Influence of Lutein Supplementation on Macular Pigment, Assessed with Two Objective Techniques

Tos T. J. M. Berendschot, R. Alexandra Goldbohm, Wilhelmina A. A. Klöpping, Jan van de Kraats, Jeannette van Norel, and Dirk van Norren

PURPOSE. Macular pigment (MP) may protect against age-related macular degeneration. This study was conducted to determine the extent of changes in the macular pigment density as a consequence of oral supplementation with lutein. A second purpose was to compare two objective measurement techniques.

METHODS. In the first technique, reflectance maps were made with a scanning laser ophthalmoscope. Digital subtraction of log reflectance maps and comparison between the foveal area and a 14° temporal site provided MP density estimates. In the second technique, spectral reflectance of the fovea was measured with a fundus reflectometer and analyzed with a detailed optical model, to arrive at MP density values. Eight subjects participated in this study. They took 10 mg lutein per day for 12 weeks. Plasma lutein concentration was measured at 4-week intervals.

RESULTS. After 4 weeks, mean blood level of lutein had increased from 0.18 to 0.90 μM. It stayed at this level throughout the intake period and declined to 0.28 μM 4 weeks after termination. Measurement of the density of MP showed a within-subject variation of 10% with MP maps and 17% with spectral reflectance analysis. MP density showed a mean linear 4-week increase of 5.3% (P < 0.001) and 4.1% (P = 0.022), respectively.

CONCLUSIONS. Supplementation with lutein significantly increased the density of the MP. Analyzing reflectance maps with a scanning laser ophthalmoscope provided very reliable estimates of MP.

M acular pigment (MP), concentrated in the central area of the retina, contains the carotenoids lutein and zeaxanthin. It protects the macular region by its capability of filtering blue light, thereby possibly decreasing photochemical light damage. In addition, MP is capable of scavenging free radicals. Cross-sectional studies have observed an inverse association between a diet with a high content of the carotenoids lutein and zeaxanthin and the prevalence of age-related macular degeneration (AMD). Although for a definite proof of this relationship a follow-up study is needed, it is of interest to know whether MP density can be modified. Two studies have been published showing a change in MP density, one by a dietary modification (consumption of spinach and/or corn) on 11 subjects and one by supplementing lutein in two subjects. Hammond et al. had some subjects fail to show a change in MP density. Both studies used heterochromatic flicker photometry to determine MP density in experienced subjects. This psychophysical method has disadvantages. It is time consuming, and its reliability depends on the understanding by the subject of the task involved. This makes the test less suitable for a naive target population. Recently, Bernstein et al. demonstrated in an animal model resonance Raman scattering as an objective method that may have future use in studying human MP density. The purpose of the present study was twofold. First, we wanted to establish the extent of changes in the MP density as a consequence of oral supplementation with lutein. Second, we wanted to compare two objective techniques for measuring MP on the basis of fundus reflectance.

METHODS

Subjects

Eight male, nonsmoking volunteers between the ages of 18 and 50 years (mean age, 40.6 years) were recruited for the study. To assure homogeneity in lutein plasma levels at baseline, a simple food-frequency questionnaire was used to exclude subjects with a high intake of lutein—that is, subjects who had eaten vegetables high in lutein, such as spinach and kale, more than four times in the 4-week period preceding the beginning of the study. Furthermore, only subjects with a relatively normal (Dutch) dietary pattern and not taking vitamin or mineral supplements were selected. The subjects took a daily dose of 10 mg of lutein, in the form of lutein diesters derived from marigolds, for a period of 12 weeks. The study was conducted according to good clinical practice, approved by the local medical ethics committee, and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects before participation in the study.
Measurement of Plasma Lutein

Blood was sampled after an overnight fast at baseline and at 4-week intervals during the study. Four weeks after the end of the lutein supplementation period, a final blood sample was taken. For lutein analyses 1 ml plasma was mixed with 1 ml ethanol (containing 16 to 32 micromoles tocopheryl acetate per liter as internal standard). After 10 minutes, 2 ml hexane was added, and the sealed tubes were vortexed for 4 minutes. After centrifugation for 10 minutes at 3000g at 4°C, the hexane layer was separated and evaporated under nitrogen at room temperature. The residue was dissolved in 0.4 ml high-performance liquid chromatography (HPLC) solvent and transferred into brown HPLC injection vials. Lutein was quantified by HPLC with a hyperchrome 3-µm column Nucleosil 120. The mobile phase consisted of acetonitrile-methylene chloride-methanol (70:15:10, vol/vol/vol), and the flow rate was 1 ml/min. An absorbance detector was used at 445 nm for detection of lutein. Limit of detection of lutein was 3.0 nM plasma. The amount of lutein present in the plasma sample was quantified by calculating the ratio of the peak height of lutein to that of the internal standard.

Measurement of MP

MP density was measured in both eyes at baseline and at 4-week intervals during the study. Four weeks after the end of the lutein supplementation period, a final measurement was taken. Two techniques were used: MP maps from scanning laser ophthalmoscopy (SLO) and spectral analysis. The methods were always applied successively in the order of SLO first, spectral analysis second. A mydriatic was used to dilate the pupil for both setups.

MP Maps from SLO. Fundus reflectance maps at 488- and 514-nm argon laser wavelengths were made with a custom-built SLO (Fig. 1). The SLO covers a retinal area of 40° × 23°, has a well-defined exit pupil 2 mm in diameter, and allows reflectance maps at different wavelengths to be grabbed within a few video frames. Blood and melanin effectively absorb light that has entered the choroid, and the major contribution to the reflectance is from the discs in the outer segments of the cones.8 This leaves the lens and the MP as the only relevant absorbers in this wavelength region. As a consequence, digital subtraction of log reflectance provides density maps of the sum of both absorbers. Figure 1C shows a typical example, calculated from the reflectance maps shown in Figures 1A and 1B. At 14°, temporal MP density is assumed to be negligible. Thus, with this site providing an estimate for the lens density, the mean MP density was calculated in a 1.5° field centered at the fovea. This field is not sampled with the Utrecht retinal densitometer.8 Briefly, a rotating wheel (14 revolutions per second) offers a sequence of 14 interference filters in the range 430 to 740 nm to enable quas simultaneous measurement of the reflectance across the visual spectrum. The illumination field was 1.8° centered at the fovea. Light reflected from the fundus was measured in a detection field of 1.5°, concentric within the illumination field. To obtain an estimate of the mean MP density in this area, spectral fundus reflectance was measured in two conditions: perpendicular, with the instrument’s entry and exit pupils aligned to the peak of the Stiles-Crawford (SC) function, and oblique, 2 mm temporal to the SC peak.11 A detailed optical model of foveal reflection was used to arrive at individual estimates of parameters as equivalent thickness of blood layer, and densities of the lens, MP, and melanin.11 In short, the incoming light is assumed to reflect, at the inner limiting membrane (ILM), discs in the outer segments of the photoreceptors and the sclera. Using known spectral characteristics of the different absorbers within the eye (lens, MP, blood, and melanin), the densities of the pigments and the percentage of reflectance at the interfaces are optimized, to fit the measured data at all wavelengths. The two spectra measurements, perpendicular and oblique, were fitted simultaneously with eight free parameters. None of the parameters was assumed unchanged between baseline and supplemented status. Only MP showed significant changes in time and will be discussed. Visual pigments were bleached, and a bite board and temple pads were used to maintain head position.

Spectral Analysis. Spectral fundus reflectance was measured with the Utrecht retinal densitometer.8 Briefly, a rotating wheel (14 revolutions per second) offers a sequence of 14 interference filters in the range 430 to 740 nm to enable quas simultaneous measurement of the reflectance across the visual spectrum. The illumination field was 1.8° centered at the fovea. Light reflected from the fundus was measured in a detection field of 1.5°, concentric within the illumination field. To obtain an estimate of the mean MP density in this area, spectral fundus reflectance was measured in two conditions: perpendicular, with the instrument’s entry and exit pupils aligned to the peak of the Stiles-Crawford (SC) function, and oblique, 2 mm temporal to the SC peak.11 A detailed optical model of foveal reflection was used to arrive at individual estimates of parameters as equivalent thickness of blood layer, and densities of the lens, MP, and melanin.11 In short, the incoming light is assumed to reflect, at the inner limiting membrane (ILM), discs in the outer segments of the photoreceptors and the sclera. Using known spectral characteristics of the different absorbers within the eye (lens, MP, blood, and melanin), the densities of the pigments and the percentage of reflectance at the interfaces are optimized, to fit the measured data at all wavelengths. The two spectra measurements, perpendicular and oblique, were fitted simultaneously with eight free parameters. None of the parameters was assumed unchanged between baseline and supplemented status. Only MP showed significant changes in time and will be discussed. Visual pigments were bleached, and a bite board and temple pads were used to maintain head position.
Statistical Analysis

To quantify the quality of the measurement techniques, we compared the within-subject variations. Both relative SD and coefficient of repeatability were calculated. To estimate the possible increase in MP density over time, we applied a statistical general linear model (GLM), with repeated-measurements analyses on MP density with both time and eye as within-subject factors. Time was included in the model as linear effect. For one of our subjects, one of the fundus reflectance measurements failed in one eye. In the GLM analysis, we used the mean of his other four MP densities (in the same eye) for this data point. For clarity, in Figures 2 and 4, mean values of left and right eyes are presented.

RESULTS

Individual response curves of plasma lutein concentration are shown in Figure 2A. All subjects showed a substantial increase in plasma lutein concentration at week 4. Mean lutein concentration, shown in Figure 2B, increased from $0.18 \pm 0.08 \text{ M}$ at baseline to $0.90 \pm 0.18 \text{ M}$ ($P < 0.001$) at 4 weeks, and it remained at this level throughout the supplementation period. Four weeks after termination, the lutein level was still elevated ($0.28 \pm 0.06 \text{ M}; P = 0.005$). For comparison, literature data are included in Figure 2B (see the Discussion section).

Figures 3A and 3B show individual response curves for the MP density with both techniques. Baseline MP density values showed a large variation between subjects. In the majority of the measurements, MP density showed an increase with time. For the relative SD there was a within-subject variation of $10\%$ with MP maps and $17\%$ with spectral analysis. The coefficients of repeatability were $0.17$ and $0.27$, respectively.

To emphasize changes in density as a result of lutein supplementation, we normalized the individual response curves for each subject by dividing each density by the mean of the five measurements (Figs. 3C, 3D). Statistical analysis yielded a linear 4-week increase in relative MP density of $5.3\%$ ($P < 0.001$), calculated from MP maps, and of $4.1\%$ ($P = 0.022$), obtained with the spectral analysis. Mean response curves for both techniques are compared in Figure 4, together with literature data.

At baseline, plasma lutein concentration showed a significant correlation with MP density, $r = 0.78$ ($P < 0.001$) determined with MP maps and $r = 0.82$ ($P < 0.001$) determined with spectral analysis, respectively (Fig. 5).
A daily dose of 10 mg lutein supplementation induced an increase in mean plasma lutein by a factor of 5 and a linear 4-week increase in relative MP density of 4% to 5%. To our knowledge, this is the first study in which the effects of intake of lutein have been assessed with objective measurement techniques. In particular, the SLO-based technique provided very reliable results. With this technique all subjects showed a significant increase in MP density. The spectral reflectance analysis provided noisier results that did not allow such a conclusion for all individuals. Figure 4 shows a comparison between our results on naive subjects and heterochromatic flicker photometry data from literature. Landrum et al. published results of a study of 20 weeks of supplementation of 30 mg lutein per day derived from marigolds in two skilled subjects who were measured four to five times week. The noise in their results seems comparable to that in our technique. They found a 4-week increase of 4.2% in relative MP density, which also compares well with our results. Hammond et al. published results of a study of 15 weeks in 11 subjects who consumed spinach and/or corn (10.8 mg of lutein). Eight subjects showed an increase in MP density, but two showed a slight decrease. We estimated a 4-week increase in relative density of 3.5% for all subjects in their study. Their mean results suggest a considerably higher noise level than that in the present study, partly due to a temporary return to baseline MP density at 8 weeks, which they attribute to interaction with other tissues that accumulate lutein. This was neither observed in this study, nor in Landrum et al. It could be due to the use by Hammond et al. of dietary intake of lutein modification (consumption of spinach and/or corn), compared with supplementing lutein in the form of lutein diesters derived from marigolds in this study and the study by Landrum et al. Hammond et al. also studied both men and women, whereas in this study and that of Landrum et al., only men were involved. In women, there is no significant correlation between MP density and lutein in the diet, and the correlation between MP density and lutein in the blood is much weaker than in men. A division between responders and nonresponders, as proposed by Hammond et al., also could be seen in the spectral analysis of the fundus reflectance presented in the current study. We wonder whether this may be an artifact of the measurement techniques. With the SLO-based technique, all subjects showed a significant increase.

Heterochromatic flicker photometry is a rather demanding technique for subjects—in particular the task of adjusting flicker at a peripheral location. This may be the cause for noisier results than those obtained with the present SLO technique. A significant improvement in heterochromatic flicker photometry, however, was recently described in a psychophysical setup that avoids Maxwellian view. In Landrum et al. lutein intake was three times as high and plasma lutein concentration was twice as high as in the present study (Fig. 2). However, their increase in MP density was similar to ours. Apparently, a dose of 10 mg per day is sufficient to provide a 4-week increase of 4% to 5%. This conclusion is supported by the still-elevated plasma lutein concentration a month after the end of supplementation, compared with baseline, accompanied with a still-increasing MP density between weeks 12 and 16 ($P = 0.03$ for the MP maps, $P = 0.42$ for the reflectance analysis). Thus, a high plasma lutein level seems to elevate MP density gradually over time. This is further corroborated by the high correlation between

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932910/)

**FIGURE 4.** Comparison of the change in relative density over time of MP, as determined with the methods of the present study and with heterochromatic flicker photometry studies by Hammond et al. ($n = 11$) and Landrum et al. ($n = 2$). Error bars: SE.

**FIGURE 5.** Correlation at baseline between plasma lutein and MP density with two methods. (A) MP maps, $r = 0.78$ ($P < 0.001$), and (B) spectral analysis, $r = 0.82$ ($P < 0.001$). Solid lines are regression lines for left and right eyes combined. Error bars: SD.
plasma lutein concentration and MP density at baseline in our (all male) subject group (Fig. 5). Considering the study design, we cannot exclude that this effect was due to factors other than plasma lutein alone. For a definitive answer, a double-blind randomized controlled trial is needed.

Because we obtained maps of MP distribution we were able to look for changes in MP distribution with lutein supplementation. An exponential decay of MP density as a function of eccentricity fitted our data well up to 4°. No changes were found between the different measurements in time.

Our results show that fundus reflectance, in particular as obtained with our custom-built SLO, can be used as a fast and objective test to obtain reliable estimates of MP density. To reduce the effect of differences in pupil size, our SLO has a well-defined exit pupil, whereas commercially available SLOs use the whole pupil plane. Further, to minimize the influence of head and eye movements on the reflectance maps, our SLO allows reflectance maps at different wavelengths to be grabbed within a few video frames, which may be difficult in other SLOs. However, both adaptations may be of minor influence, because MP density is determined by a relative comparison of two reflectance maps. We opted for using a bite board, which is costly and time consuming. It may be possible to avoid using it in larger scale studies. The Utrecht retinal densitometer uses a spot with a rather low intensity to measure spectral fundus reflectance, because it has been optimized for retinal densitometry. Therefore, to obtain an adequate signal-to-noise ratio, densitometer outputs were averaged over a 2-minute interval. Nevertheless, one subject, the fourth in Figure 5, had such low fundus reflectance in the bluish wavelength region, that the MP density, determined by analyzing this wavelength region, showed an SD of 0.40 OD and 0.28 OS. All others showed a mean SD of 0.12. This may explain the discrepancy for this particular subject between the results obtained with the MP maps and with the spectral analysis. Severe cataract lowers the intensity and gives rise to similar problems. Increasing the intensity of the measuring light may shorten the time interval for data acquisition and improve the performance of the reflectance analysis. At baseline, mean values for MP density (single pass) were 0.26 for the SLO technique and 0.47 for the reflectance analysis. Reflectance at the ILM, anterior to the MP, may lower the apparent MP density measured. This ILM reflectance is corrected for in the model. However, their reflectance shows up as an underestimate of MP density, both in the SLO technique and sources of light scatter, such as floaters, could also introduce a 0.12. This may explain the discrepancy for this one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp Eye Res. 1997;65:57–62.


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References


