The influence of supplemental lutein and docosahexaenoic acid on serum, lipoproteins, and macular pigmentation1–4

Elizabeth J Johnson, Hae-Yun Chung, Susan M Caldarella, and D Max Snodderly

ABSTRACT

Background: Lutein and docosahexaenoic acid (DHA) may protect against age-related macular degeneration (AMD). Lutein is a component of macular pigment. DHA is in the retina.

Objective: The objective of this 4-mo study was to determine the effects of lutein (12 mg/d) and DHA (800 mg/d) on their serum concentrations and macular pigment optical density (MPOD).

Design: Forty-nine women (60–80 y) were randomly assigned to placebo, DHA, lutein, or lutein + DHA supplement. Serum was analyzed for lutein and DHA (0, 2, and 4 mo). MPOD was determined (0 and 4 mo) at 0.4, 1.5, 3, and 5° temporal retinal eccentricities. Serum was analyzed for lipoproteins (4 mo).

Results: There was no interaction between lutein and DHA supplementations for serum lutein and MPOD. The lutein supplementation × DHA supplementation × month interaction was significant for serum DHA response (P < 0.05). In the lutein group, serum lutein increased from baseline at 2 and 4 mo (P < 0.001), and MPOD increased at 3.0° (P < 0.01). In the DHA group, serum DHA increased at 2 and 4 mo (P < 0.0001), and MPOD increased at 0.4° (P < 0.05). In the lutein + DHA group, serum lutein and DHA increased at 2 and 4 mo (P < 0.01), and MPOD increased at 0.4, 1.5, and 3° (P = 0.06, 0.08, and 0.09, respectively). Differences from placebo in lipoprotein subfractions were greater for the lutein + DHA group (4 mo).

Conclusions: Lutein supplementation increased MPOD eccentrically. DHA resulted in central increases. These results may be due to changes in lipoproteins. Lutein and DHA may aid in prevention of age-related macular degeneration. Am J Clin Nutr 2008;87:1521–9.

INTRODUCTION

Age-related macular degeneration (AMD) is a leading cause of blindness (1). Epidemiologic reports suggest that intake of foods rich in lutein protects against AMD (2). Lutein and its structural isomer, zeaxanthin, selectively accumulate in the retina and are particularly dense in the macular region (3) where they are main components of macular pigment (MP) (2). Lutein functions as an antioxidant (4, 5) and blue light filter and may protect the macula from light-initiated oxidative damage (6). Oxidative stress is high in the eye because of repeated exposure to light and the high rate of oxidative metabolism in the retina (2).

It is believed that cumulative oxidative damage may play an important role in the pathogenesis of AMD. We have shown that it is possible to increase MP density with lutein-rich foods (7). Others have shown that lutein supplements are also effective (8–11).

Lutein is transported in the blood by lipoproteins, with the largest amount transported by HDL and smaller amounts by LDL and VLDL (12–15). Some evidence suggests that DHA might be an especially significant carrier for the retina. For example, in the plasma most (>60%) of apolipoprotein E is associated with the HDL fraction (16). Recent evidence suggests that apolipoprotein E can be synthesized within retinal Müller cells and binds to receptors on ganglion cells (17). Thomson et al (18) recently argued that the subspecies of HDL containing apolipoprotein E supplies lipids and lipid-soluble lutein and zeaxanthin to the retina. Thus, by increasing HDL, retinal lutein levels may be concomitantly increased.

Docosahexaenoic acid (DHA) is a key fatty acid found in the retina (19, 20). Rod outer segments of vertebrate retina have a high DHA content (19, 20). Because photoreceptor outer segments are constantly being renewed, a constant supply of DHA may be required for proper retinal function. Marginal depletion may impair retinal function and influence the development of AMD. The results of epidemiologic studies support the role of DHA in prevention of AMD. In 2 prospective follow-up studies it was reported that DHA intake was inversely related to the risk of AMD (21, 22). Of note is the observation that supplemental DHA increases HDL and HDL subfractions (23–25). Therefore, DHA may, in part, decrease risk of AMD via increased transport of lutein into the macula.

The aim of this study was to determine the individual and combined effects of lutein and DHA on MP density in older women. There are limited data on the effect of lutein intake on MP density in the elderly. The effects of DHA intake on MP have not been explored. Another aim of this study was to examine the

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2 Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the US Department of Agriculture.

3 Supported by USDA grant 1950-5100-065 and by Mead Johnson Nutritional. Martek Biosciences Corporation donated the docosahexaenoic acid supplements.

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effects of DHA (with or without lutein) on serum lipoproteins and lipoprotein subfractions, because of the possibility that changes in lipoproteins might contribute to changes in MP.

SUBJECTS AND METHODS

Subjects

Fifty-seven nonsmoking women (60–80 y) were recruited from the general population for a 4-mo supplementation study. Older women were selected because they tend to have an increased risk of AMD (2). All subjects underwent a screening examination that included a medical history, a physical examination, and a routine blood clinical chemistry profile. Volunteers with any history or biochemical evidence of lactose intolerance, liver, kidney, or pancreatic disease, anemia, active bowel disease or resection, insulin-dependent diabetes, easy bruising or bleeding, bleeding disorders, hyperglycemia, hyperlipoproteinemia, or alcoholism were excluded from the study. Moreover, individuals taking mineral oil or medications suspected of interfering with fat-soluble vitamin absorption were excluded. Other exclusion criteria included current use of steroids or nonsteroidal anti-inflammatory drugs, use of antihistamine drugs, vaccinations within the previous 2 wk, and use of any nutrient supplement for the previous 2 mo or carotenoid supplements for the previous 6 mo. Smoking was not permitted during the course of the study.

All subjects were given a complete ophthalmic examination before and after the supplementation period by a practicing retina specialist who was masked to the experimental treatments received by the subjects. The examination included fundus photography and measurement of intraocular pressure in both eyes. On the basis of both the ophthalmoscopic observations and fundus photography, the examiner recorded the presence or absence of hard and soft drusen and/or retinal pigment epithelium (RPE) mottling. Eye diseases such as macular degeneration, glaucoma, and cataract or cataract surgery were exclusions for participation in the study. This study protocol was approved by the Human Investigative Review Committee of Tufts University, Tufts-New England Medical Center and the Schepens Eye Research Institute. Informed consent was obtained from all subjects.

Study design

Women were randomly assigned to one of 4 groups: placebo (P), DHA (D), lutein (L), or lutein + DHA (LD). Subjects visited the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University on days that supplements were distributed and blood was obtained [0 mo (baseline), 2 mo, and 4 mo]. Subjects were instructed to take the supplement with a nutritional energy drink (237 mL; Mead Johnson Nutritionals, Evansville, IN) but were otherwise asked to not alter their diets. This drink was provided. Supplements were provided in capsule form. The placebo supplements (one each for lutein and DHA) were identical in appearance to the test supplements. Previous studies with comparable doses of lutein and DHA have shown these levels to be safe for human consumption (7, 8, 29–31).

At baseline and 4 mo, subjects visited the Schepens Eye Research Institute (Boston, MA) for testing of MP optical density (MPOD). There were 2 visits at each time point to obtain duplicate measures of MPOD.

Serum analysis for carotenoids and fatty acids

Serum carotenoids were extracted and analyzed by HPLC by using the method described by Yeum et al (32). The fatty acid composition of serum was determined after direct transesterification and gas-liquid chromatography by procedures similar to those described previously (33).

Measurement of MPOD

MPOD in the right eye was measured by heterochromatic flicker photometry with use of a 3-channel Maxwellian view optical system as described by Hammond et al (34). A rotating sectored mirror combined 2 channels to produce the test stimulus, which alternated between a measuring wavelength strongly absorbed by MP and a comparison wavelength outside the absorption band of MP. The third channel provided a 10° background field. Ditric optics interference filters were used to set the wavelength of the background (470 nm) and comparison (550 nm) channels. A grating monochromator (Bausch and Lomb model HD426) was used to determine the wavelength of the measuring (460 nm) stimulus. The stimulus subtended 0.8° of visual angle and was centrally fixated for measurements of peak MP density. This provides a measure at 0.4° retinal eccentricity, the edge of the stimulus (34). A profile of MPOD in the temporal retina was obtained for each subject by having the subject look at a fixation point at 1.5, 3, and 5° temporal retinal eccentricities. The parafoveal reference locus was at 7° temporal retinal eccentricity. The subject’s head position was stabilized with an adjustable bite bar and headrest. MPOD was measured on 2 separate days 1–4 days apart before supplementation and on 2 separate days 1–4 days after 4 mo of supplementation. Mean values of the 2 sessions were used for the analyses. The test-retest correlation at 0.4° eccentricity was 0.9. This is also the correlation reported by Snodderly et al (35) in a group of 54 women aged 54–79 y. The total MPOD was calculated as the area under the curve for the 5 loci at which optical densities were measured (KaleidaGraph version 3.5; Synergy Software, Reading, PA).
Lipoprotein analysis

At study end (4 mo), serum was analyzed for total cholesterol, lipoproteins (VLDL, LDL, and HDL), and lipoprotein subfractions by nuclear magnetic resonance spectroscopy (36). The total volume of serum collected did not allow for analysis of lipoproteins at baseline for most subjects. The nuclear magnetic resonance method uses the characteristic methyl group signals broadcast by lipoprotein subclasses of different sizes as the basis for their quantification. Each measurement includes the concentrations of 5 subclasses of HDL (H1–H5, with larger numbers denoting larger particle size), 3 subclasses of LDL (L1–L3), and 6 subclasses of VLDL (V1–V6). Lipoprotein subclasses were grouped into large, intermediate, and small subclasses. That is, large, intermediate, and small HDLs were (H5 + H4), H3, and (H2 + H1), respectively; large, intermediate, and small LDLs were L3, L2, and L1, respectively; and large, intermediate, and small VLDLs were (V6 + V5), (V4 + V3), and (V2 + V1), respectively.

Statistical analysis

Data are presented either as the mean ± SE or as the change (mean ± SE) from baseline values. Data were log transformed before statistical analysis for normal distribution as needed. Baseline characteristics were compared among the 4 intervention groups by one-way analysis of variance (ANOVA) for continuous variables and a chi-square test for categorical variables. To compare the effects of supplementation on serum lutein and DHA responses with time, a 3-factor repeated-measures ANOVA was performed for serum lutein and DHA, or total MPOD. The baseline serum zeaxanthin concentration was different among groups (P < 0.05), but this difference was not obviously related to other variables.

Approximately one-third to one-half of our subjects had some drusen, and they were distributed fairly evenly among the groups (Table 1). During the study one subject in the LD group had an increase in the confluence of soft drusen. No other subjects had indications of ocular changes.

Compliance with consumption of the nutrient drink and supplements (days supplements consumed/total days of study) was 97%. There was no significant change in body weight within any of the 4 groups at study end. Baseline dietary intakes of the macronutrients, lutein and DHA, were not different among groups (Table 2) and did not change during the study. Dietary intakes of lutein were one-seventh to one-third lower than the study intervention of 12 mg/d, and dietary intakes of DHA were one-sixteenth to one-third lower than the study intervention of 800 mg/d.

Serum lutein

Lutein supplementation significantly increased serum lutein during the supplementation period (lutein × month interaction, P < 0.0001). DHA supplementation had no effect on serum lutein. The lutein × DHA × month interaction for serum lutein was not significant.

In a secondary analysis, we compared the lutein level at single time points with the respective baseline values in each group. Serum concentrations of lutein increased from baseline after 2 and 4 mo of lutein supplementation (with and without DHA) (P < 0.01; see Table 2).

**RESULTS**

**Subject characteristics and diets**

Fifty-seven women were admitted for this study. Eight women dropped out of the study for the following reasons: medication use (n = 1), autoimmune disease (n = 1), unknown (n = 1), aversion to study protocol (n = 4), and significant changes in lifestyle throughout the supplementation interval, including a 7-kg weight loss (n = 1). The primary complaint was aversion to consumption of the high-calorie breakfast drink. Therefore, the total number of women studied was 49. Subject characteristics at baseline are given in Table 1. There were no significant differences in baseline measures of age, body mass index (kg/m²), serum concentrations of lutein and DHA, or total MPOD. The baseline serum zeaxanthin concentration was different among groups (P < 0.05), but this difference was not obviously related to other variables.

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**Table 1**

<table>
<thead>
<tr>
<th>Subject characteristics at baseline</th>
<th>Placebo group</th>
<th>DHA group</th>
<th>Lutein group</th>
<th>Lutein + DHA group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 14)</td>
<td>(n = 11)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>68 ± 1 1 2</td>
<td>68 ± 1</td>
<td>65 ± 2</td>
<td>68 ± 1</td>
</tr>
<tr>
<td>Soft drusen size (μm)</td>
<td>2.94 ± 2.58</td>
<td>0.17 ± 0.11</td>
<td>2.36 ± 1.90</td>
<td>0.57 ± 0.46</td>
</tr>
<tr>
<td>Percentage of subjects with drusen (%)</td>
<td>40</td>
<td>50</td>
<td>33</td>
<td>36</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8 ± 3.1</td>
<td>24.5 ± 1.3</td>
<td>24.6 ± 1.5</td>
<td>27.0 ± 1.3</td>
</tr>
<tr>
<td>Serum lutein (μmol/L)</td>
<td>0.30 ± 0.05</td>
<td>0.37 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>Serum zeaxanthin (μmol/L)</td>
<td>0.051 ± 0.001</td>
<td>0.083 ± 0.002</td>
<td>0.056 ± 0.002</td>
<td>0.070 ± 0.002 1 2</td>
</tr>
<tr>
<td>Serum DHA (nmol/L)</td>
<td>31.7 ± 3.3</td>
<td>26.2 ± 2.1</td>
<td>36.5 ± 2.1</td>
<td>30.1 ± 3.7</td>
</tr>
<tr>
<td>Total MPOD (area)</td>
<td>1.05 ± 0.14</td>
<td>0.92 ± 0.09</td>
<td>1.09 ± 0.10</td>
<td>1.01 ± 0.13</td>
</tr>
<tr>
<td>Peak MPOD (0.4°)</td>
<td>0.54 ± 0.07</td>
<td>0.45 ± 0.03</td>
<td>0.63 ± 0.03</td>
<td>0.51 ± 0.06</td>
</tr>
</tbody>
</table>

1 HDA, docosahexaenoic acid; MPOD, macular pigment optical density. Baseline characteristics were compared using one-way ANOVA followed by Dunn’s test. Chi-square analysis was applied for categorical variable (the presence of drusen). Values in a row with different superscript letters differed significantly, P < 0.05.

2 ± SE (all such values).
Lutein and/or docosahexaenoic acid (DHA) were supplemented in place of the placebo. Lutein supplementation in the placebo group and each experimental group supplemented with lutein, zeaxanthin, and DHA was determined by one-way ANOVA followed by Dunn’s test for multiple comparisons. Dietary intakes of macronutrients, lutein, and DHA were not different among groups at baseline.

Figure 1. This observation retained statistical significance even after application of the Bonferroni correction (corrected limit of statistical significance: \( P < 0.05/\text{number of tested month} = 0.025 \)). No significant changes in serum lutein were observed for the P and D groups. In the L (2 and 4 mo) and LD (2 mo) groups, serum concentrations of zeaxanthin increased from baseline to 0.086 ± 0.002 and 0.085 ± 0.002 \( \mu \text{mol/L} \), respectively (\( P < 0.05 \)). Note that the lutein supplement also contained 0.5 mg zeaxanthin that presumably contributed to this increase in serum levels. However, the zeaxanthin increase in the LD group was not significant after correction for multiple testing.

### Serum DHA

The result of 3-factor repeated-measures ANOVA showed that there was an interaction between lutein × DHA × month (\( P < 0.05 \)). In D and LD groups, the serum concentration of DHA increased significantly from baseline at 2 and 4 mo (determined by paired \( t \) test, \( P < 0.0001 \)) and remained so after Bonferroni correction (corrected limit of statistical significance: \( P < 0.05/\text{number of tested month} = 0.025 \)). However, as shown in Figure 2, the addition of lutein to DHA supplementation lowered the serum DHA response. For both the D and LD groups, the greatest increases in serum concentrations of DHA occurred in the first 2 mo of the intervention.

#### Macular pigment optical density

### Changes in the spatial distribution of MP

Two-way ANOVA analysis showed that lutein and DHA had no combined effect on MP increases at all loci. The combination of lutein and DHA supplementations also had no interaction with

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**TABLE 2** Dietary intakes at baseline

<table>
<thead>
<tr>
<th></th>
<th>Placebo group ((n = 10))</th>
<th>DHA group ((n = 14))</th>
<th>Lutein group ((n = 11))</th>
<th>Lutein + DHA group ((n = 14))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (J/d)</td>
<td>6121 ± 507</td>
<td>7221 ± 582</td>
<td>7500 ± 1327</td>
<td>6706 ± 878</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>52 ± 5</td>
<td>66 ± 5</td>
<td>70 ± 12</td>
<td>67 ± 9</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>69 ± 7</td>
<td>68 ± 7</td>
<td>84 ± 15</td>
<td>70 ± 13</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>164 ± 15</td>
<td>192 ± 19</td>
<td>196 ± 39</td>
<td>182 ± 24</td>
</tr>
<tr>
<td>Lutein/zeaxanthin (mg/d)</td>
<td>2.3 ± 0.6</td>
<td>2.5 ± 0.3</td>
<td>3.5 ± 0.9</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>DHA (mg/d)</td>
<td>92 ± 32</td>
<td>143 ± 29</td>
<td>126 ± 35</td>
<td>157 ± 36</td>
</tr>
</tbody>
</table>

\( ^{1} \) All values are \( x \pm SE \). DHA, docosahexaenoic acid. Differences in baseline dietary intakes of the macronutrients, lutein, zeaxanthin and DHA were determined by one-way ANOVA followed by Dunn’s test for multiple comparisons. Dietary intakes of macronutrients, lutein, and DHA were not different among groups at baseline.

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**FIGURE 1.** Mean ± SE change from baseline in serum lutein concentrations in the placebo group and each experimental group supplemented with lutein (12 mg) and/or docosahexaenoic acid (DHA; 800 mg). Lutein supplementation × DHA supplementation × month interaction, \( P = 0.3588 \) (3-factor repeated-measures ANOVA). Lutein supplementation × month interaction, \( P < 0.001 \). DHA supplementation × month interaction, \( P = 0.7302 \). *Significantly different from baseline \((P < 0.01)\) within a group based on a paired \( t \) test.

**FIGURE 2.** Mean ± SE change from baseline in serum DHA concentrations in the placebo group and each experimental group supplemented with lutein, docosahexaenoic acid (DHA), or both. Lutein supplementation × DHA supplementation × month interaction, \( P < 0.05 \) (3-factor repeated-measures ANOVA). *Significantly different from baseline \((P < 0.05)\) within a group based on a paired \( t \) test. **Significantly different from baseline \((P < 0.0001)\) within a group based on a paired \( t \) test.
the MP locus as determined by 3-factor repeated-measures ANOVA. Lutein supplementation increased MP at 1.5 and 3 loci ($P < 0.05$). DHA supplementation did not show a significant effect on the MP increase. However, a secondary statistical analysis showed somewhat different results. The spatial pattern of increases in MPOD varied with supplementation type. In the L group, there were increases in MPOD at each retinal locus, and the increase was statistically significant at the 3° locus ($P < 0.01$; Figure 3). In the D group, there was an increase in the most central locus (0.4°; $P < 0.05$) with no significant changes in the other retinal loci (Figure 3). However, the latter observation did not retain its statistical significance after correction for multiple testing (corrected limit of statistical significance: $P_* = 0.05/\text{number of tested locus} = 0.0125$). It should be noted that because the variables tested in this study were dependent on one another to some extent, Bonferroni correction could be highly conservative and miss real differences (38). In the combination group (LD), MPOD tended to increase at the 0.4, 1.5, and 3° loci ($P = 0.06, 0.08$, and 0.09, respectively; Figure 3).

**Total macular pigment optical density**

There was no significant interaction between lutein and DHA supplementations on the total increase in MPOD. Lutein supplementation increased total MPOD ($P < 0.05$), whereas DHA had no effect. Interestingly, DHA tended to increase total MPOD in the subgroup of MP responders ($P = 0.0864$; see below).

Total MPOD significantly increased after lutein supplementation for 4 mo in the L group ($P < 0.05$; Figure 4). MPD also increased in the LD group although not significantly ($P = 0.07$). No significant changes from baseline in MPOD were observed for the P and D groups (0.071 ± 0.111 and 0.037 ± 0.068, respectively; Figure 4).

**MP responders and nonresponders**

Eight of 11 subjects in the L group and 11 of 14 subjects in the LD group had a MPOD response to lutein supplementation (>5% increase of total MPOD from baseline). The magnitude of the response was not related to BMI, age, or baseline concentrations of serum lutein, but there was a weak negative relation between soft drusen size in the eye at baseline and the increase in MPOD ($r = -0.367, P < 0.05$). Neither hard drusen size nor the presence of RPE mottling was related to the increase in MPOD. However, when we repeated statistical analysis in subjects without soft drusen, we did not observe stronger associations between supplementation and the MPOD response. This lack may be due to the smaller sample size in subgroup analysis.

**Serum lipoproteins**

The serum total cholesterol and lipoprotein concentrations after 4 mo of lutein and/or DHA supplementation or placebo are shown in Table 3. Lutein and DHA supplementations were not associated with differences in total serum cholesterol and lipoproteins among the groups.

When the individual lipoprotein subfractions were examined, we did not detect any combined effect of lutein and DHA supplementation. However, serum concentrations of lipoprotein subfractions were somewhat different in groups supplemented with DHA, and the LD group tended to have the largest differences from the P group.

For the HDL subclasses, the large HDL subclass had the greatest concentrations in all study groups ($P < 0.05$) and DHA supplementation tended to increase H5 ($P = 0.0871$; Figure 5). H3 was lower in groups fed DHA ($P < 0.05$).

For the LDL subclasses, the large LDL subclass had the highest concentrations in all study groups (Figure 5). DHA supplementation was significantly associated with lower L2 ($P < 0.05$). Interestingly the size of HDL and LDL particles (nm diameter) were larger in DHA-supplemented groups ($P < 0.05$, respectively). For the VLDL subclasses, the intermediate VLDL subclass had the greatest concentrations in all study groups. DHA supplementation lowered large ($P < 0.01$) and intermediate VLDL.

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**FIGURE 3.** Mean ± SE changes in spatial distributions of macular pigment optical density (MPOD; 4 mo change from baseline) in placebo and experimental groups supplemented with lutein, docosahexanoic acid (DHA), or both. Lutein supplementation × DHA supplementation × LOCUS interaction, $P = 0.3668$ (3-factor repeated-measures ANOVA). Lutein supplementation × DHA supplementation interaction, $P = 0.9940$, $P = 0.7282$, $P = 0.4182$, and $P = 0.2180$ at 0.4, 1.5, 3, and 5°, respectively (2-way ANOVA). Lutein supplementation, $P < 0.05$ and $P < 0.05$ at 1.5 and 3°, respectively (2-way ANOVA). *Significantly different from baseline ($P < 0.05$) based on a paired $t$ test. This difference became nonsignificant after Bonferroni correction. **Significantly different from baseline ($P < 0.01$) based on a paired $t$ test. #Different from baseline ($P < 0.1$) based on a paired $t$ test.
subfractions although the effect was not significant for intermediate subfractions \( (P = 0.0934; \text{Figure 5}) \).

**DISCUSSION**

An evaluation of the effects of supplemental lutein and DHA is timely in light of the Age-Related Eye Disease Study 2 (AREDS2) (39). AREDS2 is designed to extend the findings of AREDS, which demonstrated that oral supplementation with high-dose antioxidant vitamins and minerals (vitamins C and E, \( \beta \)-carotene, zinc, and copper) reduced the risk of progression to advanced AMD by 25\% \( (40) \). AREDS2 will test whether lutein and zeaxanthin as well as eicosapentaenoic acid and DHA also have protective effects.

The results of our study demonstrate that supplementation with daily oral doses of lutein (12 mg/d for 4 mo) is effective in increasing circulating levels of lutein as well as MPOD in older women. This increase has been demonstrated in younger subjects by our research group \( (7, 41) \) and by others \( (8, 11, 42–44) \). To date, no studies have examined these effects in older women, who may have a particular risk for advanced AMD \( (2) \). About one-fifth of subjects in the L and LD groups did not have increases in MPOD \(<5\% \) increase from baseline at 4 mo) with lutein supplementation. This result is similar to that of an earlier study we conducted in men and women using spinach as the

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Concentrations of serum total cholesterol and lipoproteins after 4 mo of supplementation with lutein, DHA, or both*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol ( \text{mg cholesterol/dL} )</td>
</tr>
<tr>
<td>Placebo ( (n=10) )</td>
<td>194 ± 8</td>
</tr>
<tr>
<td>DHA ( (n=14) )</td>
<td>209 ± 5</td>
</tr>
<tr>
<td>Lutein ( (n=11) )</td>
<td>199 ± 8</td>
</tr>
<tr>
<td>Lutein + DHA ( (n=14) )</td>
<td>201 ± 8</td>
</tr>
</tbody>
</table>

* All values are \( \bar{x} \pm \text{SE} \). DHA, docosahexaenoic acid. There was no interaction between lutein and DHA supplementations on serum concentrations of total cholesterol and lipoproteins at 4 mo determined by 2-way ANOVA. Lutein and DHA supplementation had no effect on serum total cholesterol or lipoproteins.
source of lutein (7). A MPOD response was not related to BMI, age, dietary intake of lutein, or baseline serum and macular concentrations of lutein, but there was a weak negative relation between soft drusen size in the macula of the right eye (tested eye) at baseline and the increase in MPOD ($r = -0.367$, $P < 0.05$). Further study is needed to establish the factors that determine an MPOD response to supplementation.

During the study one subject in the LD group had an increase in the confluence of soft drusen. No other subjects had indications of ocular changes. In this age group it is not surprising that an occasional subject might show changes with time that are unrelated to the supplementation, but in future studies with larger numbers of subjects the possibility of deleterious as well as beneficial retinal changes should be evaluated.

Analysis of the relation between lutein supplementation and DHA supplementation showed that there was an interaction between these 2 treatments. These results suggest that lutein lowers the DHA response. However, further study is required to confirm this suggestion.

Another new finding in this study is the unexpected effect of DHA supplementation on serum lutein and MPOD. An effect of DHA on macular pigmentation may have implications for prevention of AMD. In the prospective follow-up study of participants in the Nurses’ Health Study and the Health Professionals Follow-Up Study, it was reported that DHA intake was inversely related to risk of AMD (21). These investigators examined the associations of intake of fish (a major source of DHA) with risk of AMD and reported that participants who ate fish >4 times/wk had a lower risk of AMD than did those who consumed fish <3 times/mo (relative risk: 0.65, 95% CI: 0.46, 0.91). Similar results have been reported by others (22, 45). We measured increases from baseline in serum lutein at 2 mo for the LD group that were significant after correction for multiple testing, whereas change at this locus was negligible in the L group ($P = 0.4794$). Interestingly, the effects of lutein + DHA supplementation on the MPOD appeared to be a combination of the individual effects of lutein and DHA supplementation although this interaction was not statistically significant when determined by 2-way ANOVA. The low levels of statistical significance are due in part to the variability in MPOD response to supplementation, as we have reported previously (7, 41). An alternative interpretation is that MPOD levels increased at all eccentricities, including the 7° reference point, which would not show up in the difference between the reference locus and the other loci. However, there were no significant changes in the 7° reference point measures in any of the 4 groups, so this is an unlikely explanation.

The mechanism by which DHA increases MPOD may be its effects on the transport and uptake of lutein into the macula. Although there were no significant differences in the total serum lipoproteins among the groups, there were differences in the lipoprotein subclasses. Evaluation of serum lipoprotein subclasses has been suggested as a useful tool in assessing the risk of cardiovascular disease (46). The effects of DHA (fish oil) on these subclasses are toward an improved lipoprotein profile (47). Given that cardiovascular disease and AMD share many of the same risk factors (48, 49), these changes may help to reduce risk of AMD.

In our study, we measured the effect of lutein and/or DHA supplementation on lipoprotein subclasses at study end. DHA supplementation was associated with differences in lipoprotein subclasses. In most cases, the LD group had the greatest differences from the P group, these differences being toward a less atherogenic pattern, that is, larger HDL and LDL (50). However, most differences did not reach significance. Given that these differences could affect transport and delivery of lipids to tissue, eg, xanthophylls to the macula, it is possible that the increases in MPOD with DHA supplementation were due to a change in lipoprotein profile that promotes the uptake of xanthophyll into the retina. These effects appear to occur centrally in the macula given that the increases in both the D and LD groups did not occur for the P and L groups. The reason for the preference toward an accumulation that is central, rather than eccentric, is not known, but it could be enhanced uptake or deposition by cone photoreceptors. Given that zeaxanthin rather than lutein accumulates preferentially in the central macula (51) but that a significant portion of this accumulation originates from the conversion of lutein to meso-zeaxanthin (52), perhaps the DHA affects accumulation of this zeaxanthin and/or its conversion from lutein.

DHA may also affect macular pigmentation through interaction with lutein and the interphotoreceptor retinoid-binding protein, much like the interaction of DHA with 11-cis retinal and interphotoreceptor retinoid-binding protein (53). The DHA content in the RPE is much lower than that of the photoreceptors. Chen et al (53) proposed that the outer segment–RPE gradient of DHA drives the transfer of regenerated 11-cis retinal from the RPE back to the outer segments. This occurs by DHA inducing a rapid and specific release of 11-cis retinal from one of the interphotoreceptor retinoid-binding protein retinoid-binding sites. In a similar manner, DHA may be involved in the transport of lutein.

As a cautionary note, we recognize that the supplement group who received both lutein and DHA did not have greater increases of MPOD. Much remains to be learned about the interactions underlying tissue accumulation of the macular xanthophylls. The interactions of the various factors, including effects of DHA on lipoprotein subclasses, warrant investigation in a longitudinal manner.

In conclusion, supplementation of these elderly women with lutein alone increased MPOD eccentrically, whereas DHA supplementation alone resulted in central increases in MPOD. The combination of supplements had a combined effect on the MPOD spatial profile. DHA facilitated accumulation of lutein in the blood and macula. Some of these effects may have occurred through alterations of the lipoprotein profile by DHA.

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