of L by 33% and mean MP density by 19%. In some cases, increases in MP density during the test period were substantial (factor of two). For example, one subject increased from an optical density of 0.31 to 0.65 after only one month of dietary modification. This corresponds to a reduction in transmission of 460-nm light from 50% to 23%.

Although the increases in MP density were relatively rapid, our data indicate that the subsequent decreases must be slow. MP remained elevated after treatment was discontinued. The postdiet MP data points averaged for Figure 1 were collected from 1 to 6 months after discontinuing dietary modification. At these same time points serum L had returned to baseline.

For one male subject, we had MP data for a period of 5 years before dietary modification. During this prolonged period, this subject's MP density was maintained at an average of 0.30 (range, 0.27 to 0.35). After only 14 weeks of dietary modification, his MP density increased 50% and it has remained at this elevated level for 9 months after discontinuing the test diet. This observation implies that MP density remains relatively stable once the pigment has been successfully integrated into the retina, presumably in cell membranes.[1]

Two persons showed substantial increases in serum concentrations of L (mean increase, 31%) but had a slight decrease of 11% in MP density during the study period. One possible explanation for this finding is that these persons increased in a manner that our psychophysical technique could not measure. The psychophysical measurements are referenced to a peripheral location of 6°. If the MP density of these persons increased by nearly the same amount in the foveal center and at 6°, our method would not detect any change. However, examination of the MP spatial profiles of subjects who responded strongly (Figs. 2, 3) showed that the increased density was not uniform across the retina, but was greatest at the center and progressively decreased with retinal eccentricity. Therefore, we regard the idea of a spatially uniform increase as unlikely.

Comparing the mean data of the retinal nonresponders to the retinal responders, we found that the nonresponders' dietary intake of L and Z before the study was four times higher. Furthermore, baseline concentrations of serum L were 17% higher, and serum Z was 52% higher. In contrast, their baseline densities of MP were 30% lower. This suggests that the nonresponsiveness of their retina to dietary intake of L and Z is a stable personal characteristic.

To evaluate this possibility further, one male retinal nonresponder was given daily semipurified supplements (FloraGlo Lutein; Nature's Life, Garden Grove, CA) consisting of 40 mg of L and 4.0 mg of Z, separated into two doses, for 112 days. He was instructed to take these supplements at different times of day with a fat source. Landrum et al.[13] have demonstrated for two subjects that supplementation with semipurified supplements of L dipalmitate (30 mg/day) can produce significant increases in MP density. However, our nonresponding subject did not show an increase in MP density despite increases in serum concentrations of L by a factor of 4, and increases in serum Z by a factor of 2. These results indicate that individual characteristics identified in response to food intake may also be evident in response to purified supplements.

As a cautionary note, our data from the two retinal nonresponders suggest that dietary modification may have caused a slight decrease in their MP density. Although our sample size was not large enough to evaluate this decrease statistically, the possibility that a minority of persons could respond negatively to dietary modification has clear clinical importance and warrants further study.

Wide variation in tissue response to supplements has been reported for buccal mucosa cell during β-carotene dosing.[14] In this large study ($n = 178$), it was found that variations in tissue response were population specific. Epidemiologic studies of the effects of carotenoids on disease have not considered these kind of variations in tissue response to carotenoid intake. If carotenoids protect a tissue locally rather than systemically, assessment of a person's tissue response is crucial for evaluating the role of carotenoids in diseases that involve that tissue. Data from our study highlight the importance of knowing carotenoid concentrations in the retina when evaluating the importance of carotenoids for preventing retinal degeneration.

Elevation of MP density in patients may be beneficial for two reasons. First, if L and Z are protective against the neovascular form of AMD,[3] the probability that the patient will progress to neovascular complications could be reduced. Second, there is a substantial body of evidence that MP protects the retina and retinal pigment epithelium against light damage.[1] Increasing MP density might therefore be a useful adjunct to surgical procedures involving intense exposure of the eye to light. Harmful side effects from surgical light exposures have been documented for patients[15] and for surgeons as well.[16] By enhancing retinal protection for patients and ophthalmologists before surgery, some of the risks of surgical light exposures might be reduced.

One piece of information that is missing at present is the influence of dietary modification on carotenoid content of the retina at eccentricities beyond where MP is optically measurable. At these more peripheral locations, carotenoid content must be assayed by biochemical extractions and HPLC analyses. Obtaining this information will require animal experiments, which we are currently conducting.

Key Words

age-related macular degeneration, carotenoids, diet, macular pigment

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