**Effect of dietary lutein and zeaxanthin on plasma carotenoids and their transport in lipoproteins in age-related macular degeneration**

Weig Wang, Sonja L Connor, Elizabeth J Johnson, Michael L Klein, Shannon Hughes, and William E Connor

**ABSTRACT**

*Background:* Low dietary intakes and low plasma concentrations of lutein and zeaxanthin are associated with an increased risk of age-related macular degeneration (AMD). No studies have challenged AMD patients with a diet high in lutein and zeaxanthin.

*Objective:* The objective was to examine the effect of diets low or high in lutein and zeaxanthin on plasma carotenoids and their transport in AMD patients.

*Design:* Seven AMD patients and 5 control subjects were fed a low-lutein, low-zeaxanthin diet (1.1 mg/d) for 2 wk, which was followed by a high-lutein, high-zeaxanthin diet (11 mg/d) for 4 wk. Ten subjects continued the diet for 8 wk. Plasma and lipoprotein carotenoids were measured by HPLC.

*Results:* The high-lutein, high-zeaxanthin diet resulted in 2- to 3-fold increases in plasma concentrations of lutein and zeaxanthin and other carotenoids, except lycopene, in the AMD patients and the control subjects. With this diet, 52% of the lutein and 44% of the zeaxanthin were transported by HDL; 22% of lutein and zeaxanthin was transported by LDL. Only 20–25% of α-carotene, β-carotene, and lycopene was transported by HDL; 50–57% was transported by LDL.

*Conclusions:* The AMD patients and control subjects responded similarly to a diet high in lutein and zeaxanthin; plasma carotenoid concentrations increased greatly in both groups, and the transport of carotenoids by lipoproteins was not significantly different between the groups. This finding suggests that abnormalities in the metabolism of lutein and zeaxanthin in AMD may reside in the uptake of lutein and zeaxanthin from the plasma and transport into the retina.

**KEY WORDS** HDL cholesterol, LDL cholesterol, VLDL cholesterol, retina, age, macular degeneration

**INTRODUCTION**

Age-related macular degeneration (AMD) is the leading cause of blindness in persons aged ≥65 y and affects >6 million people in the United States (1, 2). Of the many nutritional factors associated with the risk of developing advanced AMD is a reduced dietary intake of the carotenoids lutein and zeaxanthin (3, 4). Lutein and zeaxanthin intakes of ≈6 mg/d or greater have been related to a decreased risk of AMD (3). The typical US diet contains ≈1–3 mg/d of lutein and zeaxanthin combined (3–6).

The dietary intake of lutein and zeaxanthin has been shown to influence the plasma concentrations of these carotenoids and their content in the macula in humans without AMD and in nonhuman primates (7–16). The macula of monkeys given a lutein- and zeaxanthin-free diet had more oxidative damage than did the macula of monkeys fed a diet containing these carotenoids (10). Macular pigments were not detected in primates who were fed lutein- and zeaxanthin-deficient diets. However, feeding lutein and zeaxanthin to the deficient monkeys led to some restoration of macular pigments (11, 14, 15).

Healthy human subjects given a lutein supplement, either in the form of lutein tablets or of supplemented foods, resulted in significant increases in plasma lutein concentrations (7–9, 12, 13, 16). Macular pigment density was significantly correlated with plasma concentrations and dietary intakes of lutein and zeaxanthin, although the response varied among subjects (8, 9). Donor eyes of AMD patients had a significantly lower content of lutein and zeaxanthin than did donor eyes with normal retinas (17).

Carotenoids are lipophilic plant pigments that are transported by lipoproteins in the plasma of humans and animals (18). LDL is the primary transporter of carotenes; HDL is the primary transporter of xanthophylls such as lutein and zeaxanthin. The lipoprotein transport of carotenoids in AMD patients has not been studied extensively. A recent report showed that fasting carotenoid concentrations and their distribution in lipoproteins were not significantly different between AMD patients and control subjects (19).

The objective of this study was to determine whether differences exist between AMD patients and control subjects in their plasma carotenoid responses to a diet high in lutein and zeaxanthin and in their transport of lutein and zeaxanthin. We challenged AMD patients and control subjects with a diet of whole foods high in lutein and zeaxanthin after a control period of a diet low in lutein and zeaxanthin. No previous studies of the effects of

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high-lutein, high-zeaxanthin diets on plasma carotenoid responses have been conducted in patients with AMD. Only a few studies of such effects have been conducted in healthy subjects, who showed a response to both supplements and to a diet high in lutein and zeaxanthin.

SUBJECTS AND METHODS

Subjects

Seven patients with advanced AMD with greatly reduced vision in one eye and good visual acuity (20/30 or better) in the fellow eye were recruited from the Casey Eye Institute, Oregon Health and Science University (OHSU). Five control subjects of similar ages without signs of AMD in either eye were either spouses of the AMD patients or were recruited from other OHSU clinics (Table 1). Macular status was assessed by using the Age-Related Eye Disease Study System (AREDS) to classify AMD: category 1, no macular abnormality in either eye; category 2, mild or borderline macular abnormality or AMD features; category 3, many small or few intermediate drusen or pigment abnormalities; and category 4, advanced AMD in at least one eye (20). All subjects were non-Hispanic whites. Both the AMD patients and the control subjects were elderly and had similar disease and biochemical characteristics other than AMD diagnosis. The study protocol was approved by the OHSU Institutional Review Board, and the subjects provided informed consent before the study began.

Study design

The overall design of the study is shown in Figure 1. Initially, the number of subjects was based on published data indicating that the 2 groups would be equal and our expectation was that the change in the AMD group would be only 50% of that of the control group. Power was estimated at 74% with a sample size of 18 subjects per group (total of 36 subjects). An interim analysis was carried out in 12 subjects, which indicated that, to achieve significance ($P < 0.05$), a difference in the changes between the AMD and control groups in plasma lutein with the 2 diets would have to be 8.1 $\mu$g/dL on the basis of the observed SDs of the changes in the 12 subjects. This implies that the difference in changes for the remaining 24 subjects would need to exceed 10.0 $\mu$g/dL to attain a power of 74%, which is highly unlikely given

![FIGURE 1. Study design. GCRC, General Clinical Research Center, Oregon Health and Science University.](https://academic.oup.com/ajcn/article-abstract/85/3/762/4633032)
that the observed difference in the first one-third of the study was only 2.5 μg/dL. This, coupled with the considerable amount of time and effort involved on the part of the subjects to participate in the 12-wk highly controlled feeding study, led to the decision to end the study with 12 subjects.

**Diets**

There were 2 dietary phases: a typical low-lutein, low-zeaxanthin diet (2 wk) and a high-lutein, high-zeaxanthin diet (12 wk). All food was prepared for the subjects for the first 6 wk of the study. The subjects were instructed to prepare a diet high in lutein and zeaxanthin for the last 8 wk. All diets met energy and other nutritional needs.

The diet low in lutein and zeaxanthin (∼1100 μg/d, or 1.1 mg/d) and other carotenoids was fed for 2 wk. The low-lutein, low-zeaxanthin diet was similar to a typical US diet with regard to carotenoids, protein, fat, and carbohydrate (4, 21). The diet high in lutein and zeaxanthin (∼11 000 μg/d, or 11 mg/d—10 times the amount in the low diet) was fed for 4 wk. Six AMD patients and 4 control subjects continued the high-lutein, high-zeaxanthin diet for an additional 8 wk. The subjects prepared the diet at home with considerable guidance and monitoring by registered dietitians. The subjects kept daily records of the amounts of foods that they consumed.

The diet high in lutein and zeaxanthin had a high content of fruit and vegetables and was lower in fat and higher in complex carbohydrate and fiber than was the diet low in lutein and zeaxanthin. Examples of one day of foods for each diet are given in Table 2. Because of the concern for a spillover effect on plasma and lipoprotein carotenoid concentrations from the high-carotenoid diet, the diets were not randomized; the diet low in lutein and zeaxanthin was fed first. A multivitamin containing 250 μg lutein was provided daily to all subjects, because this was the amount typically consumed by the subjects before they entered the study.

For the first 2 wk of the diet low in lutein and zeaxanthin and for the following 4 wk of the diet high in lutein and zeaxanthin, all meals were prepared by the General Clinical Research Center (GCRC), Bionutrition Department, OHSU. The subjects came to the GCRC 3 times/wk to be weighed and to eat breakfast. Foods were then packaged for the remainder of the day and the following day. On Fridays, foods were packaged for the weekend. All uneaten foods were returned to the GCRC and were weighed. Daily nutrient intakes were computed for each subject on the basis of the foods consumed. Nutrient calculations were performed by using the Nutrition Data System for Research (NDS-R, software version 4.02, Food and Nutrient Database 30, released November 1999; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) (22).

### Plasma lipids and lipoproteins

Fasting plasma samples were drawn weekly for 6 wk when food was provided and twice at 4-wk intervals during the home meal preparation. Because a 30-mg lutein, 50-g fat tolerance test was done at the beginning of the last week of each diet period, to minimize any effect of the test on carotenoid distributions in lipoproteins, data were used from the end of week 1 of the diet low in lutein and zeaxanthin and from the end of week 3 of the diet high in lutein and zeaxanthin. The data used in Figure 2 were from the end of weeks 1 and 2 of the typical low-lutein diet and weeks 1, 2, 3, 4, 8, and 12 of the high-lutein diet.

Blood was collected into tubes containing EDTA. Plasma was immediately centrifuged in polypropylene tubes by sequential
ultracentrifugation to separate lipoprotein fractions in a Beckman 50.4 Ti rotor with the use of an L8-80 M Ultracentrifuge (Beckman Coulter, Fullerton, CA) at 5 °C. Individual fractions were separated by centrifugation under the following conditions: chylomicrons at 17,000 rpm (2.9 × 10^4 g), 5 °C for 30 min, density = 1.006; VLDL at 50,000 rpm (2.7 × 10^5 g), 5 °C for 9 h and 10 min, density = 1.006; LDL at 50,000 rpm (3.1 × 10^5 g), 5 °C for 9 h and 25 min, density = 1.063. HDL was the remaining bottom portion after the LDL spin. Lipoprotein cholesterol and triglycerides were analyzed by using a Hitachi 704 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). The laboratory procedures for the plasma lipid and lipoprotein separations and determinations were in compliance with the surveillance programs of the Centers for Disease Control and Prevention in Atlanta, GA (23).

Plasma and lipoprotein lutein, zeaxanthin, and other carotenoids

Plasma and lipoprotein carotenoids were measured as described previously (13). Briefly, plasma and lipoprotein fraction samples were protected from light and stored at −80 °C until analyzed. Echinoderm in ethanol was added as an internal standard to 200 μL serum or lipoprotein fraction sample and 0.5 mL of 0.9% saline. The mixture was extracted by using 2 mL chloroform:methanol (2:1, by vol). The mixture was mixed by vortex and centrifuged and chloroform layer was removed. A second extraction was done on the mixture with the use of 3 mL hexane, which was followed by mixing by vortex and centrifugation. The hexane layer was combined with the first extraction and evaporated to dryness under nitrogen. The residue from serum was redissolved in 150 μL ethanol, mixed by vortex, and sonicated for 30 s. A 50-μL aliquot was used for the HPLC analysis (16).

A C30 carotenoid column was used for the carotenoid measurements. Carotenoids were quantified by determining peak areas in the HPLC chromatograms and calibrated against known amounts of standards. Carotenoid standards were provided by Roche Vitamins, Ltd (now DSM Nutritional Products, Parsippany, NJ). The percentage of carotenoids in each lipoprotein fraction was calculated as the absolute concentration of each fraction divided by the sum of the concentrations of all fractions. In our ultracentrifugation procedures, all the lipoprotein fractions were standardized to the same final volumes. The mass sum of each carotenoid found in the VLDL, LDL, and HDL fractions divided by the amount found in the plasma used for isolation of lipoproteins times 100 represents the recovery of each analyte. For all carotenoids, the mean recovery ranged from 80% to 100% (percentage of the sum of the analyte from various lipoprotein fractions to total plasma analyte concentration).

### Statistical analyses

Results are expressed as means (±SEM). Data were assessed by using 2-factor repeated-measures analysis of variance (SPSS, version 14.0). If the interaction (group × diet) was not significant, no subgroup analysis was performed (the effect due to time was made across groups). If a significant interaction was found, a subgroup analysis was performed with a Bonferroni correction. Differences were considered statistically significant at P < 0.05.

### RESULTS

**Diets**

Nutrient intakes were computed from actual dietary intakes for the periods in which all food was provided. Because no significant differences in the nutrient intakes during each dietary period were found between the AMD and control groups, the data were combined (Table 3). The subjects consumed ~10 times more lutein and zeaxanthin with the diet high in lutein and zeaxanthin...
than with the diet low in lutein and zeaxanthin. Except for lycopene, the subjects also consumed 3–5 times the amount of all other carotenoids because of their presence in the same foods that are high in lutein and zeaxanthin. Lycopene is found primarily in tomato products, foods that were not increased in the diet high in lutein and zeaxanthin. With the diet high in lutein and zeaxanthin, the subjects also consumed fewer calories; less saturated fat, total fat, and cholesterol; and more carbohydrate, fiber, and vitamin C. Retinol and vitamin E intakes were not significantly different between groups.

### Plasma lutein and zeaxanthin concentrations

Plasma lutein was greater in both the AMD and control groups during the diet high in lutein and zeaxanthin than during the diet low in lutein and zeaxanthin. The concentrations remained elevated throughout the 12 wk of the diet high in lutein and zeaxanthin (Figure 2). The rapidity of the increase in plasma lutein was of interest. One week after the diet high in lutein and zeaxanthin, the plasma lutein concentration had increased significantly and doubled after 2 wk. The downward drift in plasma lutein in the control group at weeks 8 and 12 occurred because 2 subjects unable to maintain their earlier intakes of lutein and zeaxanthin; home diets were assessed from food records. The response of the AMD and control subjects was not significantly different, and no significant group-by-time interaction was observed (Table 4). After 2 wk of the diet low in lutein and zeaxanthin (≈1.1 mg/d), plasma lutein was 12.79 ± 1.65 μg/dL for the control group and 9.91 ± 1.36 μg/dL for the AMD group. Likewise, plasma zeaxanthin was 2.09 ± 0.38 μg/dL for the control group and 1.94 ± 0.36 μg/dL for the AMD group. The main effect of time was significantly different for both the AMD and control groups (P < 0.05). Four weeks of the diet high in lutein and zeaxanthin resulted in a 2- to 3-fold increase in plasma lutein in both the control (26.07 ± 4.72 μg/dL) and AMD (25.72 ± 4.00 μg/dL) groups. Plasma zeaxanthin was significantly greater in both the AMD (3.57 ± 0.66 μg/dL) and the control (3.57 ± 0.87 μg/dL) groups at week 4 of the diet high in lutein and zeaxanthin diet than at week 2 of the diet low in lutein and zeaxanthin. Two control subjects had difficulty eating the high-lutein diet that was prepared for them.

Considering lutein and zeaxanthin together, the plasma was ≈80% lutein and 20% zeaxanthin with the diet low in lutein and zeaxanthin (lutein:zeaxanthin = 4:1) and 87% lutein and 13% zeaxanthin with the diet high in lutein and zeaxanthin (lutein:zeaxanthin = 6.7:1). This is comparable with the chemical analysis of the 1 d of food in which lutein was 87% and zeaxanthin was 13% for both the diets low and high in lutein and zeaxanthin (lutein:zeaxanthin = 6.7:1).

### Other plasma carotenoids

Concentrations of β-cryptoxanthin, α-carotene, β-carotene, and lycopene in plasma, LDL, and HDL after 1 wk of the diet low in lutein and zeaxanthin and after 3 wk of the diet high in lutein and zeaxanthin are shown in Table 5. No significant group-by-diet interaction was observed, except for plasma and LDL β-carotene. Although the dietary intake increased ≈5-fold in both groups, β-carotene increased significantly only in the control subjects. This finding was likely due to the fact that the AMD patients started out with high plasma concentrations of β-carotene. With the diet low in lutein and zeaxanthin, plasma β-carotene was significantly greater in the AMD group (35.75 μg/dL) than in the control group (15.14 μg/dL). Even though the mean dietary intake increased significantly from 1 to 7 mg, no significant increase in plasma β-carotene was observed in the AMD group. Plasma β-carotene increased significantly in all 5 control subjects but in only 1 AMD patient. In the AMD group, it remained unchanged in 4 subjects and decreased in 2 subjects, who had been taking 17 mg/d β-carotene as a supplement daily before the study, but who did not take it during the study.

Plasma β-cryptoxanthin and α-carotene were significantly greater with the diet high in lutein and zeaxanthin than with the diet low in lutein and zeaxanthin in both groups. Plasma lycopene concentrations were not significantly different between the diets or groups.

### Distribution of carotenoids among lipoproteins

The concentrations of the major carotenoids in plasma, LDL, and HDL after both diets are shown in Table 5. The distribution of carotenoids among lipoproteins was not significantly different between the AMD and control subjects.

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### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Plasma lutein</th>
<th>Plasma zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control subjects</td>
<td>AMD patients</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td><strong>Low L/Z diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>12.79 ± 1.65</td>
<td>9.91 ± 1.36</td>
</tr>
<tr>
<td>4 wk</td>
<td>26.07 ± 4.72</td>
<td>25.72 ± 4.00</td>
</tr>
<tr>
<td>8 wk</td>
<td>24.02 ± 3.86</td>
<td>25.96 ± 3.53</td>
</tr>
<tr>
<td>12 wk</td>
<td>22.16 ± 5.48</td>
<td>25.49 ± 3.93</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. The diets were prepared at home by the subjects (4 control subjects, 6 AMD patients) between weeks 4 and 12. There was no significant group-by-time interaction.

2 Significantly different from the low L/Z diet, P < 0.05 (adjusted for multiple comparisons with the Bonferroni correction).
Lutein, zeaxanthin, β-cryptoxanthin, and α-carotene concentrations in the VLDL fraction were significantly greater with the diet high in lutein and zeaxanthin than with the diet low in lutein and zeaxanthin in both groups (data not shown). β-Carotene in the VLDL fraction was significantly greater with the diet high in lutein and zeaxanthin than with the diet low in lutein and zeaxanthin only in the control group and did not change significantly in the AMD group. Lycopene concentrations in the VLDL fraction were not significantly different between the diets or the groups.

Lutein, zeaxanthin, and β-cryptoxanthin in the LDL fraction were significantly greater with the diet high in lutein and zeaxanthin than with the diet low in lutein and zeaxanthin in both groups (main effect of diet). β-Carotene in the LDL fraction was significantly greater with the diet high in lutein and zeaxanthin than with the diet low in lutein and zeaxanthin in the control group but not in the AMD group. No significant differences in α-carotene and lycopene were observed between the groups or diets.

Lutein, zeaxanthin, β-cryptoxanthin, and α-carotene were significantly greater in the HDL fraction with the diet high in lutein and zeaxanthin than with the diet low in lutein and zeaxanthin in both groups. β-Carotene and lycopene were not significantly different between the diets or groups.

The ratio of HDL to LDL for the various carotenoids are also shown in Table 5 for the control and AMD groups. Interestingly, the ratio decreased with the decreasing polarity of the carotenoids in both groups. The ratios for lutein in the control and AMD groups were 2.49 ± 0.43 and 3.09 ± 0.54, respectively, with the diet low in lutein and zeaxanthin. The ratios were 2.13 ± 0.51 and 2.75 ± 1.04 in the control and AMD groups, respectively, with the diet high in lutein and zeaxanthin. For zeaxanthin, the ratios in the control and AMD groups were 1.95 ± 0.47 and 2.12 ± 0.39, respectively, with the diet low in lutein and zeaxanthin and 1.61 ± 0.23 and 2.43 ± 0.92, respectively, with the diet high in lutein and zeaxanthin.

Because the carotenoid distribution patterns with the diet high in lutein and zeaxanthin were not significantly different between the AMD and control groups, the data were combined (Figure 3). HDL was the major lipoprotein transporter of lutein (52%) and zeaxanthin (44%). LDL was the major transporter of α-carotene (50%), β-carotene (55%), and lycopene (57%).

**TABLE 5**

Concentrations of the major carotenoids in plasma, LDL, and HDL after the consumption for 1 wk of the diet low (~1.1 mg/dL) in lutein and zeaxanthin (L/Z diet) in the patients with age-related macular degeneration (AMD) and the control subjects*.

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Plasma</th>
<th>LDL</th>
<th>HDL</th>
<th>HDL:LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low L/Z diet</td>
<td>High L/Z diet</td>
<td>Low L/Z diet</td>
<td>High L/Z diet</td>
<td>Low L/Z diet</td>
</tr>
<tr>
<td>Lutein (µg/dL)</td>
<td>8.35 ± 2.04</td>
<td>27.53 ± 5.67*</td>
<td>1.56 ± 0.51</td>
<td>5.93 ± 1.60*</td>
</tr>
<tr>
<td>Zeaxanthin (µg/dL)</td>
<td>2.08 ± 0.55</td>
<td>3.66 ± 1.05*</td>
<td>0.54 ± 0.18</td>
<td>1.03 ± 0.26</td>
</tr>
<tr>
<td>β-Cryptoxanthin (µg/dL)</td>
<td>5.53 ± 1.09</td>
<td>16.05 ± 3.32*</td>
<td>2.33 ± 0.37</td>
<td>6.55 ± 1.22</td>
</tr>
<tr>
<td>β-Carotene (µg/dL)</td>
<td>15.14 ± 5.50</td>
<td>36.18 ± 6.75*</td>
<td>8.72 ± 2.16</td>
<td>22.69 ± 5.57</td>
</tr>
<tr>
<td>Lycopene (µg/dL)</td>
<td>15.17 ± 2.89</td>
<td>15.26 ± 3.12</td>
<td>12.18 ± 3.93</td>
<td>8.99 ± 1.84</td>
</tr>
</tbody>
</table>

* All values are ± SEM.

**FIGURE 3.** Mean (±SEM) percentage distribution of the major carotenoids in plasma lipoproteins of the patients with age-related macular degeneration and the control group combined (n = 12) after a diet high in lutein and zeaxanthin.
zeaxanthin (week 2) and 83.99 ± 5.02 kg with the diet high in lutein and zeaxanthin (week 4); the difference was not clinically significant ($P = 0.050$).

**DISCUSSION**

The diet high in lutein and zeaxanthin, which provided intakes of 11–12 mg/d, increased plasma lutein concentrations 2- to 3-fold in both the AMD patients and the control subjects. Similar results occurred in 22 healthy subjects fed a high-vegetable diet containing 11 mg lutein and zeaxanthin (12). In another study, healthy subjects fed 12 mg lutein and zeaxanthin daily from spinach and corn had a 2-fold increase in serum lutein (13). Our study indicated that the AMD patients did not respond differently than did the control subjects when challenged with a diet high in lutein and zeaxanthin. The possibility exists that the low statistical power due to a small sample size may have limited the ability to detect a difference. However, the response of the AMD patients was comparable with that of the healthy subjects fed lutein and zeaxanthin reported in the literature.

The intervention with ≈11 mg lutein and zeaxanthin daily is about twice the recommendation for the prevention of AMD (3), which implies that the lutein and zeaxanthin status of individuals can be readily improved through the consumption of ordinary foods in the diet. The increases in plasma lutein and zeaxanthin were maintained throughout the additional 8 wk of instructed self-feeding at home. It is of interest that both the AMD patients and the control subjects were able to maintain a high intake of vegetables and fruits while preparing their own food. We were impressed at how well these older adults followed the diet high in lutein and zeaxanthin.

There are advantages of lifestyle changes over supplementation, which may have implications for any dietary intervention for AMD. For example, the greater dietary intake of all carotenoids, except lycopene, with the diet high in lutein and zeaxanthin also resulted in increased plasma concentrations of the other carotenoids. These responses were similar to those reported in healthy subjects who were fed a high-vegetable diet (12). Such a diet could have other potential benefits in terms of the prevention of cancer and cardiovascular disease. Supplements of either lutein or zeaxanthin or both without dietary modification would not have these benefits.

Per our chemical analysis, the amounts of lutein and zeaxanthin provided by the diet high in lutein and zeaxanthin were considerably lower than the amounts computed (10.2 mg compared with 14.8 mg) (Table 2). This raises the possibility that the lutein and zeaxanthin intake with the diet high in lutein and zeaxanthin might have been ≈7.5 mg and not ≈11.1 mg. However, there are generally problems associated with both chemical analysis and computer estimates of nutrients. The increases in plasma lutein in our study were comparable with those observed in other studies with intakes of 11–12 mg lutein and zeaxanthin (12, 13) and to a 3-fold increase in plasma lutein and a 2-fold increase in plasma zeaxanthin in one AMD patient and one control subject who were given a supplement containing 12 mg lutein and zeaxanthin (WE Connor, unpublished observations, 2004). Even if the analyzed values were more reflective of actual intake, ≈7.5 mg lutein and zeaxanthin would still be well above the intake of ≈6 mg that is reported to be associated with a reduced risk of AMD (3). Furthermore, with the diet high in lutein and zeaxanthin, the ratio of lutein to zeaxanthin was similar in the plasma (6.9) and the chemically analyzed aliquot of 1 d of food (6.8). This means that lutein and zeaxanthin were metabolized in the body at similar rates.

HDL was the major lipoprotein transporter of lutein (52%) and zeaxanthin (44%) with the diet high in lutein and zeaxanthin, which was similar to other published data. Clevendence and Bieri (24), who fed healthy young men a typical US diet, showed that 53% of the lutein and zeaxanthin was transported in HDL. Our results also agreed with a recent report that showed no difference in lipoprotein distribution between AMD patients and control subjects; each group had >50% of lutein associated with HDL (19).

Furr and Clark (25) hypothesized that xanthophylls are more likely to be associated with the surface of the lipoproteins, whereas the carotenes more likely exist in the lipid core of the lipoproteins because of their polarity differences. LDL in serum contributes about twice as much total surface lipid as does HDL (18). This should result in a ratio of HDL to LDL to 1:2 if lutein and zeaxanthin were correlated with the surface area of the lipoproteins. In our studies, the ratio of HDL to LDL was close to 3:1 for lutein and close to 2:1 for zeaxanthin. This ratio was similar in the AMD and control groups and after both the diets low or high in lutein and zeaxanthin. Because HDL-cholesterol concentrations decreased with the diet high in lutein and zeaxanthin, the increase in lutein and zeaxanthin in the total HDL fraction was not caused by an increase in HDL, which actually decreased. Therefore, the surface area of the lipoproteins did not explain the 3:1 and 2:1 HDL-LDL ratios we observed.

The ratio of HDL total core lipid to LDL has been estimated to be ≈1.5 in healthy persons (18), which is similar to the ratios we determined for α-carotene, β-carotene, and lycopene (Table 5). Given the HDL-LDL ratio of 3:1 for lutein and of 2:1 for zeaxanthin, the contents of lutein and zeaxanthin in HDL and LDL would not correlate with their core lipid content.

We suspect that the 3:1 or 2:1 HDL-LDL ratios of lutein and zeaxanthin are more likely to be dependent on the same specific binding or affinity of the xanthophylls to the HDL. This specific affinity of carotenoids to different lipoproteins may then control which tissues the carotenoids are distributed to. For example, those tissues high in LDL receptors, such as the prostate and adrenal glands, would be high in the nonpolar carotenoids β-carotene and lycopene, which is the case (26, 27). Carotenoids carried by HDL containing apolipoprotein E (apo E) are perhaps more efficiently delivered to the central nervous system than from LDL because apo E receptors are abundant in the central nervous system. The preferential uptake of lutein and zeaxanthin from HDL in the retina may be partly explained by the specific binding or affinity of the xanthophylls to the HDL containing apo E, similar to α-tocopherol (28–31).

The crucial role of HDL in the transport of lutein and zeaxanthin is particularly illustrated in the genetic strain of chickens known as WHAM chickens. In this species of chicken there is a mutation of the transporter ABCA1, which results in a very low HDL and an impairment in the transport of lutein and zeaxanthin (32). In particular, the WHAM retina has very low concentrations of lutein and zeaxanthin, ≈5% of the usual concentrations in a normal chicken retina. In the chicken, HDL is the major lipoprotein, so that a deficiency of HDL may significantly impair the transport of lutein and zeaxanthin.

As powerful antioxidants, lutein and zeaxanthin reduce atherosclerotic lesions in animals and reduce the progression of intima-media thickness in human carotid arteries (33). In the
retina, cholesterol accumulation occurs in Bruch’s membrane (34). The similarity of the composition between drusen (oxidized fat, cholesterol esters and protein) associated with AMD and the extracellular deposits associated with atherosclerosis (35) supports the hypothesis that AMD and atherosclerosis may have similar etiologic factors. Perhaps, the protective effects of HDL against cardiovascular disease may be attributed in part to its high content of the antioxidants lutein and zeaxanthin.

The results of this study suggest that patients with AMD may have normal mechanisms to increase plasma concentrations of lutein and zeaxanthin after a diet high in these carotenoids. Furthermore, the transport of these carotenoids was not significantly different between the AMD and control groups in that the lipoprotein HDL was the major transporter, but other lipoproteins, VLDL and LDL, also transported lesser quantities of lutein and zeaxanthin. Because there was no difference in plasma lutein and zeaxanthin concentrations and lipoprotein distribution between the AMD patients and the control group, we suggest that, if there is any defect in the metabolism of lutein and zeaxanthin in patients with AMD, the defect may reside in the uptake of lutein and zeaxanthin from the plasma and transport into the retina. This is a topic that will be the subject of future experiments.

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