Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: The LUNA study

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Abstract

Macular pigment (MP), consisting of lutein (L) and zeaxanthin (Z), is believed to protect the retina from photo-oxidative damage. The current study investigates, in terms of MP optical density (MPOD) and serum concentrations of its constituent carotenoids, response to supplemental L and Z, and co-antioxidants. An intervention (I) group, consisting of 108 subjects (mean [±SD] age: 71.5 [±7.1] years), of which 92.6% exhibited features of age-related macular degeneration (AMD), received a daily supplement consisting of 12 mg L and 1 mg Z, both provided as ester 120 mg vitamin C, 17.6 mg vitamin E, 10 mg zinc, 40 μg selenium (Ocuvite Lutein™) for a period of 6 months. MPOD was measured, by 2-wavelength autofluorescence (AF), on five occasions during the period of supplementation, and once again 3 months following discontinuation of the supplement. A control (C) group of 28 subjects (mean [±SD] age: 71.0 [±8.1] years), who received no dietary supplementation or modification, was examined at baseline and once again after a mean of 29.4 (±9.3) weeks. At baseline, mean (±SD) MPOD (at 0.5 eccentricity) was 0.504 (±0.197) and 0.525 (±0.189) in the I and C groups, respectively. There was a statistically significant increase in MPOD (at 0.5 eccentricity) for the I group (0.1 [±0.009]; p < 0.0008), whereas no significant increase was seen in the C group (0.03 [±0.02]; p > 0.05), over the period of supplementation. In order to classify supplemented subjects into quartiles, in terms of MPOD response, we calculated the difference between MPOD (at 0.5 eccentricity) at visit 6 and at baseline (visit 1). Quartile 1 (the “non-responder” quartile) displayed no increase in MPOD (at 0.5 eccentricity), in spite of rises seen in serum concentrations of L and Z. The three “responder” quartiles reached similar final plateaus of MPOD (at 0.5 eccentricity), reflected in final mean (±SEM) values of 0.59 (±0.04) optical density unit (ODU), 0.64 (±0.03) ODU and 0.64 (±0.03) ODU for quartiles 2, 3 and 4, respectively. Subjects with low baseline MPOD were more likely to exhibit a dramatic rise in MPOD, or to exhibit no rise in MPOD, in response to supplements than subjects with medium to high baseline MPOD values. Supplementation with 12 mg L and 1 mg Z, combined with co-antioxidants, resulted in an increase of MPOD at 0.5 eccentricity in a majority of subjects, including those afflicted with AMD. However, there remains a substantial proportion of subjects for whom, in spite of rises in serum concentrations of L and Z in these subjects, MPOD augmentation in response to supplemental L, Z and co-antioxidants could not be detected over the study period, thus indicating that intestinal malabsorption of these carotenoids is not responsible for the lack of a macular response to such supplements. Further, our results suggest that saturable mechanisms play a role in the retinal capture and/or stabilisation of the macular carotenoids.

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keywords: age-related macular degeneration; lutein; macular pigment; Ocuvite Lutein™; zeaxanthin

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1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world (Klaver et al., 1998). Oxidative stress, which refers to tissue damage caused by reactive oxygen intermediates (Beatty et al., 2000), and retinal damage by short-wavelength (blue) light, has been implicated in the aetio-pathogenesis of AMD.

A pigment composed of three carotenoids, lutein (L), zeaxanthin (Z) and meso-zeaxanthin (meso-Z), accumulates at the macula, where it is known as macular pigment (MP) (Beatty et al., 2001; Bone et al., 1992, 1988; Sommerburg et al., 1999). In humans, L and Z cannot be synthesised de novo and are derived entirely from diet (Malinow et al., 1980), whereas meso-Z is largely derived from retinal L (Neuringer et al., 2004). Due to its short-wavelength light screening and antioxidant properties, it is believed that MP may afford protection against the development of AMD.

Several studies have investigated the relationship between dietary and serum concentrations of L (and Z) and MP in humans, and all have demonstrated a positive relationship between these variables (Bone et al., 2000; Brady et al., 1996; Ciulla et al., 2001; Hammond et al., 2002, 1996a; Rock et al., 2002).

Non-dietary variables suspected of acting as determinants of serum concentrations of L (and Z) and/or MP optical density include: age (Beatty et al., 2001; Gellermann et al., 2002); sex (Hammond et al., 1996a); iris colour (Hammond et al., 1996b); race (Rock et al., 2002; Gruber et al., 2004); body fat (Gruber et al., 2004; Hammond et al., 2002; Nolan et al., 2004); ultraviolet light exposure (Rock et al., 2002); tobacco and drinking habits (Gruber et al., 2004; Hammond et al., 1996c; Rock et al., 2002); and genetic background (Hammond et al., 1995; Liew et al., 2005). However, the exclusively dietary origins of L and Z would suggest that dietary intake of these carotenoids represents one of the most important determinants of serum L (and Z) and MP. This notion is consistent with most cross-sectional and supplementation studies (Bone et al., 2000; Broekmans et al., 2002; Hammond et al., 1997).

The LUNA (LUtein Nutrition effects measured by Auto-fluorescence [AF]) study was designed to investigate changes in MPD in, and serum concentrations of L and Z, in response to supplements consisting of the macular carotenoids and co-antioxidants.
twice only, at baseline and at 10–50 weeks (mean ± SD: 29.4 ± 9.3 weeks) following recruitment into the study.

Eleven subjects from the I group and 2 subjects from the C group were excluded from final analysis as they failed to attend last follow up visits. Subject compliance (for the I group) was monitored by asking the subjects to bring in their empty supplement containers when finished.

2.2. Serum carotenoid assessment

Serum concentrations of L and Z were measured at baseline and at the end of the supplementation period for all supplemented subjects. Following collection of blood samples (7.5 ml), serum was separated from blood by centrifugation at 1500 × g for 15 min, and then aliquoted into two amber color tubes and stored at −70 °C until the time of analysis.

Serum carotenoids were analyzed using a method described elsewhere (Dachtler et al., 2001). A 300 μl aliquot of serum was pipetted into a light screening amber micro-centrifuge tube, and then 20 μl of internal standard (9 μg/ml β-apo-8’-carotenal in ethanol), 2 ml hexane-isopropanol 3:2 (v/v) and 1 ml 0.85% NaCl were added. This tube was vortexed for 1 min and then centrifuged at 800 × g for 2 min. The supernatant was then removed and the extraction procedure was repeated with 2 ml of hexane. The combined supernatants were evaporated under a stream of nitrogen (30 °C) and were solubilized in 200 μl of ethanol. Two-hundred microliters of this ethanolic solution was injected on a 116 liquid chromatograph (Beckman Coulter GmbH, Krefeld, Germany) and interfaced with a model 168 diode-array detector.

Samples were eluted isocratically (mobile phase: acetone/water 86:14 (v/v)) at a flow rate of 1 ml/min. The chromatographic run was monitored at an absorbance detection of 450 nm. We used a ProntoSIL 120-3-C30 reversed-phase column (3 μm particle diameter, 250 cm length × 4.6 cm (i.d.), Bischoff Analysentechnik GmbH, Leonberg, Germany) at room temperature.

This assay has been validated against the Standard Reference Material 968c (fat-soluble vitamins, carotenoids, and cholesterol in human serum) of the National Institute of Standards and Technology, Gaithersburg, USA. Also, the interassay coefficients of variation (CoVs) were determined by replicate analyses (n = 55) over a 12-month period of frozen aliquots from a normal and a pathological sample (mean concentrations shown in parentheses): for L, the CoVs were 8.2% (0.1 μg/ml) and 6.5% (0.6 μg/ml) for normal and pathological examples, respectively; and for Z, the CoVs were 9.8% (0.01 μg/ml) and 6.7% (0.06 μg/ml) for the normal and pathological examples, respectively.

L and Z standards were provided by Carl Roth GmbH & Co. KG, (Karlsruhe, Germany) and β-apo-8’-carotenal was purchased from Fluka (Deisenhofen, Germany). All solvents were of high performance liquid chromatography (HPLC)-grade and were purchased from Merck KGaA (Darmstadt, Germany). Peak area was used for quantification purposes.

2.3. Other biochemical analyses

For the I group, total cholesterol, triglycerides, low-density lipoprotein (LDL), high density lipoprotein (HDL), and zinc concentrations, were measured in serum, at baseline and at exit from the study.

Total cholesterol and triglycerides were measured using standard enzymatic methods (CHOD-PAP and GPO-PAP; Roche Diagnostics, Mannheim, Germany). HDL cholesterol was quantified using a direct enzymatic method with PEG-modified enzymes (Roche Diagnostics). LDL-cholesterol was calculated using the Friedewald formula (Friedewald et al., 1972). All measurements were performed on a Boehringer Mannheim/Hitachi automatic analyzer type 747.

Serum concentration of zinc was determined by flame atomic absorption using a Perkin–Elmer 2380 atomic absorption spectrophotometer. These methods were validated by regular analyses of control sera supplied by the national German INSTAND Proficiency Testing Program and the International Quality Assurance Program of the US Center for Disease Control and Prevention.

2.4. Measurement of macular pigment

A Heidelberg Retina Angiograph (Heidelberg Engineering, Heidelberg), modified for fundus autofluorescence (AF), was used for analysis. AF uses the fluorescence of lipofuscin that is present in the retinal pigment epithelium (RPE) cells (Delori, 2004; Delori et al., 2001b; Wüstemeyer et al., 2002).

Lipofuscin (LF) absorbs short-wavelength radiation between 400 and 580 nm, and emits its fluorescence between 500 and 800 nm, with a broad maximum at 620 nm. As MP absorbs blue-light for wavelengths shorter than 550 nm, with a peak absorbance of 460 nm (Bone and Landrum, 1984; Stockman et al., 2000), the absorption of light by MP is related to the quantity of this pigment in the retina and can therefore be used as a measure of MPOD.

The current AF-method (Delori et al., 2001b) uses two excitation wavelengths that are differentially absorbed by MP. This dual-wavelength approach takes account for the non-uniform distribution of lipofuscin in the RPE (Delori, 2001a). This method has been described in more detail elsewhere (Delori et al., 2001b; Trieschmann et al., 2006; Wüstemeyer et al., 2002) and has been used in and suggested for clinical studies before (Jahn et al., 2005; Liew et al., 2005; Wolf, 2006; Wüstemeyer et al., 2003).

During this study, MPOD measurements were performed by the primary investigator (MT) using the same testing device and protocol throughout. Pupil dilation was achieved using dilating drops containing 0.5% tropicamide and 2.5% phenylephrine. Pupil dilation was deemed satisfactory for testing when a pupilary diameter of 6 mm was achieved. Subjects had to fixate with the fellow eye, because fixation in the study eye is inhibited due to bleaching caused by the instrument’s bright light. Where the fellow eye had low vision, thus preventing fixation, the subject was requested to look straight ahead with minimal amount of motion.
The modified Heidelberg Retina Angiograph was aligned to the subject’s eye, first using infrared (IR) light, and then with the 488 nm excitation wavelength. Retinal bleaching is performed to minimize the influences of absorption of incoming light by rhodopsin. Before the first images were taken, exposure with the 488 nm wavelength was allowed for at least 20 s. The area bleached is $20 \times 20^\circ$ and mean laser power at the pupil is 240 $\mu$W. Afterwards, two movies were obtained for each of the 2-excitation wavelengths quickly before recovery. The first movie consisted of 16 images taken at 488 nm and the second consisted of 16 images taken at 514 nm.

The system software aligned the images of these two movies; this was achieved using anatomical details such as the retinal blood vessels. From this, an average image at each wavelength was generated, and processed using image analysis.

The system software is specifically designed to analyse AF images acquired with the 488 and 514 nm excitation wavelength, and to derive the optical density distribution using the 2-wavelength method. The averaged images for the 488 and 514 nm excitations are first aligned to each other, and a map of the relative densities are calculated for each location of the field, $F$, (each pixel) as:

$$D_F(460) = \frac{1}{K(488) - K(514)} \log \frac{I_F(488)}{I_F(514)}$$

where $D_F(460)$ is the optical density of the MP for 460 nm and at the location $F$, $I_F(488)$ and $I_F(514)$ are the intensities of the uniform excitation light at the retina for 488 and 514 nm, respectively. The extinction coefficients, $K(488) = 0.781$ and $K(514) = 0.255$, were used (Stockman et al., 2000) to scale down the MP density as if it was measured by a single wavelength at peak absorption, thus giving a value for $1/[K(488) - K(514)]$ of 1.90. However, the constants supplied by Stockman et al. (2000) was correct for the wavelengths used in the 2-wavelength AF technique, it should be borne in mind that the use of 514 nm instead of a wavelength longer than 540 nm, restricts the low end of the range of MP densities that one can measure reliably. This is because the errors associated with the noise in each image will also be scaled by the same factor 1.9. However, the proximity of both wavelengths has the advantage of making it more likely that the assumptions of the method are not violated (Delori et al., 2001b; Trieschmann et al., 2006). Indeed, it minimizes spectral variation of other fluorophores and absorbers that may interfere with the measurement.

The centre of the fovea is defined by the software as the centre of a Gaussian distribution fitted to the relative density map. Manual intervention is sometimes needed if the distribution is not symmetrical. In these cases, anatomical landmarks such as blood vessels are used to define the centre in the follow up measurements. The software then calculates the mean relative density in concentric annuli (1 pixel wide) to produce the radial distribution of $D_F(460)$ at eccentricities between 0 and 153 pixels (about 6° of visual angle). The profile generally plateaus for eccentricities larger than about 4°, and the reference P (defining the offset for no MP) was selected at an eccentricity of 6°.

For this study, we present the mean MP density averaged along the area of an annulus with retinal eccentricity of 0.5° and width of 1 pixel. We avoided using the peak density (in the centre of the fovea) as used in many previous studies, because of the variability associated with a 1 pixel measurement.

2.5. Reproducibility, test—retest and inter-examiner variability

The reproducibility and inter-examiner/inter-sessional variability of the 2-wavelength AF-method (for the 0.5° measure) has been reported elsewhere (Trieschmann et al., 2006). In brief, reproducibility was expressed in terms of a reliability ratio, which was 0.94, calculated for 20 subjects using five repeated measures within a given session. Test—retest variability and inter-examiner variability has also been assessed, in 24 subjects. The mean difference (±SD) between sessions (measurement 1 [performed by examiner 1] minus measurement 2 [performed by examiner 2]) was 0.002 (±0.042) ODU.

2.6. Statistical analysis

The statistical package SAS for Windows (version 8.2) was used for analysis. Descriptive comparisons were made using the standard $t$-tests for continuous variables and chi-square tests for categorical variables. Pearson correlation coefficients were used to assess the strength of the association between MPOD (at 0.5° eccentricity) and potential determinants. Skewedly distributed factors, such as L, Z or triglycerides, were logarithmically transformed to achieve more normally distributed values. Differences between baseline and follow up measurements within the I and C groups were assessed using paired $t$-tests, while comparisons between groups employed unpaired statistical methods. The impact of confounders and multiple risk factors were evaluated by linear regression analyses. A $p$-value of <0.05 was considered statistically significant.

3. Results

3.1. Baseline findings

The baseline characteristics in the I group are shown in Table 1. The I and C groups were similar in terms of age [mean age (±SD): I group = 71.5 (±7.1); C group = 71.0 (±8.1) years; $p = 0.77$] and sex (male:female ratio = 40:68 [37:63%] for the I group; male:female ratio = 12:16 [43:57%] for the C group; $p = 0.6$). Mean (±SD) MPOD at 0.5° eccentricity for the I group was 0.504 (±0.197) ODU and was statistically comparable to the mean (±SD) MPOD at 0.5° eccentricity for the C group, which was 0.525 (±0.189) ODU ($p = 0.6$).

Of the 108 subjects in the I group, 5 (4.7%) were current cigarette smokers, 63 (58.9%) had a history of hypertension,
11 (10.3%) had diabetes mellitus, 20 (18.7%) had a record of coronary heart disease, and 3 (2.8%) had recovered from a stroke. Also, 33 (30.8%) of subjects in the I group reported that they were currently taking lipid-lowering drugs.

In the I group, mean (±SEM) MPOD at 0.5° eccentricity for females was 0.52 (±0.03) ODU, which was higher than the mean (±SEM) MPOD at 0.5° eccentricity for males [0.48 (±0.02) ODU]; however, this difference was not statistically significant (p = 0.30).

Similarly, there was no significant difference between current cigarette smokers and non-cigarette smokers in terms of MPOD at 0.5° eccentricity [mean (±SEM) MPOD at 0.5° eccentricity of current cigarette smokers: 0.55 (±0.10); non-cigarette smokers: 0.50 (±0.02); p = 0.64]. MPOD levels at 0.5° at baseline did not reveal a significant relationship to age (Fig. 1); older subjects (over 70 years of age) had lower MPOD at 0.5° eccentricity than younger subjects (51–69 years) [mean (±SEM) MPOD at 0.5° eccentricity of older subjects was 0.48 (±0.02) ODU versus 0.54 (±0.03) ODU for younger subjects]. However, this difference did not reach statistical significance (p = 0.17).

There was a positive and significant relationship between serum concentrations of L and Z at baseline and MPOD at 0.5° eccentricity (serum L: r = 0.21, p = 0.03; serum Z: r = 0.21, p = 0.03). Also, baseline measurements of triglycerides were significantly and inversely related to baseline MPOD at 0.5° eccentricity (r = −0.3, p = 0.001), with a positive relationship found between baseline measurements of serum HDL and MPOD at 0.5° eccentricity, which approached statistical significance (r = 0.18, p = 0.06) (Table 2).

### 3.2. Response to supplemental L, Z, and co-antioxidants

#### 3.2.1. MPOD

At the last follow up visit, 97 (89.8%) and 26 (92.9%) were investigated in the I and C groups, respectively. After an initial decline, albeit non-significant (p = 0.74), in the group mean MPOD at 0.5° eccentricity for the I volunteers by visit 2, MPOD at 0.5° eccentricity rose, and continued to rise, until visit 6 (3 months following discontinuation of the supplement) (Fig. 2).

The mean (±SEM) difference in MPOD at 0.5° eccentricity between baseline and visit 6 was 0.1 (±0.009) ODU and 0.03 (±0.02) ODU for the I and C groups, respectively, and this rise was significant for the I group only (p < 0.001). Fig. 2 illustrates mean values for all observations available at each visit. By contrast, and as indicated at the beginning of the paragraphs, only n = 97 subjects of the I group attended visit 6 and therefore the difference between visits 6 and 1 can only be calculated for 97 subjects.

The rise in MPOD at 0.5° eccentricity for the I group was significantly stronger than that seen in the C group (p < 0.0008).

#### 3.2.2. Responders and non-responders in terms of MPOD

In order to classify supplemented subjects into quartiles, in terms of MPOD response, we calculated the difference between MPOD at 0.5° eccentricity at visit 6 (highest values during follow up) and at baseline (visit 1) (Fig. 3). Following elimination of subjects who failed to attend for full follow up visits, 92 (85.2%) were included in the I group for this analysis.

Subjects in the lowest quartile of retinal response (quartile I) had an average (±SEM) MPOD at 0.5° eccentricity of 0.49

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### Table 1

Baseline characteristics for supplemented subjects (intervention group)

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
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<tbody>
<tr>
<td></td>
<td>n = 108 (mean ± SD)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.5 ± 7.1</td>
</tr>
<tr>
<td>Male:female (%)</td>
<td>37.4:62.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 ± 4.0</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>206 ± 38.5</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>58.7 ± 17.9</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mg/dl)</td>
<td>147.3 ± 38.4</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) (non-fasting)</td>
<td>152.5 ± 87.2</td>
</tr>
<tr>
<td>Serum lutein (µg/ml)</td>
<td>0.158 ± 0.143</td>
</tr>
<tr>
<td>Serum zeaxanthin (µg/ml)</td>
<td>0.018 ± 0.013</td>
</tr>
<tr>
<td>MPOD at 0.5°</td>
<td>0.504 ± 0.197</td>
</tr>
</tbody>
</table>

MPOD, macular pigment optical density; HDL, high density lipoprotein.

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### Table 2

Baseline MPOD at 0.5° eccentricity with respect to potentially confounding variables for supplemented subjects (intervention group)

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>−0.05</td>
<td>0.58</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>−0.08</td>
<td>0.40</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>−0.08</td>
<td>0.44</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>0.18</td>
<td>0.06</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) [log transformed]</td>
<td>−0.30</td>
<td>0.001**</td>
</tr>
<tr>
<td>Lutein (µg/ml)</td>
<td>0.21</td>
<td>0.03*</td>
</tr>
<tr>
<td>Lutein (µg/ml) [log transformed]</td>
<td>0.23</td>
<td>0.017*</td>
</tr>
<tr>
<td>Zeaxanthin (µg/ml)</td>
<td>0.21</td>
<td>0.03*</td>
</tr>
<tr>
<td>Zeaxanthin (µg/ml) [Log transformed]</td>
<td>0.18</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**p < 0.01, *p < 0.05.

MPOD, macular pigment optical density.
(±0.049) ODU at baseline, and exhibited no significant increase up to visit 6 [0.47 (±0.051) ODU; p = 0.98]. In the other three quartiles, a significant augmentation of MPOD at 0.5° eccentricity was seen, in spite of differences between baseline values for MPOD at 0.5° eccentricity for these quartiles.

The three responder quartiles reached similar final plateaus of MPOD at 0.5° eccentricity, reflected in final mean (±SEM) values of 0.59 (±0.04) ODU, 0.64 (±0.03) ODU and 0.64 (±0.03) ODU for quartiles II, III and IV, respectively (Fig. 3). When expressed in terms of percentage of baseline MPOD at 0.5° eccentricity, quartile I (the non-responder quartile) actually exhibited a loss of 3.1% from the baseline value, whereas rises of 11.9, 23 and 50.5% were seen in quartiles II, III and IV, respectively.

Of note, quartiles based on MPOD response (at 0.5° eccentricity) to supplements were statistically similar in terms of: age: (p = 0.34); baseline serum L: p = 0.67; baseline serum Z: p = 0.81; body mass index (BMI): p = 0.59; sex p = 0.32; smoking habits: p = 0.37. However, baseline HDL was significantly higher in the quartiles with low or no response [quartile I: baseline HDL (±SEM) = 66.6 (±3.3) mg/dl; quartile II: 61.6 (±2.9) mg/dl; quartile III: 53.9 (±3.1) mg/dl; quartile IV: 53.7 (±3.0) mg/dl; p = 0.013]. The use of anticholesteremic agents was more common in quartiles with a greater MPOD response than in those with a poorer response (45.8% in quartile IV) but this association did not reach statistical significance (p = 0.13).

3.2.3. Changes in MPOD with respect to changes in serum L (and Z)

Following supplementation, mean (±SEM) serum L (and Z) concentrations increased significantly from baseline to visit 5 [baseline: mean serum L (±SEM) = 0.16 (±0.015) µg/ml; visit 5: mean serum L (±SEM) = 0.593 (±0.042); p < 0.001; baseline: mean serum Z (±SEM) = 0.018 (±0.001) µg/ml; visit 5: mean serum Z (±SEM) = 0.022 (±0.001); p = 0.007]. Interestingly, subjects in quartile I who exhibited no detectable increase in MPOD at 0.5° eccentricity, showed a significant increase in serum L and in serum Z that was statistically similar to the increases seen in the three ‘responder’ quartiles (Table 3).

Finally, increases in MPOD at 0.5° eccentricity were not correlated with changes in serum concentrations of Z over the study period (r = 0.08; p = 0.44), nor were they related to changes in serum concentrations of L during this period of supplementation, for the entire intervention group (r = −0.01, p = 0.98).

In quartile I, changes in serum L (and Z) were unrelated to changes in MPOD (serum L: r = −0.0621, p = 0.7682; serum Z: r = −0.0711, p = 0.7355). Similarly, no significant relationship was observed between changes in MPOD and changes in serum concentrations of L or Z for quartiles II and III (p > 0.05, for all). However, in quartile IV the changes in MPOD correlated positively and significantly with changes in serum Z (r = 0.5772, p = 0.0025), but not with changes in serum L (r = 0.3177, p = 0.1217), over the study period.

3.2.4. Baseline HDL and serum concentrations of L and Z

For supplemented subjects, there was a positive and significant relationship between baseline serum concentrations of HDL and baseline serum concentrations of Z (r = 0.2735, p = 0.0044), but not with baseline serum concentrations of L (r = 0.1251, p = 0.1990). Changes in serum concentrations of L and Z, in response to supplements, were unrelated to
baseline serum concentrations of HDL (serum L: \( r = 0.0338, \ p = 0.7435 \); serum Z: \( r = -0.085, \ p = 0.4104 \)).

When these relationships are analyzed for subgroups classified according to macular response to supplemental L, Z and co-antioxidants, we observed that, for the “responder” quartiles II, III and IV, neither baseline concentrations of serum L (or Z), nor changes in serum concentrations of L (or Z) in response to supplements, were related to baseline concentrations of serum HDL (\( p > 0.05 \), for all). However, for the “non-responder” quartile (quartile I), there was a positive and significant relationship between baseline serum concentrations of HDL and baseline serum concentrations of Z (\( r = 0.5705, \ p = 0.0029 \)), whereas there was an inverse relationship, which approached statistical significance, between baseline serum concentrations of HDL and the observed increase in serum concentrations of Z (\( r = -0.3960, \ p = 0.05 \)) over the period of supplementation. In contrast, there was no demonstrable relationship between baseline serum concentrations of HDL and baseline, or changes in, serum concentrations of L (\( p > 0.05 \), for all).

4. Discussion

4.1. Baseline findings

The LUNA (LUtein Nutrition effects measured by Auto-fluorescence) study was designed to report on the effects of dietary supplementation with macular carotenoids, and co-antioxidants, on serum concentrations of L and Z and on MPOD.

Consistent with other studies (Berendschot et al., 2000; Bone et al., 2000; Burke et al., 2005; Curran-Celentano et al., 2001; Hammond et al., 1996a; Johnson et al., 2000), we found a significant and positive relationship between baseline serum concentrations of L (and Z) and MPOD. However, the significant and positive relationship between MPOD at 0.5° eccentricity and serum HDL that we demonstrated is a novel finding, and somewhat consistent with a previous report on a large number of subjects (\( n = 376 \)) that described a weak, albeit non-significant, relationship between MPOD and HDL in men (\( r = 0.07; \ p > 0.05 \)), although, in that study this relationship proved to be negative (but also non-significant) for women (Broekmans et al., 2002). Whilst the hydrocarbon carotenoids are found largely in the LDL fraction of the lipoprotein profile, L and Z are equally distributed between HDL and LDL (Alves-Rodrigues and Shao, 2004; Goulinet and Chapman, 1997; Parker et al., 1999). Therefore, given the positive association between serum L (and Z) concentrations and MPOD that we and others have reported, it is perhaps unsurprising that MPOD displays a significant and positive relationship with serum concentrations of HDL.

In the LUNA study, baseline triglycerides were inversely and significantly related to baseline MPOD at 0.5° eccentricity for males and for females, consistent with the findings of Broekmans et al. (2002) in men only (\( r = -0.18; \ p < 0.05 \)), but those investigators actually report a significant and positive relationship between these variables in women (\( r = 0.17; \ p < 0.05 \)). To our knowledge, no other published study has investigated the relationship between these parameters. Levels of thiobarbituric acid-reactive substances (TBA-RS), which are seen as an indicator of lipid peroxidation, have been shown to be positively correlated with plasma triglycerides and negatively correlated with plasma carotenoids. Indeed, the levels of TBA-RS in plasma are thought to accurately reflect the level of peroxidizable substrate, which are modified by the presence of carotenoids (Franke et al., 1994). Therefore, the positive association between TBA-RS and triglycerides, and the parallel and negative association between these variables and serum concentrations of L and Z, could explain the significant inverse relationship between triglycerides and MPOD that we demonstrated.

4.2. Response to supplemental L, Z, and co-antioxidants

4.2.1. MPOD

The mean increase in MPOD at 0.5° eccentricity in the LUNA study was 0.1 (±0.009) ODU for the I group, which represents a mean increase of 15.9% from baseline values. The percentage changes of MPOD at 0.5° eccentricity in the I group between visits were, after an initial decline at visit 2 (loss of 1.8% between visits 1 and 2), 3.2% from visits 2 to 3, 7.8% from visits 3 to 4, 1.8% from visits 4 to 5 and 4.1% between visits 5 and 6 (interval between visits 1 and 5 was 6 weeks, whereas there was a 3 month interval between visits 5 and 6). These findings are broadly consistent with those reported by previous investigators. Berendschot et al. (2000) \((n = 8, \ 10 \ mg \ L/day, \ fundus \ reflectance \ maps \ at \ 488 \ and \ 514 \ nm \ argon \ laser \ wavelengths) \) demonstrated a mean linear 4-week increase in MPOD of 5.3%. Landrum et al. (1997) \([n = 2, \ 30 \ mg \ L/day, \ heterochromatic \ flicker \ photometry (HFP)] \) reported an increase in MPOD of 4.2% after 4 weeks of supplementation, and also reported that MPOD continued to rise for 40–50 days after discontinuation of supplements, and then reached a plateau. The Lutein Antioxidant Supplementation Trial (LAST) study \((n = 90, \ 10 \ mg \ L/day, \ HFP) \) demonstrated an increase in mean MPOD of approximately
In our study, the increase in MPOD occurred somewhat later than in some of these previous studies, and continued until at least 3 months following discontinuation of supplements. Certainly, our findings are consistent with previous reports of persistently augmented MPOD following discontinuation of supplements or dietary modification, and suggest a very slow turnover of carotenoids within the retina (Bone et al., 2003; Johnson et al., 2000b). In other words, although relatively high doses of L and Z might be required to augment MPOD, it would appear that a lower maintenance dose may suffice to maintain the elevated levels of MP (Bone et al., 2003).

In our control group, only a slight and non-significant increase in MPOD at 0.5° eccentricity was detected. Again, these data are in keeping with previous reports which have shown that MPOD is stable over long periods of time in the absence of dietary modification or supplementation, in spite of fluctuations in serum concentration of L and Z (Jahn et al., 2006; Nolan et al., 2006).

In one quartile of the supplemented subjects in our study we did not detect a rise in MPOD values with the method used, although serum concentrations of L and Z did rise significantly in these subjects, and to a statistically similar level to the responder quartiles. These findings suggest that malabsorption, or impaired transport within serum, of the macular carotenoids is not responsible for the failure of supplements to augment MPOD in “retinal non-responders”, but is consistent with the view that impaired capture and/or stabilisation of L and Z within the retina may contribute to a poor or absent macular response to supplemental L, Z, and co-antioxidants in some subjects.

Quartile I of our study corresponds with Hammond’s et al. (1997) “retinal non-responders”; however, subjects in this group did not differ from the “retinal responder” groups in terms of variables putatively associated with MPOD, such as serum concentrations of L (and Z), BMI, age, sex, and cigarette use, thus suggesting that the benefits of supplemental L and/or Z, if any, could be extended to subjects with some or all of these attributes.

Indeed, the only baseline variable that was significantly associated with response to supplements in terms of MPOD at 0.5° eccentricity was HDL, which was found in significantly higher concentrations in the quartiles with low or no macular response. These findings could, possibly, be explained by the use of anticholesteremic agents. For example, if HDL is important in terms of transport of L and/or Z, reflected in its significant and positive association with MPOD at baseline, then the use of anticholesteremic agents (which increase HDL) might be expected to result in a greater response to supplemental L and/or Z, as demonstrated here (albeit not to a significant level). Indeed, quartile IV (highest responders) had low MPOD at 0.5° eccentricity at baseline, and 45.8% of them were using lipid-lowering therapy on a long term basis, consistent with this hypothesis. However, our finding that highest baseline HDL is associated with poor retinal response (as it was significantly higher in the low or no response quartiles) is not readily explained.

To our knowledge, no study to date has investigated the relationship between MPOD at baseline and response to supplemental L and Z, although one study did comment that the increase in MPOD following supplementation was related to increases in serum concentrations of L and Z, but not with baseline MPOD (Bone et al., 2003). We found that the greatest increase in MPOD at 0.5° eccentricity in supplemented subjects (quartile IV) was seen in those with the lowest baseline MPOD; however, quartile I (“retinal non-responders”) also had baseline values in the lowest third of the range of MPOD at 0.5° eccentricity (mean: 0.48 ODU). In other words, it appears that subjects with low baseline MPOD are more likely to exhibit a dramatic rise in MPOD, or no rise in MPOD, in response to supplemental L and Z. A reasonable explanation to account for these apparently contradictory findings may rest on the dichotomy of the response, which, in turn, reflects the dichotomy of causality of low baseline MPOD in these subjects. In other words, “retinal responders” with low baseline MPOD may simply have deficient dietary intake of L and Z, whereas “retinal non-responders” with low baseline MPOD may have impaired mechanisms which govern the capture and/or stabilisation of the macular carotenoids within the retina.

At visit 6, MPOD values at 0.5° eccentricity for the “responder quartiles” were very similar, in spite of different baseline values. This finding suggests that the mechanisms underlying retinal capture and/or stabilisation of the macular carotenoids are saturable at the level of supplementation used in the LUNA study, consistent with the view that the retinal uptake of L and Z is mediated by specific xanthophyll-binding proteins (XBPs), which have been shown to be saturable (Bernstein et al., 1997; Yemelyanov et al., 2001).

4.2.2. Serum response to supplemental L, Z, and co-antioxidants

In the LUNA study, supplemental L, Z and co-antioxidants resulted in an increase in serum L from 0.16 μg/ml at baseline to 0.593 μg/ml at visit 5, when supplementation was stopped, representing an approximately fourfold increase. Earlier studies have reported fivefold (Berendschot et al., 2000), sixfold (Koh et al., 2004) and 10-fold (Landrum et al., 1997) increases in serum concentrations of L following supplementation with this carotenoid. In our study, the I group failed to exhibit a significant relationship between the observed increase in MPOD and the observed increases in serum concentrations of L and Z.
The lack of augmentation of MPOD at 0.5° eccentricity following supplementation in one quartile of the proband does not appear to be caused by malabsorption, or impaired transport of the carotenoids within serum, because the increases in serum concentrations of L and Z in this quartile were statistically comparable to the increases seen in the responder quartiles. In fact, the “non-responder” quartile exhibited the highest response in terms of the observed increase in serum concentrations of L and Z (Table 3). Although this tendency towards a greater serum response in “retinal non-responders” was insignificant, it is tempting to hypothesise that tissue uptake of supplemented macular carotenoids is poorer in retinal non-responders than in retinal responders, thus resulting in greater circulating concentrations of the macular carotenoids.

Our finding that the highest responder quartile (IV) was unique in terms of its significant and positive relationship with observed increases in serum Z, but not with serum L, is a novel and interesting finding, and may simply reflect the fact that Z is the dominant carotenoid at 0.5° eccentricity. However, it is also possible that the mechanisms governing the capture and stabilisation of circulating macular carotenoids differs for L and Z.

4.2.3. Baseline HDL and serum concentrations of L and Z

The relationships between serum HDL and serum concentrations of the macular carotenoids that we observed, at baseline and following supplementation, are difficult to interpret. Briefly, HDL was positively and significantly related to serum Z (but not L) at baseline. However, subgroup analysis reveals that the “retinal non-responders” probably accounted for this finding, which was not replicated for any of the “retinal responders” quartiles. However, this positive and significant relationship between baseline HDL and baseline serum Z, and the inverse relationship between baseline HDL and changes in serum Z following supplementation (of borderline significance), amongst “retinal non-responders” appear somewhat contradictory, and warrant discussion. It may be that an inability of “retinal non-responders” to capture circulating macular carotenoids in adipose and other tissues, results in an excessive increase in serum concentrations of circulating levels of Z in these subjects, which will, clearly, result in an inverse relationship between the rise in serum Z following supplementation and the unchanging concentration of HDL in these subjects.

Of note, this is the first study to use the two wavelength AF technique of measuring MP in an AMD population. It is important to note, however, that our use of this technique for such patients makes the assumption that Delori et al.’s (2001b) established validity of this two wavelength AF technique for measuring MP in the normal population, by measuring their MP spectral absorbance curves and showing that they matched the L and Z in vitro absorbance curves, holds true for patients with AMD. Indeed, a study designed to test our assumption in this regard is warranted, and should be supported.

5. Conclusion and summary

The Age-Related Eye Disease Study (AREDS) has furnished the ophthalmic community with convincing evidence that antioxidant supplements are beneficial to patients with at least intermediate AMD (AREDS, 2001). Low dietary intake and/or serum levels of L and Z were associated with subtypes of AMD in some, (Gale et al., 2003; Mares-Perlman et al., 2001; Seddon et al., 1994; Snellen et al., 2002), but not all (Cardinal et al., 2005; Dasch et al., 2005; Mares-Perlman et al., 1995; VandenLanegen et al., 1998), studies. Given the anatomic, biochemical and optical properties of MP, there is a biologically plausible rationale to suggest that supplemental L and/or Z may delay, prevent, or modify the course of AMD.

The LUNA study has demonstrated that supplementation with 12 mg L and 1 mg Z, both provided as ester, 120 mg vitamin C, 17.6 mg vitamin E, 10 mg zinc, 40 μg selenium (Ocuvit Lutein™) results in a significant augmentation of MPOD at 0.5° eccentricity in a majority of subjects, including those afflicted with AMD, consistent with the findings of Cullu and Hammond (2004) who found higher MP densities in subjects with established AMD who supplemented L. The importance of these findings rests on the fact that the beneficial effects of these antioxidants, if any, can also be extended to subjects with established disease.

However, we were unable to demonstrate augmentation of MP in a substantial proportion of subjects over the study period, in spite of the expected and observed rises in serum concentrations of L and Z, in response to supplemental L, Z and co-antioxidants. Also, subjects with low baseline MPOD were more likely to exhibit a dramatic rise in MPOD, or to exhibit no rise in MPOD, in response to supplements than subjects with medium to high baseline MPOD values. Finally, our study also suggests that saturable mechanisms may govern the retinal capture and/or stabilisation of L and Z, and may explain, at least in part, some of our findings.

References


