The Respective Relationships between Lipoprotein Profile, Macular Pigment Optical Density, and Serum Concentrations of Lutein and Zeaxanthin

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PURPOSE. The dietary carotenoids lutein (L) and zeaxanthin (Z) are transported on lipoproteins in the serum. The mechanism of delivery of L and Z to the macula, where they constitute macular pigment (MP), is poorly understood. This study was an investigation of the respective relationships between serum lipoprotein profile, MP optical density (MPOD), and serum L and Z.

METHODS. Three hundred two healthy subjects were recruited; 211 (69.9%) were women. Demographic and health details were recorded. Fasting blood samples were taken for lipoprotein analysis by spectrophotometric assay and L and Z analysis by high-performance liquid chromatography. MPOD was measured by heterochromatic flicker photometry.

RESULTS. The mean ± SD (range) age of all subjects was 48 ± 11 (21–66) years. There was a statistically significant and positive association between serum L concentration and both serum cholesterol concentration (r = 0.239, P < 0.001) and serum HDL concentration (r = 0.324, P < 0.001), but not with serum LDL concentration (r = 0.095, P = 0.101). There was a statistically significant but inverse association between serum triglyceride concentration and total MPOD (r = −0.118, P = 0.044). There was no significant association between MPOD and serum cholesterol concentration or serum HDL concentration (P > 0.05).

CONCLUSIONS. The findings are consistent with the hypothesis that HDL is important for the transport of L in serum. The mechanism(s) whereby L and Z are captured by the macula and whether the serum (apo)lipoprotein profile is important in the transfer of the carotenoids from serum to retina merit further study.

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Lutein (L), zeaxanthin (Z), and meso-zeaxanthin (meso-Z) are hydroxycarotenoids that constitute the macular pigment (MP) in humans. L and Z are solely of dietary origin, whereas meso-Z is generally not found in the diet, but is understood to be primarily formed in the retina after biochemical isomerization from L.1–2 There is a growing body of evidence to suggest that MP may prevent and/or delay the progression of age-related macular degeneration (AMD), which is the leading cause of age-related blindness in the developed world.3–5 MP is found in its highest concentration in the central macula, where it functions as a powerful antioxidant and acts as a filter of actinic short-wavelength blue light, thus limiting (photo-)oxidative damage to retinal cells.6 These properties of MP are believed to be the mechanism(s) whereby it protects against AMD.

It is known that L and Z are transported in serum on circulating lipoproteins. Some studies have reported that L and Z are relatively equally distributed between low-density lipoprotein (LDL) and high-density lipoprotein (HDL) molecules.6,7 However, other studies have reported that HDL is the preferential carrier of the MP carotenoids in plasma.8–10 In a recent study, Connor et al.11 have shown that Wisconsin hyperalpah mutant (WHAM) chicks, which have a genetic mutation resulting in a very low circulating HDL concentration, accumulate only 6% of the retinal L when compared with that in control chicks, suggesting an important role for HDL in the transport of L in serum and/or its capture by the retina.

The uptake of the MP carotenoids into the retina is poorly understood. Bhosale et al.12,13 have identified specific xanthophyll-binding proteins, of which the Pi isoform of glutathione S-transferase (GSTP1) has a high affinity for Z and meso-Z, but not for L.14 Recently, Bhosale et al.15 have also identified a human retinal L-binding protein (HR-LBP), purified from peripheral human retina. The paucity of available data on the transport of L and Z in serum and their capture and uptake into the retina prompted us to investigate the relationship between the lipoprotein profile and MP optical density (and the serum concentrations of its constituent carotenoids).

METHODS

Subjects

Three hundred and two subjects were recruited for this study, which was performed in the Macular Pigment Research Group (MPRG) laboratory at Waterford Institute of Technology, Ireland. The subjects were recruited by local advertisement in various media. The study was approved by the Research Ethics Committee of Waterford Institute of Technology, and the subjects were required to sign an informed consent document before participation. All experimental procedures adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria for participation were age between 20 and 70 years, no clinical evidence of ocular disease, no dietary supplementation with MP carotenoids, and visual acuity 20/40 or better. The following information was recorded for each subject: demographic details; family history of AMD (confirmed by letter from the diagnosing ophthalmologist); personal smoking history; dietary intake of L and Z, assessed with a validated 170-item food frequency questionnaire (FFQ). Examination included visual acuity (Snellen and LogMAR); body mass index (BMI; calculated as kg/m²); MP optical density measurement by customized heterochromatic flicker photometry (cHFP) with a macular...
densitometer; and screening for ocular disease by nonmydriatic fundus photography with a nonmydriatic auto fundus camera (AFC-210; Nidek, Gamagori, Japan). Twelve-hour fasting blood samples were obtained to quantify serum concentrations of L and Z by high performance liquid chromatography (HPLC), and lipoprotein levels by spectrophotometric assay.

Food Frequency Questionnaire

Dietary intake of L and Z was assessed by a self-administered, semiquantitative FFQ developed by the Scottish Collaborative Group (SCG) at the University of Aberdeen (Scotland, UK). It is the primary method used in epidemiologic studies to assess dietary intake of various nutrients and foods most commonly consumed in a Western diet.16 This FFQ was developed in the Scottish Heart Health Study; it has been validated against weighed-food records and biomarkers; and its validity, in terms of quantifying dietary intake of L and Z (separately), has been confirmed in studies by O’Connell et al. and others.17-19

The questionnaire is designed to assess a subject’s dietary intake over the preceding 2 to 3 months. Although subjects were assessed at different time points throughout the year, it has been shown that month-to-month serum concentrations of L and Z and MP optical density are relatively stable over a 24-month period.20 The FFQ consists of 170 foods and drinks, grouped into 21 sections. A standard portion or measure for each type of food or drink is specified, and subjects are required to indicate how many portions they consumed per day and how often they consumed each type of food, ranging in frequency from “rarely or never” to “7 days per week.” The questionnaire includes a color photograph depicting examples of standard food measures and an example of how to fill out the questionnaire. The questionnaire was completed by the subject in the presence of the primary investigator (EL), after detailed instructions for the required task. It took between 20 and 30 minutes to complete.

Completed FFQs were entered into a spreadsheet (Access; Microsoft, Redmond, WA) developed for analysis purposes by the SCG. Nutrient analysis was performed with a program (Visual Basic for Access; Microsoft) using food composition data based on McCance and Widdowson’s The Composition of Foods.21 Dietary intake of L and Z was calculated on the basis of food composition data from the U.K., European, and U.S. data sources, by using standard principles or criteria for the matching of food items and standardized recipes or manufacturer’s ingredient information, where necessary.22-25

Measurement of MP Optical Density

MP optical density was measured psychophysically by cHFP, a technique that has been validated against the absorption spectrum of MP in vitro.26 HFP is based on the fact that MP absorbs short-wavelength blue light, with peak absorption occurring at a wavelength of 458 nm. The subject is required to make isoluminance matches between two flickering lights: a green light (not absorbed by MP) and a blue light (with peak absorption occurring at a wavelength of 458 nm). The luminance of the test target. When an isoluminant (null-flicker) match has matches between these flickering lights. The luminance of the green and a blue-background test field (wavelength, 468 nm) that saturates the S-cone pathway. A minimum of three null-flicker readings were recorded for each subject at each of the test loci (0.25°, 0.5°, 1°, 1.75°, and 7° retinal eccentricities). Measurement of MP optical density at these points of retinal eccentricity enabled us to plot the spatial profile of MP across the macula. For each subject, we then calculated the area of MP optical density under the spatial profile, using the trapezoidal rule, as follows: total MP optical density = \[ \frac{\text{MP optical density at 0.25°} + \text{MP optical density at 0.5°}}{2} \times 0.25° \]

Total MP optical density gives a better measurement of the quantity of MP across the macula than an individual measurement at a single point of retinal eccentricity. The MP optical density measurement was performed under conditions of dimmed light (ambient illuminance, 4 lux, as measured with a light meter; ILM 350 Lux Meter; Iso-Tech, Champaign, IL, at a viewing distance of 18.5 in. [47 cm]).

The major advantage of cHFP over standard HFP instruments is that the flicker frequency of each test target is customized for each subject, minimizing the variance between consecutive measurements and thus increasing the accuracy and ease of use of the test. A more detailed description of this instrument has been published by Nolan et al.27 Further information on the technique and advantages of cHFP have been published by Nolan et al.28

Blood Sample Collection

A 12-hour fasting blood sample was collected from each subject in a 4-mL tube (Z Serum Sep Clot Activator Vacutette Greiner Bio-One GmbH, Kremsmünster, Austria) at the beginning of the study visit. The whole blood sample was immediately refrigerated at 2°C to 8°C, before centrifugation at approximately 1800g for 10 minutes. Centrifugation was performed within 4 hours of phlebotomy. After centrifugation, the supernatant serum sample was aliquotted into 1.5-mL amber (light-sensitive) microcentrifuge tubes (Brand GmbH, Wertheim, Germany) and stored at −70°C before analysis.

Serum L and Z Analysis

Serum L and Z were quantified by using reversed-phase HPLC. We used an LC system (1200 series; Agilent Technologies Ireland Ltd., Dublin, Ireland) with a C18 column (Merck-Hitachi, Darmstadt, Germany) and a UV detector (Hitachi, Tokyo, Japan). The mobile phase consisted of 0.1% acetic acid and acetonitrile, and the flow rate was 1 mL/min. The column temperature was 40°C, and the injection volume was 10 µL. The retention times for L and Z were 2.7 minutes and 5.2 minutes, respectively. The detection wavelength was set at 210 nm. The concentration of L and Z was calculated using a standard curve of known concentrations. The assay was linear over the range of 0.01 to 50 µM. The coefficient of variation for the within-run precision was <5%, and the coefficient of variation for the between-run precision was <10%.
Ireland) with photodiode array detection at 295, 325, and 450 nm. A 5-μm analytical/preparative 4.6 × 250-mm specialty reversed-phase column (201TP; Vydac, Hesperia, CA) was used with an in-line guard column. The mobile phase consisted of 97% methanol and 3% tetrahydrofuran and was degassed with an in-line degasser. The flow rate was 1 mL/min, and the total run time was 15 minutes. All carotenoid peaks were integrated and quantified (Chem Station software; Agilent).

A 400-μL aliquot of serum was pipetted into a 1.5-mL clear microcentrifuge tube. Ethanol (300 μL; containing 25 μg/mL butylated hydroxytoluene; BHT) and 200 μL internal standard (ethanol containing 25 μg/mL α-tocopherol acetate) were added to each tube. Heptane (500 μL) was then added, and the samples were vortexed vigorously for 1 minute, followed by centrifugation at approximately 225 g for 5 minutes. The resulting heptane layer was retained and transferred to a second labeled 1.5-mL light-sensitive microcentrifuge tube, and a further heptane extraction was performed. The combined heptane layers were immediately evaporated to dryness under a stream of nitrogen by using a sample concentrator (Techne; Bibby-Scientific Ltd., Stone, UK). These dried samples were reconstituted in methanol (200 μL), and a 150-μL sample was injected for HPLC analysis. The mean (±SD) percentage of recovery of our internal standard (ethanol containing 25 μg/mL α-tocopherol acetate) was 94% ± 4%. The concentration of L and Z (in milligrams per milliliter) was adjusted accordingly for each assay.

DSM Nutritional Products (Basel, Switzerland) provided the L and Z standards that were used to generate standard curves for quantification of these carotenoids. This assay was validated against the National Institute of Standards and Technology (NIST) standard before analysis.

Serum Lipoprotein Analysis

Lipoprotein analysis was performed (ACE Clinical Chemistry System; Alfa Wassermann, Woerden, The Netherlands), with reagents and consumables supplied by Randox Laboratories Ltd. (Antrim, UK). The ACE Clinical Chemistry System is an automated random access analyzer that is used for in vitro diagnostic purposes in the quantification of the constituents of blood and other fluids. It is fully automated, being directed by an internal computer and accessed via a DOS-based user interface (Microsoft). Analyses were performed on 200-μL serum samples by spectrophotometric analysis at 37°C. The analysis module uses a holographic diffraction grating spectrophotometer to measure absorbance at 16 different wavelengths. Measurements for each assay are recorded at wavelengths and times that are preprogrammed for each test. Sample results are calculated as specified by the test parameters. A wide variety of manufacturer-validated tests are preprogrammed into the system, and additional test applications can be programmed as required. System reagents are identified via computer-coded labels on the base of each bottle. During analysis, samples and reagents are automatically pipetted into disposable cuvettes in a 48-position reaction wheel. The system initiates an assay by transferring specific amounts of sample and reagent into a cuvette. The cuvette contents are mixed, incubated, and then optically analyzed at the times and wavelengths specified in the programmed test parameters. On completion of each test, used cuvettes are ejected into a waste container and new cuvettes are loaded onto the reaction wheel from the cuvette hopper.

The protocol we used for lipoprotein analysis included daily, two-level quality-control assessments, and duplicate analyses on 50 (16.6%) samples, to ensure the accuracy of the results.

Statistical Analysis

Commercially available statistical software (SPSS, ver. 17; SPSS, Chicago, IL) was used for analysis of the results. A graphics software package (SigmaPlot version 8.0; Systat, San Jose, CA) was used for graphical presentation of the results. Data are presented as the mean ± SD throughout. Associations were investigated by using a general linear model approach, with Pearson correlation, partial correlation, Pearson χ² testing, and the independent samples t-test, as appropriate. Correlations are Pearson r, unless otherwise noted. The level of statistical significance was set at the standard P < 0.05.

RESULTS

The anthropometric and lifestyle data for all subjects included in this study are detailed in Table 1. One hundred twenty-one (40.1%) subjects had a clinically confirmed family history of AMD. On average, subjects with a positive family history were significantly older (mean ± SD age, 51 ± 8 years) than were those with a negative family history of AMD (mean ± SD age, 46 ± 12 years), and they reported a higher number of years smoking (12.53 ± 4.62 years) than were subjects with a negative family history of AMD (9.11 ± 2.48 years). The difference in age was statistically significant (t-test, P < 0.05). The difference in years smoking was also statistically significant (t-test, P < 0.05). There were no statistically significant differences in the variables of sex, BMI, MPOD, and smoking pack-years between subjects with a positive family history of AMD and those with a negative family history of AMD.

**Table 1.** Anthropometric and Lifestyle Data for All Subjects and with Respect to the Family History of AMD

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Entire Group (n = 302)</th>
<th>Negative Family History of AMD (n = 181)</th>
<th>Positive Family History of AMD (n = 121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>48 ± 11</td>
<td>46 ± 12</td>
<td>51 ± 8</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>91 (30.1%)</td>
<td>63 (34.8%)</td>
<td>28 (23.1%)</td>
</tr>
<tr>
<td>Female</td>
<td>211 (69.9%)</td>
<td>118 (65.2%)</td>
<td>93 (76.9%)</td>
</tr>
<tr>
<td>BMI*</td>
<td>26.75 ± 4.58</td>
<td>26.54 ± 4.38</td>
<td>27.02 ± 4.87</td>
</tr>
<tr>
<td>MPOD 0.25°†</td>
<td>0.48 ± 0.21</td>
<td>0.47 ± 0.21</td>
<td>0.50 ± 0.20</td>
</tr>
<tr>
<td>MPOD 0.5°†</td>
<td>0.38 ± 0.17</td>
<td>0.37 ± 0.17</td>
<td>0.39 ± 0.18</td>
</tr>
<tr>
<td>MPOD 1°†</td>
<td>0.24 ± 0.13</td>
<td>0.24 ± 0.13</td>
<td>0.24 ± 0.13</td>
</tr>
<tr>
<td>Total MPOD</td>
<td>0.72 ± 0.43</td>
<td>0.70 ± 0.43</td>
<td>0.73 ± 0.44</td>
</tr>
<tr>
<td>Smoking, pack-years‡</td>
<td>5.72 ± 11.30</td>
<td>6.45 ± 12.53</td>
<td>4.62 ± 9.11</td>
</tr>
<tr>
<td>Serum L, μg/mL</td>
<td>0.089 ± 0.047</td>
<td>0.081 ± 0.043</td>
<td>0.101 ± 0.051</td>
</tr>
<tr>
<td>Serum Z, μg/mL</td>
<td>0.016 ± 0.011</td>
<td>0.015 ± 0.011</td>
<td>0.017 ± 0.010</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>5.406 ± 1.098</td>
<td>5.265 ± 1.086</td>
<td>5.616 ± 1.086</td>
</tr>
<tr>
<td>Serum HDL, mmol/L</td>
<td>1.495 ± 0.379</td>
<td>1.465 ± 0.350</td>
<td>1.540 ± 0.416</td>
</tr>
<tr>
<td>Serum LDL, mmol/L</td>
<td>3.214 ± 0.922</td>
<td>3.184 ± 0.965</td>
<td>3.259 ± 0.857</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.304 ± 0.613</td>
<td>1.348 ± 0.626</td>
<td>1.239 ± 0.590</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* BMI, body weight in kilograms divided by height in meters squared (kg/m²).
† MPOD, MP optical density. There were no statistically significant differences in MP optical density at any degree of eccentricity between the groups (independent-samples t-test: P > 0.05, for all).
‡ Pack-year calculation = (the number of cigarettes smoked per day × number of years smoking)/20.
There was a significantly smaller proportion of male subjects (23.1%) in the family group with a positive history of AMD than in the group with a negative family history (34.8%; Pearson correlation, \( r = -0.118, P = 0.044 \)); however, although losing statistical significance, the directionality of the observed associations remained at the other degrees of retinal eccentricity (\( P > 0.05 \)). There was no statistically significant association between MP optical density at any degree of retinal eccentricity and serum cholesterol concentration, serum HDL concentration, or serum LDL concentration (Pearson correlation, \( P > 0.05 \), for all).

When we analyzed the study sample according to family history of AMD, the inverse association between MP optical density and serum triglyceride concentration remained statistically significant only in subjects with a positive family history of AMD (\( r = -0.238, P = 0.009 \) for total MP optical density). The association between MP optical density at each degree of retinal eccentricity and serum triglyceride concentration (partial correlation, \( r = -0.115, P = 0.049 \)) was not statistically significant for total MP optical density (partial correlation, \( r = -0.116, P = 0.049 \); however, although losing statistical significance, the directionality of the observed associations remained at the other degrees of retinal eccentricity (\( P > 0.05 \)).

MP Optical Density Associations

There was a statistically significant inverse association between MP optical density at each degree of retinal eccentricity and serum triglyceride concentration (\( r = -0.15, P = 0.009 \) for total MP optical density; Fig. 2). After adjustment for age, sex, BMI, cigarette smoking, serum HDL concentration, and serum concentrations of L and Z, this inverse association remained statistically significant only at 1° retinal eccentricity (partial correlation, \( r = -0.116, P = 0.049 \)) and for total MP optical density (\( r = -0.118, P = 0.044 \)); however, although losing statistical significance, the directionality of the observed associations remained at the other degrees of retinal eccentricity (\( P > 0.05 \)).

When we compared total MP optical density and MP optical densities at each degree of retinal eccentricity with a family history of AMD, with adjustment for age, sex, serum L, and serum cholesterol, we did not find any significant difference in MP optical densities between the two AMD family history groups (partial correlation, \( P > 0.05 \), for all). A summary of the significant relationships between MP optical density, serum L and Z, and serum lipoproteins, detailed in the following sections, is provided in Table 2.

### Table 2. Summary of Results, Showing the Significant Relationships between MP Optical Density (and Serum Concentrations of L and Z) and Serum Lipoproteins, for All Subjects

<table>
<thead>
<tr>
<th></th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP optical density 0.25°</td>
<td>(-0.133; 0.022)</td>
<td>0.025; 0.663</td>
<td>0.019; 0.742</td>
</tr>
<tr>
<td>Partial correlation; significance*</td>
<td>-0.104; 0.078</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP optical density 0.5°</td>
<td>(-0.157; 0.007)</td>
<td>0.042; 0.471</td>
<td>0.098; 0.093</td>
</tr>
<tr>
<td>Partial correlation; significance*</td>
<td>-0.106; 0.071</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP optical density 1°</td>
<td>(-0.132; 0.023)</td>
<td>0.054; 0.353</td>
<td>-0.005; 0.934</td>
</tr>
<tr>
<td>Partial correlation; significance*</td>
<td>-0.116; 0.049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP optical density 1.75°</td>
<td>(-0.122; 0.036)</td>
<td>0.020; 0.756</td>
<td>0.019; 0.741</td>
</tr>
<tr>
<td>Partial correlation; significance*</td>
<td>-0.108; 0.067</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total MP optical density</td>
<td>(-0.15; 0.009)</td>
<td>0.020; 0.756</td>
<td>0.031; 0.59</td>
</tr>
<tr>
<td>Partial correlation; significance*</td>
<td>-0.118; 0.044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum L</td>
<td>(-0.167; 0.004)</td>
<td>0.239; &lt;0.001</td>
<td>0.324; &lt;0.001</td>
</tr>
<tr>
<td>Partial correlation; significance†</td>
<td>-0.087; 0.138</td>
<td>0.202; 0.001</td>
<td>0.28; &lt;0.001</td>
</tr>
<tr>
<td>Serum Z</td>
<td>(-0.103; 0.075)</td>
<td>0.117; 0.042</td>
<td>0.139; 0.016</td>
</tr>
<tr>
<td>Partial correlation; significance‡</td>
<td>0.052; 0.377</td>
<td>0.025; 0.666</td>
<td>-0.025; 0.666</td>
</tr>
</tbody>
</table>

Significant results are in bold (\( n = 302 \)).

* Control variables: age, sex, BMI, smoking, serum HDL, serum L, serum Z.
† Control variables: age, sex, BMI, smoking, total MP optical density, serum Z, dietary L, dietary Z.
‡ Control variables: age, sex, BMI, smoking, total MP optical density, serum L, dietary L, dietary Z.
**FIGURE 3.** Serum L concentration versus total MP optical density for all subjects ($n = 302$).

**FIGURE 4.** Serum cholesterol concentration versus serum L concentration for all subjects ($n = 302$).

**FIGURE 5.** Serum HDL concentration versus serum L concentration for all subjects ($n = 302$).

**FIGURE 6.** Serum triglyceride concentration versus serum L concentration for all subjects ($n = 302$).

**FIGURE 7.** Serum Z concentration versus total MP optical density for all subjects ($n = 302$).

**FIGURE 8.** Serum cholesterol concentration versus serum Z concentration for all subjects ($n = 302$).
Serum HDL concentration versus serum Z concentration for all subjects (n = 302).

![Graph showing Serum HDL concentration versus serum Z concentration](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932968/)

FIGURE 9. Serum HDL concentration versus serum Z concentration for all subjects (n = 302).

retinal eccentricity (and total MP optical density) and serum cholesterol concentration, serum HDL concentration, or serum LDL concentration remained statistically insignificant for each family history group (P > 0.05, for all).

**Serum L Associations**

There was a statistically significant and positive association between serum L concentration and MP optical density at each degree of retinal eccentricity (r = 0.261, P < 0.001 for total MP optical density; Fig. 3). There was a statistically significant and positive association between serum L concentration and serum cholesterol concentration (r = 0.239, P < 0.001; Fig. 4) and between serum L concentration and serum HDL concentration (r = 0.324, P < 0.001; Fig. 5). There was a statistically significant but inverse association between serum L concentration and serum triglyceride concentration (r = −0.167, P = 0.004; Fig. 6). Of note, there was no statistically significant association between serum concentrations of L and LDL (r = 0.095, P = 0.101). After adjustment for age, sex, BMI, total MP optical density, cigarette smoking, dietary intakes of L and Z, and serum Z concentration, the observed positive associations remained significant; however, the observed inverse association between serum concentrations of L and triglyceride lost statistical significance (partial correlation, P > 0.05), although the directionality of the association remained the same.

When we analyzed the study sample according to family history of AMD, the observed positive and significant associations persisted in both groups, but the inverse association between serum concentrations of L and triglyceride regained significance in those with a positive family history of AMD (r = −0.195, P = 0.032).

**Serum Z Associations**

There was a statistically significant and positive association between serum Z concentration and MP optical density at each degree of retinal eccentricity, with the exception of 1.75° (r = 0.154, P = 0.007 for total MP optical density; Fig. 7). There was a statistically significant and positive association between serum Z concentration and serum cholesterol concentration (r = 0.117, P = 0.042; Fig. 8) and between serum Z concentration and serum HDL concentration (r = 0.139, P = 0.016; Fig. 9). After adjustment for age, sex, BMI, total MP optical density, cigarette smoking, dietary intakes of L and Z, and serum L concentration, the observed associations lost statistical significance (partial correlation: P > 0.05), and the directionality of the observed association with serum HDL concentration actually reversed (r = −0.25, P = 0.666). We observed an inverse association between serum Z concentration and serum triglyceride concentration, but this association did not reach statistical significance (r = −0.103, P = 0.075). Of note, there was no statistically significant association between serum concentrations of Z and lipoproteins were attenuated to non-significance in each group (P > 0.05, for all in each family history group), although the directionality of the observed trends remained the same.

**DISCUSSION**

This study was designed to investigate the respective relationships between lipoprotein profile, MP optical density, and serum concentrations of L and Z in 302 healthy adult subjects. We were prompted to conduct this study because there is inconsistency in the published literature with respect to the reported associations between lipoprotein profile and L and Z in serum. To our knowledge, this is the first study in which the relationship between lipoprotein profile and MP optical density has been examined.

We found that there was a statistically significant inverse association between serum triglyceride concentration and MP optical density and an inverse association between serum triglyceride concentration and serum L concentration in subjects with a positive family history of AMD. There have been no previous reports on the association between serum triglyceride concentration and either MP optical density or serum concentrations of L and/or Z. Elevated serum triglyceride concentration is an element of an undesirable lipoprotein profile and represents a risk for cardiovascular disease.31,32 Since there is an inverse association between serum triglyceride concentration and serum HDL concentration, one could expect an inverse association between serum triglyceride concentration and serum L, since HDL appears to be the most important lipoprotein involved in the transport of L in serum. This expected inverse association was observed in this study in subjects with a positive family history of AMD. In other words, it appears that individuals who have an elevated serum triglyceride concentration and a concurrently reduced serum HDL concentration may have a related and reduced capacity to transport L in serum, possibly explaining the observed inverse association between serum triglycerides and MP optical density.

In this study sample, there was a positive and significant association between serum HDL concentration (and serum cholesterol concentration) and serum L and Z concentrations, although the statistical significance of these associations persisted only for serum L concentration when we analyzed the study sample divided according to family history of AMD. The attenuation of the statistical significance of this association in subjects without a family history of AMD may simply reflect a loss of statistical power, particularly in view of the findings of Cardinault et al.,13 who reported no difference in the percentage of carotenoids present in each lipoprotein subspecies between AMD subjects and controls. Of note, there was no significant association observed between MP optical density and either serum cholesterol concentration or serum HDL concentration. We also found that there was no association between serum LDL concentration and MP optical density (or serum concentrations of its constituent carotenoids). These findings suggest that a desirable
lipoprotein profile (higher serum HDL, lower serum LDL, and lower serum triglyceride concentrations) is associated with greater serum L concentration. However, the impact of lipoprotein profile on the capture and/or stabilization of these carotenoids at the macula, where they comprise MP, is less clear from the data.

We did not directly measure the lipoprotein particle concentration of these carotenoids in serum, nor did we measure lipoprotein subspecies, as did Goulinet et al., who fractionated HDL and LDL subspecies on the basis of their hydrated density by gradient ultracentrifugation. They found that serum L and Z (combined) were relatively equally distributed between HDL and LDL. More importantly, they found that there was a progressive decrease in the concentration of these carotenoids with increasing density (and decreasing lipoprotein particle size) from light to dense LDL. They also found that most of the macular carotenoid transport by LDL was accounted for by the most abundant subspecies: LDL3 (intermediate LDL) and LDL4 (dense LDL). This is highly relevant to the transport of L and Z in serum, as LDL3 and LDL4, despite being the most abundant subspecies of LDL in that study, had reduced particle concentrations of these carotenoids compared with less dense LDL subspecies, making them more vulnerable to oxidation. However, the lack of an association in our study between LDL and L and/or Z in serum contrasts with some previous reports. LDL is the primary component of total cholesterol and has been reported in various studies to transport lipoproteins, particularly LDL. The analogous mutation in humans results in Tangier disease, which is characterized by a similar deficiency in circulating HDL concentration. In their study, involving 24 WHAM chicks and 24 control chicks, Connor et al. found that 1-day-old WHAM chicks had only 9% of the L concentration in plasma when compared with control chicks and only 6% of the retinal concentration found in the control chicks (the corresponding concentrations of Z were 6% and 9%, respectively). After a high-L diet for 28 days, there was a significant increase in the plasma and retinal concentrations of L in the WHAM and control chicks, but the increases were still greatly inferior in the WHAM chicks when compared with those in the control chicks and, furthermore, the concentrations still did not reach the levels observed in the 1-day-old control chicks. The observations of Connor et al. suggest an important role for HDL in the transport of L and Z in serum and/or their incorporation into the retina and are consistent with our current findings.

The weaknesses of our observational study are, first, that we did not assess lipoprotein subspecies, as did Goulinet et al., and second, that we did not assess the carotenoid content of lipoprotein subspecies, as did both Goulinet et al. and Connor et al. Also, we did not exclude subjects from this study on the basis of lipidemic status or on the basis of statin use. However, there is no evidence to suggest that statin use affects the physiology of carotenoids in humans or that it affects MP optical density. Furthermore, we feel that including all subjects regardless of lipidemic status or statin use represents an important and necessary first step before any investigation of the possible role that statin use may have on MP optical density and/or serum concentrations of its constituent carotenoids.

In conclusion, we found a significant and positive association between serum HDL concentration and serum concentra-
tions of L, and we observed a significant but inverse association between serum triglyceride concentration and both serum L concentration and MP optical density in those with a positive family history of AMD, in a cohort of healthy adult subjects. These findings are consistent with the view that AMD and cardiovascular disease share important antecedents.54–56 Our findings also suggest that HDL plays an important role in the transport of the macular carotenoids in serum and/or their delivery to the retina. This study will inform the design of further investigation into the association of lipoprotein profile, MP optical density, and serum carotenoid concentrations, which should include assessment of lipoprotein subspecies concentrations of L and Z.

References


