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LAST II: Differential temporal responses of macular pigment optical density in patients with atrophic age-related macular degeneration to dietary supplementation with xanthophylls

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KEYWORDS
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Responder;
Nonresponder;
Optical density units

Abstract

BACKGROUND: Age-related macular degeneration (ARMD) is the leading cause of vision loss in aging Western societies. The objective of the Lutein Antioxidant Supplementation Trial (LAST) was to determine whether specific dietary interventions increased macular pigment optical density (MPOD) and visual function in patients with atrophic ARMD. The current objective of LAST II is to discern those specific characteristics that increase MPOD, i.e., that might differentiate a responder from a nonresponder.

METHODS: The LAST study was a prospective, 12-month, randomized, double-masked, placebo-controlled trial conducted at an urban midwestern Veterans Administation Hospital from August 1999 to May 2001. Ninety patients with atrophic ARMD entered the study and were assigned randomly to 1 of 3 groups. Patients in group 1 received 10 mg lutein; in group 2, 10 mg lutein in combination with vitamins, minerals, and antioxidants; and in group 3, maltodextrin placebo. Changes in macular MPOD over time were evaluated. Characteristics potentially influencing MPOD included age, weight (body mass index), initial baseline values of macular pigment, and combining xanthophylls with other nutrients.

RESULTS: MPOD increased with supplementation and declined slightly without supplementation (regression slopes not equal to zero in supplemented groups, $P < 0.02$). The highest increases in MPOD over time occurred in patients with lower baseline values of MPOD. Statistically significant increases in MPOD density were observed in the lutein group for patients with baseline MPOD $\leq$0.3 optical density units and up to 0.2 optical density units in the lutein plus antioxidant group. Further analysis found that none of the subjects' eyes in the lowest quartile of baseline MPOD were in the lowest quartile for change in MPOD.

CONCLUSION: Noteworthy is the observation that those individuals with lowest MPOD, and in greatest need of supplementation, were also most likely to benefit from either the lutein or the lutein plus antioxidant supplementation. For those individuals who responded to supplementation, their macular pigment optical density had not ceased to increase at 12 months' duration of supplementation. The inference is that if a deficiency in macular pigment optical density is accurately diagnosed, effective interventions should be able to re-establish this prophylactic barrier.

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Age-related macular degeneration (ARMD) is the leading cause of untreated vision loss in aging Western societies. It accounts for 45% of all visual disability in the United States and is increasing not only in Western but in Asiatic societies, possibly reflecting consequences of contemporary dietary changes.[1-4] In addition to age, other risk factors

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include both behavioral characteristics and physiological characteristics, smoking representing the former and gender, cardiovascular health, and genetics the latter.[5-10] Studies using animal models have found that oxidative and photo-oxidative stress can induce apoptosis.[11,12] Carotenoids (lutein and zeaxanthin) have been found recently to protect against both apoptosis and mitochondrial loss of photoreceptors in rats.[13] The first Age-Related Eye Disease Study (AREDS) demonstrated the ability of antioxidants and zinc to slow progression of ARMD and loss of visual acuity in selected cases.[14]

Epidemiologic studies suggest that diets rich in antioxidants and the xanthophyll macular pigments, lutein and zeaxanthin, are inversely correlated with the prevalence of the disease.[5,15] Smaller studies indicate the macular pigments lutein and zeaxanthin, both alone and in combination with omega-3 fatty acids, appear to be able to reduce the prevalence and may slow the progression of macular degeneration.[16-22] The xanthophylls are versatile low/high-$\mathbf{P}_{O_2}$ antioxidants, strongly absorb blue light, and can influence fluidity of cellular membranes.[23-25] Some balance of these characteristics is inferred to govern their influence on ARMD. The direct clinical effects of intervention are the topics of the massive AREDS II study being undertaken by the National Eye Institute (NEI).

These still tentative observations, nonetheless, have led investigators to quantify the levels of macular pigment, and to track the influence on it with respect to dietary intervention or supplementation. Numerous methods have been described for measuring the levels of macular pigment in vivo.[23,26,27] They include reflectance, scattering, absorbance, and absorbance re-emission methods. Some methods measure the mean levels of total xanthophylls over the macula, whereas others provide information about their spatial distribution. Some definitive confirmation of the variation in levels of macular pigments has been seen in small-scale studies by High Pressure Liquid Chromatography (HPLC) of analysis of human retinas obtained from eye banks.[17] The inverse correlation of xanthophyll level with ARMD is suggestive of a mechanistic relation.

If increased levels of macular pigment can provide some protection from the multiple causes of ARMD, then it is important to ascertain effective means for enhancing their levels, especially for populations found to be at greater risk of the disease.[28,29] The LAST study provided an opportunity for understanding the influence of intervention on both macular pigment and visual function.[30]

LAST II, in contrast, is concerned with differential temporal response to lutein and the factors that might affect the structural replenishment of macular pigment. Such factors include age, weight (body mass index [BMI]), initial baseline values of macular pigment, and the inclusion of a mixture of additional carotenoids/antioxidants.

Materials and methods

Subjects and study design

The LAST was a prospective, 12-month, randomized, double-masked, placebo-controlled trial conducted at an urban midwestern Veterans Administration Hospital from August 1999 to May 2001. The Institutional Review Board of Hines Department of Veterans Affairs Medical Center approved the study protocol in June 1999. Written informed consent was obtained from each subject before participating in the study.

Patients with atrophic ARMD were referred by ophthalmologists at 2 Chicago-area veterans' medical facilities. Eligibility included diagnosis of atrophic ARMD (ICD9 362.51) by stereo bio-ophthalmoscopy, and at least 1 vision-degrading ARMD-associated visual abnormality associated with ARMD in 1 or both eyes such as depressed contrast sensitivity, abnormal photostress glare recovery, or Amsler grid deficits. Subjects were excluded if they had undergone recent (within 6 months) cataract or retinal surgery, were taking photosensitizing drugs, or did not meet ophthalmic/visual entrance criteria. One hundred nine subjects were registered with 19 excluded because they were fundus positive but had no psychophysical abnormalities (n = 9), voluntarily withdrew during baseline workup (n = 6), received ARMD laser treatment (n = 2), or had preretinal membrane (n = 1) or Alzheimer's disease (n = 1).[29]

Procedures

A total of 90 subjects participated in the initial screening during which demographic data (gender; age; years with ARMD diagnosis; cigarette, alcohol, caffeine use; BMI), nutritional status (multivitamin use, Harvard School of Public Health Food Frequency Intake Questionnaires), ocular data (iris color, lens opacity classification system [LOCS] 3 cataract grade, AREDS disease stage, macular pigment optical density [MPOD]), and visual data (visual acuity, contrast sensitivity function [CSF], glare recovery, Amsler grid defect count) were collected.[29]

Subjects who participated in the study were randomly assigned to 1 of 3 groups. Subjects in group 1 (L) received 10 mg lutein per day; subjects in group 2 (L/A) received 10 mg lutein per day plus a broad spectrum of antioxidants in a preparation including vitamins, minerals, amino acids, and bioflavonoids; subjects in group 3 received a maltodextrin placebo. The 10-mg dose was extrapolated from spinach pilot case series data as previously described[30,31]; the non-esterified (free alcohol) Floraglo® lutein is chemically identical to that found in spinach. Subjects were encouraged not to alter their diets and were provided an integrated instruction sheet/questionnaire/Amsler grid to monitor changes in vision over time.[32] Subjects returned for follow-up visits at 4, 8, and 12 months, during which time MPOD and visual measures were repeated.

Although several measures of visual function were reported in LAST, the single observation of interest here was the effect of the interventions on MPOD. Macular pigment was measured by heterochromatic flicker photometry (MacularMetrics, Rehoboth, Massachusetts), following the manufacturer's protocol. Subjects matched a 460 nm/540 nm flickering stimulus for perceived brightness at both 1° and 7° extrafoveally, in which the macular pigment is expected to be virtually zero. The reported macular pigment optical density (measured in density units [du]) was the mean of 4 readings in each eye at each time period taken at 1 sitting by 1 technician.

Statistical methods and data analysis

The statistical analyses were computed using PROC MIXED in SAS Version 9.1 for Windows (SAS Institute, Cary, North Carolina). The rate of increase in MPOD was estimated using a longitudinal growth model with random slopes and intercepts. Special contrast statements were included to compare the intercepts and slopes among the treatment groups. Three analysis-of-covariance models were also fit to predict change in MPOD from baseline to 12 months controlling for baseline MPOD density, age, and BMI. A statistically significant difference was defined as $P < 0.05$. No subjects were excluded from the data analysis, and no missing data were imputed. The estimation method permitted the analysis of all available data, even for subjects with missing values. For the longitudinal growth model, a total of 720 observations were possible (90 subjects * 2 eyes * 4 visits), and, of these, 572 observations (79.4%) were not missing and were used in the analyses. For the analysis of covariance models with baseline MPOD and age, 180 observations were possible (90 subjects * 2 eyes), and 125 (69.4%) were not missing and were used in the analysis. For the analysis of covariance model with BMI, 116 of 180 observations (64.4%) were not missing and were used in the analysis.

Results

Subjects

Ninety subjects participated in the study. All but 4 subjects were men, with an average age of 74.7 with a standard deviation of 7.4 years. Seventy-six of 90 patients completed the 12-month trial.

Macular pigment optical density growth rates

The results from a linear growth model for MPOD are represented in Figure 1. The points on this plot are the estimated means at each time point. The predicted values of MPOD for each group at a given month during supplementation are given by the following linear regression equations:

- Lutein: MPOD = 0.22145 + (0.00762 * months)
- Lutein + Antioxidants: MPOD = 0.19110 + (0.00652 * months)
- Placebo: MPOD = 0.23739 + (−0.00092 * months)

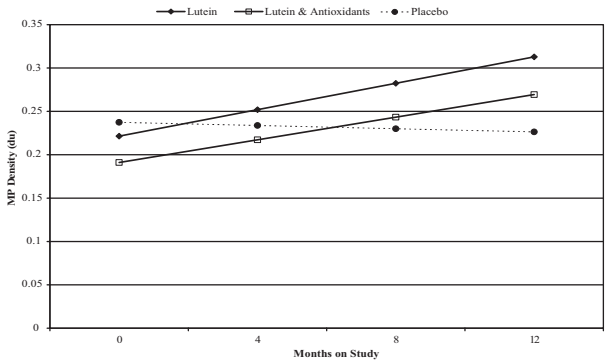

Figure 1 The estimated means of MPOD values for each group at a given month during supplementation; illustrates the greater increase in density values over time for the 2 supplemented groups.

MPOD increased with supplementation and declined slightly without supplementation. Statistically significant differences in intercepts were observed between lutein plus antioxidant and placebo groups ($P < 0.0384$), with lutein plus antioxidant subjects having lower baseline levels of MPOD than placebo subjects. Statistically significant differences in slopes were also present, indicating group differences in MPOD over time. MPOD increased at significantly greater rates in the supplemented groups than in the placebo group ($P = 0.0007$ and $P = 0.0166$ for lutein and lutein plus antioxidant groups, respectively), and these increases in the supplemented groups were significantly different from zero ($P < 0.0001$ and $P = 0.013$, respectively). Interestingly, differences in the rates of increase in the lutein versus lutein plus antioxidant groups were not statistically significant ($P = 0.73$). The slight decrease in MPOD over time observed in the placebo group, which was not statistically different from zero, may be attributable to an insufficient washout period between cessation of existing vitamin supplements and the start of the study.[30] At 8 and 12 months, the average MPOD for lutein was significantly higher than that for placebo ($P = 0.0157$ and $P = 0.0010$, respectively). Although the lutein plus antioxidant group had a similar rate of average increase in macular pigment to the lutein group, the amount of measured MPOD in lutein plus antioxidant group at each time-point was not statistically different from that found in the placebo group, an artifact of the lower baseline value for the lutein plus antioxidant group as seen in Figure 1.

MPOD change from baseline—controlling for baseline MPOD density

The results of the analysis of covariance for change in MPOD from baseline to 12 months, controlling for baseline density, are presented in Figure 2. Estimated mean change in MPOD is shown in increments of 0.05 units for baseline

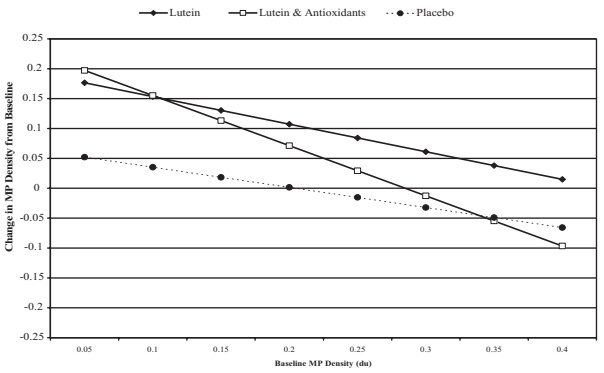

Figure 2 The estimated mean change in MPOD values from baseline to 12 months, controlling for baseline density levels. Lower baseline values were correlated with higher mean increases in MPOD over time for both the lutein and lutein plus antioxidants groups. This is most evident when comparing low MPOD (i.e., 0.05 du) with high MPOD (i.e., 0.40 du).

density. Statistically significant differences in intercepts were observed between supplemented and placebo groups ($P = 0.013$ and $P = 0.026$ for lutein and lutein plus antioxidants, respectively), consistent with the findings presented above that supplements produced greater increases in MPOD over the course of the study than placebo. The relationship between baseline MPOD and change in MPOD from baseline to 12 months was statistically similar across all treatment groups (e.g., equal slopes): lower baseline values of MPOD were associated with greater increases in MPOD from baseline to 12 months. At mean levels of baseline MPOD (0.22 du), statistically significant differences in change from baseline density were observed between lutein and placebo groups (lutein mean, 0.100; placebo mean, −0.004; $P = 0.0003$). The change in MPOD for subjects in the lutein group was statistically significantly different from zero (indicating an increase) up to 0.3 du baseline density and was statistically significantly different from placebo up to 0.4 du baseline density. The change in MPOD for subjects in the lutein plus antioxidant group was not statistically different from zero at mean levels of baseline density, but was statistically different from zero (indicating an increase) up to 0.2 du baseline density. However, change in MPOD for lutein plus antioxidant subjects was not statistically different from placebo at ≥0.2 du baseline density. These results further support the benefit of supplementation for increasing MPOD and suggest a possible small advantage of supplementing with lutein alone. The change in MPOD for subjects in the placebo group differed statistically from zero (indicating a decrease) at 0.35 du baseline MPOD and higher, again consistent with an insufficient washout period between cessation of prestudy supplements and the baseline measures.

Figure 3 further shows the relationship between baseline MPOD and expected change in density from baseline to 12 months in the supplemented groups. Subject eyes in the lower quartile of baseline MPOD in both supplement groups were much more likely to be in the upper quartile for change in density than subject eyes that were not in the lower quartile at baseline. In fact, none of the 43 subject eyes in the lowest quartile of baseline MPOD were in the lowest quartile for change in MPOD. Controlling for the baseline quartile of MPOD, there were no statistically significant differences ($P = 0.5164$) between lutein and lutein plus antioxidant groups in the percentage of eyes in the upper quartile for change in MPOD from baseline (a $2 \times 2 \times 2$ Cochran Mantel-Haenszel test of differences). These findings suggest that Lutein supplementation (with or without antioxidants) may be of most benefit to patients with low MPOD density. However, there was a statistical trend for subject eyes in the lowest quartile of baseline MPOD to have a greater increase to 12 months on the lutein supplement versus the lutein plus antioxidant supplement ($P = 0.077$). The fact that the structural data correlate with beneficial visual function found in the LAST study is implicit; however, actual visual function variables were not evaluated in this study. Further investigation of the interaction between antioxidants and lutein is also warranted.

MPOD change from baseline—controlling for age and BMI

The results of the analysis of covariance model for change in MPOD from baseline to 12 months, controlling for age, are summarized in Table 1 and Table 2. None of the intercepts or slopes were significantly different from zero, indicating that change in MPOD did not vary significantly with age. In addition, there were no differences between the individual group estimates of intercepts and slopes, indicating no group differences in the relationship between age and change in MPOD.

The results of the analysis of covariance model for change in MPOD from baseline to 12 months controlling for BMI, are summarized in Table 3 and Table 4. For the supplemented groups, none of the intercepts or slopes were significantly different from zero, indicating that change in MPOD did not vary significantly with BMI. For the placebo group, the slope was slightly negative and statistically

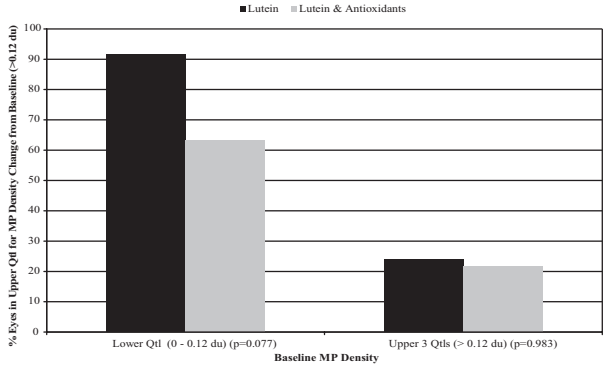

Figure 3 The inverse relationship between baseline MPOD and expected change in density from baseline to 12 months in supplemented groups. The lower baseline density is associated with greater increases in density over time.

Table 1 Analysis of covariance estimates of intercepts and slopes: Change in MPOD controlling for age

Effect	Group	Estimate	Standard error	DF	*t* value	Pr > [t]
Intercepts	Lutein	0.3100	0.2565	41	1.21	0.2338
	Lutein + antioxidants	0.3289	0.3095	39	1.06	0.2944
	Placebo	0.3357	0.2332	39	1.44	0.1579
Slopes	Lutein	−0.0029	0.0034	41	−0.83	0.4087
	Lutein + antioxidants	−0.0034	0.0042	39	−0.80	0.4282
	Placebo	−0.0045	0.0031	39	−1.47	0.1494

Note: DF = degrees of freedom; *t* value = *t*-test statistic; Pr > [t] = *P* value.

significantly different from zero, indicating a tendency for subjects with higher baseline BMI to have greater decreases in MPOD than subjects with lower baseline BMI. However, there were no statistically significant differences in intercepts or slopes between groups. In both analyses of covariance (for age and BMI, respectively), there were statistically significant group differences in mean MPOD change over time in favor of supplementation. These results are consistent with the overall conclusion that supplementation is effective for increasing MPOD.

Discussion

This report is a follow-up to the original LAST report, which looked at only "completers."[29] This report represents a more conservative statistical approach, making use of a set of generalized estimate equations established from a dataset of MPOD from all the eyes on which there were data. As with our original publication, MPOD was found to increase with both lutein supplementation regimens, regardless of whether additional antioxidants, including other carotenoids, were present in the formulation.

Our pilot data and the initial LAST study showed that enhancing the protective macular pigment layer, through diet or supplementation, resulted in improvement in visual function in elderly male patients. That the greatest rate of change in MPOD occurred in those subjects with lowest initial MPOD suggests that the ability to measure MPOD efficiently in the clinical setting is of paramount importance. This observation correctly emphasizes the current need for a reasonably priced commercial instrument capable of providing accurate, reproducible, noninvasive, rapid-capture assessments of the level and distribution of macular pigment. In our hands, measurement of MPOD by a trained individual using heterochromic flicker photometry (HFP), took at least 30 minutes for both eyes for only a single annular eccentricity. We believe the most promising methods are Raman or Spectral. These methods have the potential to record all eccentricities simultaneously and display a topographic MPOD density map. Otherwise, standard HFP information is best acquired in real time and processed and analyzed subsequently off line, after the patient has completed his examination.

An ongoing debate concerns the relationhip between subject age and MPOD. Our finding of no statistically significant correlation with age is in agreement with that of other laboratories, including the largest study to date of macular pigment measurements in a sample population of more than 1698 women age 53 to 86 years.[33] Another reported risk factor for ARMD, weight (BMI), was not found for this population to impede the positive effects of dietary intervention. These obsevations suggest that in future studies one might wish to expand the population database by investigating broader ranges of age and body mass. In addition, because the LAST population was predominantly male, it would be appropriate to assess these influences on macular pigment for a predominantly elderly female population, a population that is reported to be at greater risk of ARMD.[34,35]

Conclusion

For this population drawn from a predominantly male pool of ARMD patients from a veterans hospital, it was learned that intervention by dietary supplementation with lutein,

Table 2 Analysis of covariance estimates of differences in intercepts and slopes: Change in MPOD controlling for age

	Estimates				
Label*	Estimate	Standard error	DF	*t* value	Pr > [t]
int 1 vs int 2	−0.0189	0.4020	76.6	−0.05	0.9626
int 1 vs int 3	−0.0257	0.3467	79.6	−0.07	0.9411
int 2 vs int 3	−0.0068	0.3875	72.5	−0.02	0.9861
slope 1 vs slope 2	0.0005	0.0055	76.5	0.09	0.9324
slope 1 vs slope 3	0.0016	0.0046	79.3	0.35	0.7274
slope 2 vs slope 3	0.0011	0.0052	71.5	0.22	0.8245

Note: DF = degrees of freedom; *t* value = *t*-test statistic; Pr > [t] = *P* value.

* 1, 2, and 3 correspond to lutein, lutein plus antioxidants, and placebo, respectively.

Table 3 Analysis of covariance estimates of intercepts and slopes: Change in MPOD controlling for body mass index

Effect	Group	Estimate	Standard error	DF	*t* value	Pr > [t]
Intercepts	Lutein	0.2573	0.1619	35	1.59	0.1209
	Lutein + antioxidants	0.0966	0.2490	37	0.39	0.7003
	Placebo	0.1952	0.0991	38	1.97	0.0561
Slopes	Lutein	−0.0058	0.0057	35	−1.02	0.3133
	Lutein + antioxidants	−0.0002	0.0082	37	−0.02	0.9804
	Placebo	−0.0080	0.0038	38	−2.12	0.0402

Note: DF = degrees of freedom; *t* value = *t*-test statistic; Pr > [t] = *P* value.

either alone or in combination with other vitamins and minerals and antioxidants, results in continuous increase in macular pigment optical density over the course of an entire year. The selected method of measurement, HFP, was capable of providing an adequately accurate and reproducible method for this assessment. Nonetheless, there is a clear need for a less expensive clinical, not research, instrument capable of more facile and accurate evaluations, with more rapid and more complete capture of the entire macular profile. To achieve these goals, it might be possible to capture the information locally in the clinic but process it centrally, perhaps through an internet site.

The most significant implications from this analysis are that (1) those individuals in greatest need of supplementation, having the lowest levels of measured macular pigment optical density, comprise the population with the greatest increase in MPOD; (2) it appears macular pigment has not yet reached a plateau for the responding groups within a year, so the level of lutein might be expected to continue to increase for some period of time beyond 1 year, and therefore continued higher dose supplementation may be beneficial for the full duration; (3) in this study there was no apparent impediment to increasing macular pigment for individuals with 2 of the purported risk factors for ARMD, BMI and age; and (4) as determined by other workers, bioavailability of lutein may in some part be influenced by the nutrients accompanying it at the time of ingestion.

Table 4 Analysis of covariance estimates of differences in intercepts and slopes: Change in MPOD controlling for body mass index

	Estimates				
Label*	Estimate	Standard error	DF	*t* value	Pr > [t]
int 1 vs int 2	0.1607	0.2970	63	0.54	0.5903
int 1 vs int 3	0.0621	0.1898	58.6	0.33	0.7445
int 2 vs int 3	−0.0986	0.2680	48.5	−0.37	0.7146
slope 1 vs slope 2	−0.0056	0.0100	65	−0.56	0.5773
slope 1 vs slope 3	0.0022	0.0068	61.5	0.32	0.751
slope 2 vs slope 3	0.0078	0.0090	51.8	0.86	0.3945

Note: DF = degrees of freedom; *t* value = *t*-test statistic; Pr > [t] = *P* value.

* 1, 2, and 3 correspond to lutein, lutein plus antioxidants, and placebo, respectively.

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