Light Exposure and Macular Pigment Optical Density

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**Purpose.** Biochemical research has demonstrated that lutein and zeaxanthin, the two macular carotenoids, are bleachable pigments. Further, evidence suggests that exposure to UV light can degrade plasma carotenoid levels in vivo. The present study investigated the effects of exposure to normal levels of light on the levels of lutein and zeaxanthin in the retina.

**Methods.** The optical density of macular pigment (MPOD) was measured in two male subjects under four different light-adaptation conditions for 20 days. Heterochromatic flicker photometry was used to measure MPOD at 0.5° eccentricity.

**Results.** The four conditions of light adaptation did not significantly affect MPOD. As in previous studies, however, a significant day-to-day difference was observed for both subjects.

**Conclusions.** The results suggest that lutein and zeaxanthin levels in the eye are unaffected by light and oxidation throughout the day. This justifies current research methods in which MPOD measures are made regardless of the time of day. However, significant between-day variance indicates that multiple MPOD measures may be necessary to evaluate lutein and zeaxanthin levels in the retina accurately. (Invest Ophtalmol Vis Sci. 2003;44:306–309) DOI:10.1167/iovs.01-1191

Lutein and zeaxanthin are naturally occurring yellow pigments called carotenoids. Similar to β-carotene, these pigments are found in various colored fruits and green leafy vegetables. Of the 40 to 50 carotenoids typically consumed in the human diet, lutein and zeaxanthin, in particular, are deposited in the macular region of the retina. These pigments are collectively referred to as the macular pigment (MP).

The MP has been postulated to improve acuity through the amelioration of the effects of chromatic aberration and to preserve and protect the central retina. The latter hypothesized functions are based on the following two actions. First, MP absorbs light in the harmful short-wave end of the spectrum and dissipates it as heat before it renders damage to the foveal tissue. Second, the macular carotenoids are antioxidants, able to prevent free-radical reactions that can damage cellular components.

Although several investigators have referred to the macular carotenoids as photostable or unbleachable pigments (or at least not as rapidly bleachable in the same sense as the retinal photopigments), several biochemical studies have demonstrated that lutein and zeaxanthin can be bleached rapidly in vitro by photolysis and by reaction with various oxidizing solutions. The bleaching rate, the loss in absorption as a function of incubation time, is similar for lutein and zeaxanthin in the presence of many radical species (e.g., peroxyl radicals). This result seems to indicate that their antioxidant effectiveness is similar. However, lutein and zeaxanthin have slower bleaching rates than other carotenoids, such as β-carotene and lycopene. Siems et al. suggested that the macular carotenoids' slower bleaching rate may explain their exclusivity in the retina. Roe demonstrated that carotenoids could be photodegraded in vivo. She found that plasma carotenoid levels among her female subjects were significantly lower after 11 days of UVA and UVB light treatment. The daily exposure was equivalent to approximately 8 minutes of sunlight. White et al. observed similar effects in both male and female subjects who were consuming a controlled diet. In both of these studies, however, it is unclear what carotenoids were measured.

Previous investigators have observed day-to-day differences in the optical density, or amount, of macular pigment (MPOD) in individual subjects, where MPOD is defined at 460 nm, the wavelength of peak absorption. Despite the unknown source of this variance, researchers have assumed that MP levels do not fluctuate throughout the day. In fact, numerous investigations have compared the MPOD of subjects without regard to the time of day the measurements were performed. If free radicals and light can bleach or deplete lutein and zeaxanthin in the eye, it may explain some of the difference found day to day. Furthermore, it suggests that macular pigment may decrease throughout the day as a result of light exposure and free radical quenching. Hammond et al. proposed that similar effects may explain why individuals with light-colored irises (blue, gray) tend to have lower MPOD than individuals with dark-colored irises (brown, black). Compared with dark irises, light irises transmit more light, thereby exposing the MP to greater levels of photostress. The present study was designed to investigate the acute effects of normal light exposure on MPOD.

**Methods**

**Subjects**

Two male graduate students (27 and 30 years old), well trained in psychophysics, participated in the experiment. Both subjects were aware of the experimental hypothesis and informed consent was obtained before initiation of the experiment. The experimental procedures adhered to the Declaration of Helsinki.

**Measurement of MPOD**

The in vivo assessment of the MPOD takes advantage of the fact that lutein and zeaxanthin are deposited in the inner plexiform and photoreceptor-axonal layers. Consequently, MP filters light before it reaches the photopigments. Because the absorbance of the macular carotenoids is from 400 to 520 nm, retinal loci screened by macular pigment have a reduced sensitivity to short-wave stimuli compared with other loci where no MP exists. Several studies have demonstrated that MPOD is highest in the fovea and declines to an optically undetectable point between 6° to 8° eccentricity. Thus, if it is assumed that the fovea and parafovea have the same spectral sensitivities, then a decrease in foveal short-wave sensitivity would indicate the presence of MP. This assumption has been demonstrated to hold when the testing conditions favor detection by the mid- and long-wave cones.

In the present study, heterochromatic flicker photometry (HFP) was used to measure MPOD (see Werner et al.). HFP conducted...
light (3356 cd/m²) for 5 minutes (condition B). The light source was placed over the sealed patch. The two eye patches were worn for one subject and 8 hours for the other. Both subjects wore the eye patch was sealed to the face with medical tape, and a second eye patch.

The experiment consisted of measurements on 20 nonconsecutive days in each subject. The experiment was conducted in 29 calendar days for one subject and 33 calendar days for the other. The night space-averaged luminance of 18 cd/m² emanating from the book (condition D) was preceded by 15 minutes of dark adaptation to reproduce the same cone equilibrium before the first measurement. For each MPOD measure, the method of adjustment was calculated by taking the log ratio between the mean foveal and mean parafocal values. The start times for each session were consistent throughout the experiment. Because diet has been shown to affect MPOD, the subjects followed a restricted diet to reduce this source of variability. One week before the experiment started, and throughout its duration, the subjects avoided foods high in lutein and zeaxanthin (e.g., spinach). Also, on experiment days, the first three sessions were conducted while the subjects fasted, and the final session followed an invariant lunch.

Results

Figure 1 shows that MPOD did not vary significantly as a result of light history. No deviations from normality or homoscedasticity were obvious from visual inspection of the data. A separate one-way analysis of variance (ANOVA) for each subject indicated that the four conditions of light adaptation did not affect MPOD (subject 1 [S1]; F = 0.195, P = 0.898; subject 2 [S2]; F = 1.476, P = 0.227).

Given the possibility that MP is replenished in prolonged darkness and then bleaches rapidly after exposure to light (i.e., the stimulus from the first measurement value of the day), an analysis was performed on MPODs calculated from only the first foveal and parafoveal values from each trial. The data are virtually identical with those presented in Figure 1. A one-way ANOVA for each subject, based only on these data, showed that MPOD was not significantly different after the four conditions of light adaptation (S1; F = 0.541, P = 0.655; S2; F = 0.751, P = 0.524), suggesting that MPOD was not affected by the test stimulus immediately after removal of the eye patch.

In Figure 2 the mean MPOD (n = 4) on each experiment day is presented for both subjects. As in previous studies, day-to-day differences in MPOD were observed. One-way ANOVAs revealed that these differences were statistically significant in both subjects (S1; F = 2.207, P = 0.07; S2; F = 2.19, P = 0.02). If these observed day-to-day differences were in fact fluctuations in MPOD, it might be expected that the values obtained with the foveal target would significantly vary across days, whereas values obtained with the parafoveal target would remain invariant. A one-way ANOVA was performed on each subject’s daily foveal (n = 32) and parafoveal (n = 32) energy settings for HFP. According to the four separate
ANOVAs, the energy necessary to eliminate flicker for both the foveal and parafoveal targets significantly varied in subject 1 ($S_{\text{fovea}}$: $F = 4.728, P < 0.0001$; $S_{\text{parafovea}}$: $F = 7.507, P < 0.0001$) and subject 2 ($S_{\text{fovea}}$: $F = 7.795, P < 0.0001$; $S_{\text{parafovea}}$: $F = 7.130, P < 0.0001$). Thus, it does not appear that MPOD varies from day to day.

A Pearson's $r$ correlation between the two subjects' daily mean MPOD measures was performed to assess the possibility that an artifact of the apparatus contributed to the variation in MPOD across days. No significant correlation was obtained ($r = 0.299, P = 0.242$), suggesting that day-to-day differences were not a consequence of an optical system artifact.

**DISCUSSION**

In the present study, MPOD remained stable throughout the day, regardless of a variety of light exposures within normal daily experience. This suggests that the macular carotenoids are unbleachable or photostable in the natural retinal environment. It may be that lutein and zeaxanthin in the eye can be degraded by light of greater intensity than the lights used in the current experiment. Both subjects, however, were regularly exposed to substantial, albeit unquantified, amounts of natural daylight before the fourth session of each experiment day. Two studies have shown that ultraviolet light can significantly reduce plasma carotenoid levels.\(^{18,19}\) Although the effects reported by these studies were measured after 11 days of skin exposure, sunlight was avoided by subjects in both studies, and

![Figure 1](image1.png) **FIGURE 1.** Mean MPOD for each of four conditions of light exposure with associated SEs. MPOD was determined using a 458-nm test.

![Figure 2](image2.png) **FIGURE 2.** Mean MPOD at 458 nm and SEs for each experiment day, averaged across conditions.
the daily exposure was the equivalent of only 8 minutes of sunlight. Unfortunately, these investigators did not specify which carotenoids were affected by the exposure to ultraviolet light. In vitro, sunlight can photodegrade lutein and zeaxanthin. After 4 hours, Siems et al.\textsuperscript{15} observed significant reductions in the concentration of these carotenoids. Despite these findings, the retina is not commonly exposed to direct sunlight. Further, the eye environment may affect the bleaching rate of the macular pigment.

Alternatively, the macular pigment may photodegrade throughout the day but be rapidly replaced by lutein and zeaxanthin circulating in the blood or from other body stores. This may be unlikely, given the time (weeks) necessary to increase MPOD by either dietary increases of lutein and zeaxanthin or from other body stores. This may be unlikely, given the time (weeks) necessary to increase MPOD after supplementation, then, may involve altering a homeostatic mechanism that regulates the concentrations of these two carotenoids in the retina.

Similar to several previous studies, day-to-day variation in MPOD was observed in well-trained subjects. Landrum et al.\textsuperscript{26,28} suggested that such variation is the result of measurement error, as opposed to fluctuations in MPOD. In the present study, however, neither subjects’ MPOD significantly varied within experiment days. This suggests that between-day variation may not be due to measurement error, but possibly to day-to-day fluctuations in subjects’ sensitivity to flicker\textsuperscript{5} or relative spectral sensitivity. However, potential measurement error, such as changes in subjects’ criterion of sensation luminance, may vary between days, but remain consistent within a day. Until the source of this variation is realized, multiple MPOD measures may be necessary to evaluate accurately lutein and zeaxanthin levels in the retina.

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References