Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin

ABSTRACT

Background: Macular pigment (MP), concentrated in the central area of the retina, contains the carotenoids lutein and zeaxanthin. A low MP density could be a risk factor for age-related macular degeneration. Little information is available regarding MP density in relation to serum lutein and zeaxanthin and adipose lutein concentrations in a general population.

Objective: The objective was to investigate the associations between MP density and serum lutein, serum zeaxanthin, and adipose lutein, taking into account potential confounders in a population.

Design: Volunteers (n = 376) aged 18–75 y were recruited. In a cross-sectional design, serum (n = 376) and adipose tissue (n = 187) were analyzed for carotenoids, and MP density was measured by spectral fundus reflectance.

Results: Mean MP density in the total study group was 0.33 ± 0.15. MP density was 13% higher in men than in women (P < 0.05). Serum and blood concentrations of α-tocopherol, vitamin C, and all carotenoids except lycopene were significantly higher in women. Adipose lutein concentrations were also significantly higher in women than in men. Regression models showed a positive significant association between MP density and serum lutein, serum zeaxanthin, and adipose lutein concentrations in men after adjustment for age, but no relation in women. In men, serum lutein remained significantly associated with MP density after adjustment for age, total cholesterol, body mass index, and smoking.

Conclusion: The associations between MP density and serum lutein, serum zeaxanthin, and adipose lutein concentrations are stronger in men than in women.

KEY WORDS Macular pigment, lutein, zeaxanthin, carotenoids, macular degeneration, Netherlands

INTRODUCTION

Age-related macular degeneration (AMD) is the main cause of visual impairment in Western countries; it affects the central region of the retina at which visual acuity is the greatest, the macula lutea. Macular pigment (MP) possibly acts as a blue-light filter to protect the macular region against photodestruction by light (1). In addition, MP can scavenge free radicals (2). The carotenoids lutein and zeaxanthin are the predominant pigments in this area (3). Some observational epidemiologic studies showed a reduced risk of AMD in subjects with a higher intake of lutein and zeaxanthin or higher plasma concentrations of lutein and zeaxanthin (4–6). Other epidemiologic studies did not show significant reductions in the risk of AMD in connection with high serum concentrations or dietary intakes of lutein and zeaxanthin (7–10). MP density was associated with the preservation of visual sensitivity in another study (11). It is probable that MP can protect against AMD, but further research is necessary (12).

If it is shown that MP density has a protective effect against AMD, it would be important to understand the effect of several factors on MP density and on the relation between MP density and serum concentrations of lutein and zeaxanthin, a possible biochemical indicator of the intake of carotenoids (13).

In previous studies, MP density was positively related to dietary intake (14) and serum concentrations of lutein and zeaxanthin (14, 15). A dietary intervention study showed that an increase in the intake of lutein and zeaxanthin from foods could increase MP density (16). Two other studies reported a change in MP density through lutein supplementation (17, 18). In one study, some subjects failed to show a change in MP density after increasing their dietary intake of lutein and zeaxanthin (19).

Previous studies showed an influence of several factors, eg, sex and smoking, on the relation between MP density and serum concentrations of lutein and zeaxanthin (14, 20). In addition, a significant positive correlation was found in men between MP density and adipose lutein concentration (16), another possible biochemical indicator of carotenoid intake (13). In contrast, a negative correlation between adipose lutein and MP density was reported in women. These associations have been evaluated only in small study samples. We determined the relation between MP...
density and serum lutein and serum zeaxanthin in 376 volunteers, adjusting for possible confounding factors. The relation between adipose tissue lutein concentrations and MP density was determined in 187 volunteers, after adjustment for possible confounding factors.

SUBJECTS AND METHODS

Subjects

The 380 subjects in our study were recruited from a pool of volunteers at the TNO Nutrition and Food Research and through advertising in local and regional newspapers and on television. The cross-sectional data analyzed here were gathered during the baseline measurements of an intervention study designed to test an unrelated hypothesis. The advertising was aimed at recruiting people with a general interest in participating in nutrition studies. Respondents who expressed potential interest (n = 6900) received a form with the inclusion and exclusion criteria for the study and a questionnaire requesting data on daily fruit and vegetable consumption and lifestyle factors. This questionnaire was designed for the ranking and selection of subjects according to their intake and not for quantitative measurements. It was a simple questionnaire on the intake of fruit and vegetables, including fruit juices, over the previous month. This questionnaire contained 8 items about fruit and vegetable intake, including fruit juices, in days or weeks. In addition, it included 8 questions about portion size, 4 questions about food item specification, and 1 question about vitamin or mineral supplements.

The major inclusion criterion was an age of 18–75 y. The main exclusion criteria were pregnancy or lactation, trying to become pregnant, serum cholesterol > 7.5 mmol/L or serum triacylglycerol > 2.3 mmol/L if not stable as a result of treatment for hypercholesterolemia or hyperlipidemia, anticoagulant therapy, and vegetarian or vegan status.

A total of 2734 questionnaires were returned. Respondents in the highest quintile of fruit and vegetable consumption and those in the lowest quintile (calculated in the total group) were selected. In the lowest stratum, vitamin supplement users were excluded (n = 127).

Finally, we invited 547 respondents from the highest quintile and 775 respondents from the lowest quintile for an oral briefing. At this briefing, volunteers were informed about the blood sampling and MP density measurements and about an extra procedure (ie, a biopsy of adipose tissue) that would be conducted in a subgroup of the study population. The volunteers were told that this extra procedure could be painful in persons with little adipose tissue on the buttocks, and they had the opportunity to refuse the procedure. After the oral briefing, 589 volunteers were interested in participating in the study and were screened. Informed consent was obtained from all subjects.

During the screening, 54 volunteers were excluded because they did not meet the criteria for serum cholesterol or triacylglycerol concentrations. Many volunteers (n = 122) withdrew during the screening. A total of 413 volunteers were given an entry number; 33 of that group withdrew from the study before it began.

On day 1, 380 volunteers (179 men, 201 women) entered the study. This group consisted of 216 subjects from the low fruit and vegetable intake group and 164 subjects from the high fruit and vegetable intake group. We randomly selected volunteers from whom to obtain an adipose tissue sample, taking sex and age into account. If initially selected volunteers refused this measurement, other volunteers were randomly selected.

The final study group (95% whites) comprised 177 men aged 42 ± 15 y and 199 women (premenopausal and postmenopausal) aged 41 ± 13 y; 120 men (68%) and 144 women (72%) were nonsmokers. We obtained an adipose tissue sample from 89 men aged 43 ± 15 y and from 98 women aged 42 ± 14 y. Of this subgroup of volunteers, 63 men (71%) and 68 women (69%) were nonsmokers.

The study was performed according to the guidelines for good clinical practice of the International Conference on Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use, and it was approved by a medical ethics committee. The study was conducted at the Department of Nutritional Physiology of the TNO Nutrition and Food Research, Zeist (Netherlands), and the measurements were performed from 29 June 1998 to 16 September 1998.

Blood and adipose tissue sampling

Blood samples were obtained from subjects between 0800 and 0930 after the subjects had fasted overnight. For the analysis of carotenoids and α-tocopherol, blood was collected in tubes containing clot activator and gel (Vacutainer; Becton Dickinson and Co, Franklin Lakes, NJ). These tubes were immediately placed in a closed box for storage, to avoid the breakdown of carotenoids by ultraviolet light. The tubes were centrifuged within 15–30 min after collection at ≈2000 × g for 10 min at ≈4 °C to obtain serum. After centrifugation, serum was removed and stored at ≈−80°C. All serum handling before storage was done in subdued light.

For the analysis of vitamin C, blood was collected in tubes containing lithium heparin (Becton Dickinson). A 0.5-mL aliquot of blood was added to 2 mL metaphosphoric acid (50 g/L; Mallinckrodt Baker, Deventer, Netherlands) before freezing to preserve the vitamin C concentration during storage. This mixture was stored at ≈−80°C.

For the analysis of total cholesterol, LDL cholesterol, HDL cholesterol, and triacylglycerol, blood was collected in tubes containing clot activator and gel (Becton Dickinson). Tubes were centrifuged within 15–30 min after collection at ≈2000 × g for 10 min at ≈4 °C to obtain serum. After centrifugation, the serum was removed and stored at ≈−20°C.

Subcutaneous adipose tissue (=50 mg) was obtained by needle aspiration from the lateral buttock (21) by use of a 16-gauge needle attached to a plastic container into which the tissue was collected by connecting a vacuum tube. Samples were kept in the plastic container, placed immediately on dry ice, and stored at ≈−80°C.

Chemical analyses

Serum samples

On the day of analysis, serum samples were thawed and mixed well. Samples were deproteinized by adding a 500-mL sample to 500 mL ethanol (Nedalco, Bergen op Zoom, Netherlands) containing tocol [3,4-dihydro-2-methyl-1-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol; Matreya, Pleasant Gap, PA] as an internal standard. After the samples were mixed by vortex, they were allowed to stand for 15 min at room temperature to complete the precipitation of proteins. Subsequently, 1.0 mL n-hexane (Merck, Darmstadt, Germany) was added and mixed by vortex for 4 min. After centrifugation (2000 × g, 10 min, 4 °C), 0.5 mL of the upper hexane layer was evaporated to dryness. The residue was dissolved in 0.5 mL of a mixture of methanol (Rathburn Chemicals, Walkerburn, United Kingdom), acetonitrile (Biosolve, Valkenswaard,
Netherlands), and dichloromethane (Mallinckrodt Baker). A 50-μL portion was injected on the HPLC system. The quality of the measurements was assessed by analyzing plasma quality-control samples in each batch. The CVs for the analysis of lutein, zeaxanthin, β-cryptoxanthin, all-trans lycopene, α-carotene, β-carotene, and α-tocopherol were 3.7%, 11.5%, 8.3%, 6.2%, 14.9%, 5.8%, and 2.8%, respectively (at mean concentrations of 0.16, 0.03, 0.11, 0.31, 0.05, 0.25, and 20.9 μmol/L in the quality-control samples, respectively). The HPLC system consisted of the following equipment: a Waters 2690 solvent delivery-injection system (Waters, Etten-Leur, Netherlands), a Waters 996 diode array, and a Jasco 920 fluorescence detector (Jasco Benelux, Maarssen, Netherlands). System control and data acquisition and processing were performed with the use of MILLENNIUM® chromatography manager, version 3.05 (Waters).

Separation was obtained by 2 C18 reversed-phase columns (250 × 4.6 mm, 5 μm) in series, thermostatically controlled at 30°C. The samples were eluted by use of a gradient consisting of methanol, acetonitrile, 2-propanol (Mallinckrodt Baker), and milliQ (Millipore, Etten-Leur, Netherlands) at a flow rate of 1.5 mL/min.

This HPLC system allows simultaneous detection of retinoids, tocopherols, and carotenoids and their isomers (Figure 1). In addition, the diode array data provide spectral information for confirmation of the identity of the analytes.

**Biopsies of adipose tissue**

About 50 mg adipose tissue for biopsy was transferred to glass tubes and weighed accurately. After the addition of 0.4 mL 10% sodium ascorbate solution (Sigma-Aldrich Chemie, Steinheim, Germany) and 2.0 mL of 1.5 mmol/L ethanolic KOH solution (Merck), the samples were saponified for 30 min at 80°C. Subsequently, the samples were cooled to room temperature, 2.0 mL diisopropyl ether (Merck) was added, and the samples were mixed vigorously. Of this extract, 2.5 mL was mixed by vortex with 2.5 mL water for 4 min. The upper layer of diisopropyl ether was isolated and evaporated to dryness. The residue was dissolved in 0.1–0.2 mL of a mixture of methanol, acetonitrile, and dichloromethane. A 50-μL portion was injected on the HPLC system, the same system used for the serum samples. In each series, quality-control samples consisting of a mixture of butter and olive oil were analyzed to check for reproducibility. The concentration of the quality-control sample was 0.39 μmol/kg lutein with a CV of 6.8%.

**Blood samples**

On the day of analysis, blood samples were thawed and mixed well. Vitamin C was extracted from whole blood by the use of metaphosphoric acid (Mallinckrodt Baker). Samples were allowed to stand for 20 min at 4°C to precipitate the protein and oxidize ascorbic acid to dehydroascorbic acid. After centrifugation, samples were derivatized with 1,2-diaminobenzene (Merck) to ascorbic acid to dehydroascorbic acid. After centrifugation, samples were derivatized with 1,2-diaminobenzene (Merck) to ascorbic acid to dehydroascorbic acid. After centrifugation, samples were derivatized with 1,2-diaminobenzene (Merck) to ascorbic acid to dehydroascorbic acid.

Vitamin C was extracted from whole blood by the use of metaphosphoric acid (Mallinckrodt Baker). Samples were allowed to stand for 20 min at 4°C to precipitate the protein and oxidize ascorbic acid to dehydroascorbic acid. After centrifugation, samples were derivatized with 1,2-diaminobenzene (Merck) to ascorbic acid to dehydroascorbic acid. After centrifugation, samples were derivatized with 1,2-diaminobenzene (Merck) to ascorbic acid to dehydroascorbic acid. After centrifugation, samples were derivatized with 1,2-diaminobenzene (Merck) to ascorbic acid to dehydroascorbic acid.

**Measurement of macular pigment density**

MP density was measured in the right eye, if possible. The subjects’ pupils were dilated with tropicamide 0.5% (Chauvin, Brussels). Spectral fundus reflectance was measured with the Utrecht retinal densitometer (22). Briefly, a rotating wheel (14 revolutions/s) offered a sequence of 14 interference filters in the range 430–740 nm to enable quasisimultaneous measurement of the reflectance across the visual spectrum. Light reflected from the fundus was measured in a detection field of 1.5°, concentric within the illumination field of 1.8°. To obtain an estimate of the mean MP density in this area, spectral fundus reflectance was
measured under 2 conditions: 1) with the instrument’s entry and exit pupils aligned to the peak of the Stiles-Crawford function and 2) 2 mm temporal to the Stiles-Crawford peak. A detailed optical model of foveal reflection was used to arrive at individual estimates of values such as equivalent thickness of blood layer and densities of the MP, lens, and melanin (22). In short, the incoming light was assumed to reflect at the inner limiting membrane, the disks in the outer segments of photoreceptors, and the sclera. With the use of known spectral characteristics of the different absorbers within the eye (lens, MP, melanin, and blood), the densities of the pigments and percentage reflectance at the interfaces were optimized to fit the measured data at all wavelengths. A previous study using the same method showed a within-subject CV of 17% (18).

Measurement of potential confounders and baseline characteristics

Iris color and smoking status were determined because of their previously documented associations with MP density (20, 23). Iris color was graded by a set of 4 standard photographs, providing a 5-grade classification system (24). Blue and gray irises were classified as grade 1 or 2. These grades were distinguished by assessing the proportion of total iris area with brown or yellow pigment. A green iris was assigned a grade of 2 or 3. These grades depended on the extent of brown or yellow pigment in the iris. A predominantly brown iris was assigned a grade of 3, 4, or 5, depending on the intensity of the yellow or brown pigment (24).

Smoking status was determined with a questionnaire. Subjects were classified into 6 categories ranging from nonsmoking category 0 to > 15 cigarettes/d (category 5).

Serum triacylglycerols were analyzed by enzymatic hydrolysis with subsequent enzymatic determination of the liberated glycerol by colorimetry (commercially available kit; Boehringer, Mannheim, Germany). Total cholesterol was analyzed by enzymatic conversion to a stable chromogen, which can be detected easily by colorimetry. HDL cholesterol was analyzed by precipitation with polyethylene glycol, centrifugation and enzymatic detection were analyzed by colorimetry, and LDL cholesterol was calculated by use of the formula of Friedewald et al (25).

Furthermore, age was determined, body weight and height were measured, and body mass index (BMI) was calculated. Body weight was measured while the subjects were wearing indoor clothing and no shoes. Height was measured while the subjects were shoeless. BMI was calculated as body weight (kg)/body height squared (m²).

Statistical analyses

The statistical software package SAS/STAT (version 6; SAS Institute, Cary, NC) was used for data analysis. We used t tests to estimate the differences in serum carotenoids, vitamins, MP density, and continuous baseline characteristics between men and women, smokers and nonsmokers, and those with high and low fruit and vegetable intakes. Analysis of variance was used to evaluate differences in MP density between grades of iris color. If we found significant differences between these grades, Scheffe’s procedure was used for multiple comparisons. Pearson’s correlation coefficients were calculated between baseline characteristics and both serum lutein concentrations and MP density. Correlation coefficients, adjusted for total cholesterol, were calculated between serum lutein, MP density, and other carotenoids and vitamins. Nonnormally distributed variables were log transformed.

Chi-square tests were used to evaluate possible differences in the numbers of men and women in the categories of baseline characteristics (iris color, smoking status, and fruit and vegetable consumption). Linear regression (general linear models procedure) was used to evaluate the association between MP density and serum lutein, serum zeaxanthin, and adipose lutein concentrations.

First, 2 linear models with the independent variables serum lutein or zeaxanthin, adjusted for age, were fitted (model 1a and model 1b). Then, a model with both serum lutein and zeaxanthin, adjusted for age, was performed (model 1c); this was our basic model. After inclusion of the sex variable and the lutein-by-sex or zeaxanthin-by-sex interaction term in the models, these interaction terms were significant (P < 0.05). This resulted in final general linear models for men and women separately. Variables that could influence the association between serum lutein and MP density—smoking status and BMI—were investigated as potential confounders. The models were adjusted for each potential confounder separately. The regression coefficient of serum lutein, after adjustment for each potential confounder, was compared with the regression coefficient of serum lutein with the basic model (model 1c) for men and women. If the change in the magnitude of the regression coefficient was > 10%, the confounder was added to the model. Total cholesterol was also included in the model, because serum carotenoids were related to serum lipid concentrations. We also corrected for other carotenoids and vitamins to evaluate whether they were confounders.

Mean serum lutein concentrations were calculated across quartiles of lutein divided by total cholesterol in men and women. Because of the heterogeneity of the female group (premenopausal and postmenopausal), a regression analysis to evaluate the association between serum lutein and MP density was performed among women aged ≥ 50 y. Previous procedures were also used to analyze the association between adipose tissue lutein concentration and MP density. If the P value was < 0.05, the regression coefficients of the variables were considered significant. Regression diagnostics were used to examine the residuals and influential points and to test the model assumptions.

Two volunteers squinted too much for proper measurement of the MP density. Furthermore, one measurement was not approved by the analyst of the MP density measurement, and one subject was withdrawn from the study before MP density measurement. Data from these 4 subjects were not included in the statistical analyses. Eight subjects were measured in the left eye instead of the right eye. Their measurements were included in the analysis because there is a good correlation between the 2 eyes (26). From the 187 adipose tissue sample obtained, the lutein concentrations of 21 samples were below the lower limit of quantification. One-half of the limit of detection value was assigned to these samples, and these data were included in the analyses. Data for 177 men and 199 women were used in the regression analyses after adjustment for age. Because data were missing data for some covariables, data for only 175 men and 197 women were used in the multiple variable regression analyses after adjustment for age, total cholesterol, BMI, and smoking status. In the 2 regression models for adipose lutein concentrations adjusted for age or for age, BMI, and smoking status, 187 values (89 men, 98 women) were used.

RESULTS

Study group characteristics

Characteristics of the study group at baseline and correlations between serum lutein and MP density are presented in
Tables 1 and 2, respectively. The 10th, 50th, and 90th percentiles of serum zeaxanthin concentrations were 0.03, 0.05, and 0.08 μmol/L. Iris color was not classified in all participants, but most of the participants had blue or gray irises (grade 1) (Table 1). In men, we found significantly higher serum lutein concentrations in nonsmokers than in smokers (grade 1) (Table 1). In both men and women, MP density in the total study group was 0.33 ± 0.15 (range: 0.02–0.80). Mean MP density was 13% higher in men than in women (P < 0.001). The correlation between serum lutein and adipose tissue were higher in women than in men (P < 0.001). Serum lutein concentrations were significantly correlated with blood vitamin C, α-tocopherol, and other serum carotenoids, except for lycopene, in both men and women after adjustment for total cholesterol.

### Macular pigment density
Mean MP density in the total study group was 0.33 ± 0.15 (range: 0.02–0.80). Mean MP density was 13% higher in men than in women (P = 0.02) (Table 1). In both men and women, MP density did not differ significantly between smokers and nonsmokers.

### Tables 1 and 2

### Table 1
Baseline characteristics of volunteers and serum lutein concentrations and macular pigment (MP) density per category

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total group (n = 177 M, 199 F)</th>
<th>Smoker (n = 120 M, 144 F)</th>
<th>Yes (n = 57 M, 55 F)</th>
<th>Fruit and vegetable consumption</th>
<th>Low (n = 116 M, 98 F)</th>
<th>High (n = 61 M, 101 F)</th>
<th>Iris color</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.16 ± 0.07</td>
<td>0.17 ± 0.07d</td>
<td>0.15 ± 0.06</td>
<td>0.16 ± 0.06d</td>
<td>0.18 ± 0.07</td>
<td>0.19 ± 0.06</td>
<td>Grade 1: blue or gray (n = 101 M, 115 F)</td>
</tr>
<tr>
<td>Women</td>
<td>0.19 ± 0.08</td>
<td>0.19 ± 0.09</td>
<td>0.17 ± 0.08</td>
<td>0.16 ± 0.06d</td>
<td>0.21 ± 0.09</td>
<td>0.36 ± 0.17</td>
<td>Grade 2: blue, gray, or green (n = 28, 24 F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 3: green or light brown (n = 10 M, 15 F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 4: brown (n = 13 M, 13 F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 5: dark brown (n = 7 M, 5 F)</td>
</tr>
<tr>
<td><strong>Serum lutein (μmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total group</td>
<td>0.16 ± 0.07</td>
<td>0.17 ± 0.07d</td>
<td>0.15 ± 0.06</td>
<td>0.16 ± 0.06d</td>
<td>0.18 ± 0.07</td>
<td>0.19 ± 0.06</td>
<td>Grade 1: blue or gray (n = 101 M, 115 F)</td>
</tr>
<tr>
<td>Smoker (n = 120 M, 144 F)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 2: blue, gray, or green (n = 28, 24 F)</td>
</tr>
<tr>
<td>Yes (n = 57 M, 55 F)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 3: green or light brown (n = 10 M, 15 F)</td>
</tr>
<tr>
<td>Fruit and vegetable</td>
<td>Low (n = 116 M, 98 F)</td>
<td>0.16 ± 0.06d</td>
<td>0.17 ± 0.07</td>
<td>0.17 ± 0.07h</td>
<td>0.19 ± 0.07d</td>
<td>0.22 ± 0.12d</td>
<td>Grade 1: blue or gray (n = 101 M, 115 F)</td>
</tr>
<tr>
<td>consumption</td>
<td>High (n = 61 M, 101 F)</td>
<td>0.18 ± 0.07</td>
<td>0.21 ± 0.09</td>
<td>0.28 ± 0.14</td>
<td>0.36 ± 0.17</td>
<td>0.50 ± 0.19d</td>
<td>Grade 2: blue, gray, or green (n = 28, 24 F)</td>
</tr>
<tr>
<td>Iris color</td>
<td>Grade 1: blue or gray (n = 101 M, 115 F)</td>
<td>0.16 ± 0.07</td>
<td>0.19 ± 0.09</td>
<td>0.35 ± 0.14</td>
<td>0.31 ± 0.14</td>
<td></td>
<td>Grade 1: blue or gray (n = 101 M, 115 F)</td>
</tr>
<tr>
<td></td>
<td>Grade 2: blue, gray, or green (n = 28, 24 F)</td>
<td>0.15 ± 0.06</td>
<td>0.19 ± 0.06</td>
<td>0.33 ± 0.15</td>
<td>0.33 ± 0.15</td>
<td></td>
<td>Grade 2: blue, gray, or green (n = 28, 24 F)</td>
</tr>
<tr>
<td></td>
<td>Grade 3: green or light brown (n = 10 M, 15 F)</td>
<td>0.17 ± 0.07</td>
<td>0.17 ± 0.07</td>
<td>0.32 ± 0.17</td>
<td>0.28 ± 0.18</td>
<td></td>
<td>Grade 3: green or light brown (n = 10 M, 15 F)</td>
</tr>
<tr>
<td></td>
<td>Grade 4: brown (n = 13 M, 13 F)</td>
<td>0.17 ± 0.07</td>
<td>0.19 ± 0.06</td>
<td>0.28 ± 0.14</td>
<td>0.30 ± 0.13</td>
<td></td>
<td>Grade 4: brown (n = 13 M, 13 F)</td>
</tr>
<tr>
<td></td>
<td>Grade 5: dark brown (n = 7 M, 5 F)</td>
<td>0.19 ± 0.06</td>
<td>0.22 ± 0.12</td>
<td>0.50 ± 0.19</td>
<td>0.32 ± 0.15</td>
<td></td>
<td>Grade 5: dark brown (n = 7 M, 5 F)</td>
</tr>
</tbody>
</table>

### Table 2
Correlation between continuous variables at baseline and macular pigment (MP) density or serum lutein in 376 volunteers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>R for lutein</th>
<th>R for MP density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>42.6 ± 15</td>
<td>0.26</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5 ± 3.5</td>
<td>0.05</td>
<td>−0.24</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.82 ± 0.08</td>
<td>−0.30</td>
<td>−0.13</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>81.4 ± 13.2</td>
<td>−0.21</td>
<td>−0.23</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.39 ± 0.96</td>
<td>0.20</td>
<td>−0.10</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.82 ± 0.96</td>
<td>0.15</td>
<td>−0.08</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.39 ± 0.31</td>
<td>0.21</td>
<td>0.07</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>1.20 ± 0.63</td>
<td>−0.01</td>
<td>−0.18</td>
</tr>
</tbody>
</table>

* n = 177 M and 199 F unless indicated otherwise.
* ± SD.
* Correlation between serum lutein and baseline characteristics.
* Correlation between MP density and baseline characteristics.
* P < 0.05.
* n = 176 M.
* Significantly different from men: 7 P < 0.001, 10 P < 0.01.
* P < 0.06.
* n = 198 F.
TABLE 3
Serum and adipose tissue carotenoid, serum α-tocopherol, and blood vitamin C concentrations in men and women and the correlations with serum lutein and macular pigment (MP) density

<table>
<thead>
<tr>
<th>Variable</th>
<th>Concentration</th>
<th>R for lutein</th>
<th>R for MP density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Serum lutein (μmol/L)</td>
<td>0.16 ± 0.07</td>
<td>0.19 ± 0.08</td>
<td>—</td>
</tr>
<tr>
<td>Serum zeaxanthin (μmol/L)</td>
<td>0.05 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.64 ± 0.07</td>
</tr>
<tr>
<td>Serum β-cryptoxanthin (μmol/L)</td>
<td>0.17 ± 0.09</td>
<td>0.22 ± 0.13</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>Serum all-trans lycopene (μmol/L)</td>
<td>0.35 ± 0.18</td>
<td>0.37 ± 0.18</td>
<td>0.001 ± 0.14</td>
</tr>
<tr>
<td>Serum α-carotene (μmol/L)</td>
<td>0.06 ± 0.06</td>
<td>0.08 ± 0.06</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>Serum β-carotene (μmol/L)</td>
<td>0.33 ± 0.19</td>
<td>0.40 ± 0.25</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td>Serum α-tocopherol (μmol/L)</td>
<td>26.0 ± 5.5</td>
<td>27.6 ± 6.6</td>
<td>0.23 ± 0.07</td>
</tr>
<tr>
<td>Blood vitamin C (μmol/L)</td>
<td>40.2 ± 12.2</td>
<td>49.9 ± 13.4</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Adipose tissue lutein (μmol/kg wet wt)</td>
<td>0.32 ± 0.17</td>
<td>0.47 ± 0.31</td>
<td>0.46 ± 0.07</td>
</tr>
</tbody>
</table>

1n = 177 M and 199 F. Lutein, β-cryptoxanthin, α-carotene, β-carotene, and α-tocopherol in serum and adipose tissue are not normally distributed; variables log transformed before t test and correlation analyses.

2X ± SD.

3Significantly different from women, P < 0.05.

4P < 0.05.

5P < 0.05.

6n = 89 M and 98 F.

or between those with high and low fruit and vegetable consumption. In men, MP density of iris color grade 5 was significantly higher than that of iris color grade 4 (Table 1). MP density correlated significantly with BMI (men), body weight (men), LDL cholesterol (women), and triacylglycerol (both sexes) (Table 2).

In men, MP density correlated positively with serum lutein and zeaxanthin (both P < 0.05), β-cryptoxanthin, α-carotene, and β-carotene (all P < 0.05) after adjustment for total cholesterol. Vitamin C was correlated significantly with MP density in men and women. The partial correlation between MP density and α-tocopherol adjusted for total cholesterol was significant in women. Serum lutein and zeaxanthin were not associated with MP density in women (Table 3). Adipose tissue lutein concentrations were positively associated with MP density in men but not in women (Table 3).

Regression modeling

Serum lutein and zeaxanthin

Serum lutein and zeaxanthin concentrations appeared to be relevant predictors of MP density in men after adjustment for age (models 1a and 1b; Table 4). In the model in which serum lutein and zeaxanthin were both included, serum lutein predicted MP density in men, but zeaxanthin did not (model 1c; Table 4). In women, serum lutein, zeaxanthin, or both were not associated with MP density (models 1a, 1b, and 1c; Table 4).

BMI changed the magnitude of the regression coefficient of serum lutein in both men and women and was therefore included in the adjusted models. Smoking status changed the magnitude of the regression coefficient of serum lutein in women and was therefore included in the adjusted models. Total cholesterol was included in the multivariate model because of the correlation between serum lipids and serum carotenoids. From the multivariate model with serum lutein and zeaxanthin (model 2c; Table 4), we conclude that a serum lutein increment of 0.1 μmol/L is associated with a 0.064 higher MP density (18% of the mean) in men after adjustment for age, total cholesterol, BMI, and smoking status.

In women, serum lutein and zeaxanthin concentrations were not associated with MP density in the adjusted models (models 2a, 2b, and 2c; Table 4). We calculated mean MP density values across quartiles of lutein divided by total cholesterol. In men, MP density

TABLE 4
Multivariate regression models to evaluate the association between serum lutein, zeaxanthin, or both and macular pigment (MP) density

<table>
<thead>
<tr>
<th>Model</th>
<th>β (SE)</th>
<th>R²</th>
<th>β (SE)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td></td>
<td></td>
<td>β</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1a</td>
<td>0.081 (0.017)</td>
<td>0.12</td>
<td>0.016 (0.013)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 1b</td>
<td>0.227 (0.062)</td>
<td>0.07</td>
<td>0.062 (0.046)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 1c</td>
<td>0.071 (0.022)</td>
<td>0.12</td>
<td>0.009 (0.017)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 2a</td>
<td>0.080 (0.018)</td>
<td>0.17</td>
<td>0.022 (0.014)</td>
<td>0.05</td>
</tr>
<tr>
<td>Model 2b</td>
<td>0.241 (0.065)</td>
<td>0.14</td>
<td>0.068 (0.049)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2c</td>
<td>0.064 (0.023)</td>
<td>0.18</td>
<td>0.016 (0.017)</td>
<td>0.05</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>0.091 (0.083)</td>
<td>0.034 (0.061)</td>
<td>0.034 (0.061)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1Model 1a: MP density = serum lutein (0.1 μmol/L) + age (y) (n = 177 M, 199 F); Model 1b: MP density = serum zeaxanthin (0.1 μmol/L) + age (y) (n = 177 M, 199 F); Model 1c: MP density = serum lutein (0.1 μmol/L) + serum zeaxanthin (0.1 μmol/L) + age (y) (n = 177 M, 199 F); Models 2a, 2b, and 2c: adjusted for age (y), BMI (kg/m²), total cholesterol (mmol/L), and smoking status: 0: no smoking; 1: <1 cigarette/d; 2: 1–5 cigarettes/d; 3: 6–10 cigarettes/d; 4: 11–15 cigarettes/d; 5: >15 cigarettes/d.

2P < 0.001.

3P < 0.010.
In women, vitamin C was positively associated with MP density (interaction term < 0.01). To evaluate whether these fluctuations influenced individual plasma carotenoids fluctuate by the phase of the menstrual cycle which included both premenopausal and postmenopausal women:ISON among women is the heterogeneity of the female group, especially in the postmenopausal period. To compare the subjects from whom we took an adipose tissue sample and the total group, we evaluated the association between serum lutein and MP density in the subgroup in whom we had collected adipose tissue samples (89 men, 98 women). After adjustment for age, total cholesterol, BMI, and smoking status, the coefficients of serum lutein were higher in this subgroup of men (β = 0.088, SE = 0.026, P < 0.01; n = 89) and this subgroup of women (β = 0.049, SE = 0.024, P = 0.04; n = 97) than in the total groups of men and women. In this subgroup, the associations between serum lutein and MP density in women were weaker than those in men. The serum lutein-by-sex interaction term was not significant, which suggests that the associations were not significantly different between men and women. We evaluated the difference in CIs of the regression coefficients of serum lutein between the subgroup of women from whom we obtained adipose tissue samples and the remaining women (n = 101). The regression coefficients of serum lutein in these 2 groups were 0.049 (95% CI: 0.0020, 0.096) and 0.0025 (95% CI: −0.033, 0.038), respectively. The exclusion of samples below the detection limit did not change the results.

**DISCUSSION**

This study was conducted to assess the cross-sectional associations between the serum carotenoids lutein and zeaxanthin and MP density. In addition to biomarkers reflecting short-term (serum) intake of carotenoids, we evaluated, as a biomarker of long-term intake, the associations of adipose tissue lutein and MP density.

Serum and adipose concentrations of lutein are significantly higher in women, but their MP density is lower than that of men. The relations of serum lutein, serum zeaxanthin, and adipose tissue lutein with MP density are stronger in men than in women.

The strength of this study was the large study group, which enabled us to compare men and women. MP density was assessed with an objective noninvasive measuring technique, in contrast...
with most other studies, which used a technique that was rather demanding for subjects (14, 16, 17, 19, 20).

There were several limitations of this study that may have attenuated its results. We aimed to select Dutch volunteers with high and low fruit and vegetable intakes, so that a wide range of carotenoid intakes were included. At the end of the study, the range of serum lutein concentrations in men and women was 0.06–0.41 and 0.06–0.65 μmol/L, respectively. These ranges are similar to those for male and female subjects in the Netherlands: 0.07–0.42 and 0.08–0.51 μmol/L, respectively (28). However, serum lutein concentrations in subjects from the United States or southern Europe (Spain and Italy) are higher than those in subjects from the Netherlands (10, 14, 28), and therefore, in a global context, the range in serum carotenoids in the present study is narrow in comparison with the differences in carotenoid concentrations between populations. This difference in serum lutein between populations may reflect a lower intake of supplements, fortified foods, or lutein-containing fruit and vegetables among people in this study than among other study populations.

We did not perform duplicate MP density measurements in each subject. A previous study showed a within-subject CV of 17% (18). This measurement error could have attenuated the associations between concentrations of carotenoids in serum and adipose tissue and MP density. A possible source of bias was the ability of subjects to consent or to refuse the adipose tissue sampling. Compared with the men from whom an adipose tissue sample was not taken, the men from whom such a sample was taken had a somewhat higher MP density; however, no differences in other characteristics, including BMI, were observed between the groups of men and women with and without adipose tissue data. We evaluated the association between serum lutein and MP density in the subgroup from whom we had collected adipose tissue samples and compared it with the associations in the total study group. In women, the differences in these associations between the total group and subgroup are difficult to explain and may have been due to chance or selection bias.

In contrast with other studies (20, 29), we found no associations between smoking status and MP density. A possible explanation for this discrepancy is that smoking status was classified by the number of cigarettes smoked per day. We had no information on smoking duration or smoking history. The covariate “iris color” was not associated with MP density in the total population, contrary to the results of 2 studies that found significant differences in MP densities among iris color grades (23, 29). Most of our volunteers had an iris color of grade 1 (blue or gray). However, we found a positive association between iris color and MP density in men. This was largely attributable to men with an iris color of grade 5 (dark brown) who had a high MP density (0.50 ± 0.19). We found no association with age. Another study found a slight decline in MP density with age (29).

A previous study also found stronger correlations between plasma lutein and MP density for men (r = 0.62, P < 0.005) than for women (r = 0.3, P < 0.05) (14). The baseline results of a study with lutein supplementation showed that plasma lutein concentrations were highly correlated with MP density (r = 0.82, P < 0.001) in men (18). Our results are in line with the sex differences found in other studies, but our correlations were generally weaker. Past studies with sources of extra lutein have shown an increase in MP density in women (19). The narrow range of serum lutein concentrations in the present study relative to past studies may explain the weaker associations between serum lutein and MP density in this study. In cross-sectional studies, variables are determined at one point in time. Therefore, it is difficult to assess the temporal relation between nutritional factors—eg, serum lutein—and the dependent variable—in this case, MP density. Furthermore, our study group consisted of both premenopausal and postmenopausal women, and serum carotenoids fluctuate by the phase of the menstrual cycle (27, 30). Therefore, serum lutein concentrations in women may be a poorer reflection of past lutein intake. However, serum lutein was not associated with MP density in postmenopausal women. A comparison of MP density values across quartiles of lutein divided by total cholesterol showed a linear relation in men but not in women. In women, MP density was higher in the last quartile of serum lutein adjusted for total cholesterol. This could suggest that, in women, serum lutein is associated with MP density only at high serum lutein concentrations.

Blood vitamin C concentrations were positively associated with MP density in women. Vitamin C is abundant in the eye, including the retina and the lens. Vitamin C may participate with other antioxidants in electron transfer reactions to reduce oxidative stress in the eye, but the exact mechanism is not evident (31). We hypothesize that, because of this antioxidant capacity, vitamin C is positively associated with MP density. It could spare the antioxidants lutein and zeaxanthin in the MP. Furthermore, this positive association between vitamin C and MP density could reflect the fact that vitamin C and carotenoids exist in the same foods. Therefore, vitamin C could function as a marker of carotenoid intake. Observational epidemiologic studies are inconclusive as to the role of vitamin C in the prevention of AMD (4, 31).

Concentrations of fat-soluble vitamins in adipose tissue and in plasma (serum) can be used as markers of dietary intake. Because both take into account variations in absorption and metabolism, it may be better to classify the markers as markers of internal dose rather than as markers of dietary intake (32). The results of the association between adipose tissue concentrations and MP density were in line with the associations between serum lutein concentrations and MP density in both men and women. Lutein and zeaxanthin are dynamic components of tissues, and therefore the metabolism of lutein may differ between men and women (16). A previous study showed a negative correlation between MP density and adipose tissue lutein in women but a positive correlation in men (16). However, that study was limited by a small number of subjects (13 women, 8 men). In our study, in which we collected a larger number of adipose tissue samples (75 men, 91 women), we found a positive association between adipose tissue lutein and MP density in men and a slightly negative association in women (Figure 3). Because of this slight negative association, our findings do not contradict the suggestion that adipose tissue competes with MP for carotenoids more effectively in women than in men (16). As far as we know, the exact mechanism of lutein and zeaxanthin uptake in the retina has remained elusive.

In summary, men had lower concentrations of serum and adipose tissue lutein but a higher MP density. From these findings, we conclude that a serum lutein increment of 0.1 μmol/L is associated with a 0.064 higher MP density (18% of the mean) in men. We suggest, therefore, that dietary intervention to increase MP density may have an effect in men. In women, no significant association between serum lutein and MP density was found in the total group of women. In the subgroup of women who gave an adipose tissue sample, a positive association between serum lutein and MP density was found, but this association was weaker than
that in men. Adipose tissue could be an important factor in the association between serum lutein and MP density. Carotenoid concentrations in adipose tissue have to be taken into account to elucidate the influence of lutein intake on MP density. If MP density has a protective effect against AMD, our results in the total population agree with the notion that men are at lower risk of AMD than are women (12). Dietary intervention studies are necessary to investigate the influence of lutein intake on MP density and AMD in men and women.

We thank the volunteers who participated in the study; Hanny Leezer, Eric Busink, Wilfred Sieling, Inge van den Assum, Jose Jacobs, Soesila Sukhraj, Harm Korthals, and all other staff of the Metabolic Research Unit and laboratories, who assisted in the organization of the study and analyses of the blood samples and adipose tissue samples; Agnes Franssen, who measured MP density; Cor Kistemaker for the check on statistical analyses; and Astrid Kruizinga for performing data management.

REFERENCES