Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density1–3

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ABSTRACT

Background: Lutein and zeaxanthin are the only carotenoids in the macular region of the retina (referred to as macular pigment [MP]). Foods that are rich in lutein and zeaxanthin can increase MP density. Response to dietary lutein and zeaxanthin in other tissues has not been studied.

Objective: The objective of this study was to examine tissue responses to dietary lutein and zeaxanthin and relations among tissues in lutein and zeaxanthin concentrations.

Design: Seven subjects consumed spinach and corn, which contain lutein and zeaxanthin, with their daily diets for 15 wk. At 0, 4, 8, and 15 wk and 2 mo after the study, serum, buccal mucosa cells, and adipose tissue were analyzed for carotenoids, and MP density was measured.

Results: Serum and buccal cell concentrations of lutein increased significantly from baseline during dietary modification. Serum zeaxanthin concentrations were greater than at baseline only at 4 wk, whereas buccal cell and adipose tissue concentrations of zeaxanthin did not change. Adipose tissue lutein concentrations peaked at 8 wk. Changes in adipose tissue lutein concentration were inversely related to the changes in MP density, suggesting an interaction between adipose tissue and retina in lutein metabolism. To investigate the possibility of tissue interactions, we examined cross-sectional relations among serum, tissue, and dietary lutein concentrations, anthropometric measures, and MP density in healthy adults. Significant negative correlations were found between adipose tissue lutein concentrations and MP for women, but a significant positive relation was found for men.

Conclusion: Sex differences in lutein metabolism may be an important factor in tissue interactions and in determining MP density. Am J Clin Nutr 2000;71:1555–62.

KEY WORDS Carotenoids, lutein, zeaxanthin, macular pigment, adults

INTRODUCTION

Some epidemiologic reports (1) suggest that dietary intake of foods that are rich in carotenoids, especially lutein and zeaxanthin, protects against age-related macular degeneration (AMD). However, multiple factors, such as genetics, sunlight exposure, and cigarette smoking, are probably involved (1). The protection by lutein and zeaxanthin may be explained by the fact that these carotenoids are selectively accumulated in the retina and are particularly dense in the macula (1). In the macula, these carotenoids are referred to as macular pigment (MP). By preventing light-initiated oxidative damage to the retina and retinal pigment epithelium, MP may protect against age-related deterioration (2). Thus, an increase in MP density may retard age-related changes that lead to AMD. Supplemental lutein and zeaxanthin are currently being sold and promoted for their ability to protect the retina from oxidative damage. Experimental data show that purified supplements (3) and foods rich in lutein and zeaxanthin (4) can increase MP density. For reasons that are not clear, the response to dietary lutein and zeaxanthin in macula varies among individuals (4). Moreover, it is not clear how intake of lutein and zeaxanthin affect other tissues in the body while MP is increasing. Body fat is of particular interest, because adipose tissue is a major storage organ for carotenoids as a result of the partition of carotenoids into fat (5, 6). Furthermore, serum concentrations of carotenoids tend to reflect recent intakes of carotenoids, whereas adipose tissue concentrations of carotenoids are a better indicator of long-term intakes. Also, adipose tissue makes up a large portion of the total body weight. Given the large range among people in the amount of body fat, adipose tissue and body composition may have a strong influence on the distribution of carotenoids within the body.

In our experiments, we examined the effects of consuming foods rich in lutein and zeaxanthin on tissue concentrations of lutein and zeaxanthin. Furthermore, we evaluated cross-sectional relations between lutein and zeaxanthin concentrations in the retina, serum, adipose tissue, buccal mucosa cells (BMCs), and diet, along with indexes of body composition, because these...
2 carotenoids provide useful information on carotenoid status with relatively simple sampling methods. Evaluation of such relations is important for the understanding of overall lutein and zeaxanthin dynamics in vivo and also for determining factors that govern the response of MP density to dietary lutein and zeaxanthin.

SUBJECTS AND METHODS
Study 1: serum and tissue responses to dietary lutein and zeaxanthin

Subjects

Healthy adults (4 women and 3 men aged 33–54 y) were recruited from the general population for participation in this study. The subjects had no history of small-bowel disease, alcoholism, pancreatic disease, atrophic gastritis, hyperlipidemia, type 1 diabetes, kidney stones, or bleeding disorders. The study protocol was approved by the Human Investigative Review Committee of Tufts University and the New England Medical Center. Informed consent was obtained from all subjects. Smoking was not permitted during the study.

Spinach and corn feeding

Frozen spinach and corn were purchased in a single lot. Spinach (60 g/d) and corn (150 g/d) were distributed to the subjects in 1-wk allotments. Spinach and corn were chosen because, relative to other fruit and vegetables, they have high amounts of lutein and zeaxanthin, respectively, and are common in the US diet. The subjects were asked to eat the spinach and corn with a meal or a fat source, or both, while maintaining their usual dietary habits for the duration of the 15-wk study. One subject was given only 60 g spinach/d because of difficulties with the consumption of corn associated with diverticulosis. The carotenoids in the food supplements were measured in 4 samples each of spinach and corn by using the modified official method of analysis of the Association of Official Analytical Chemists (7) and analyzed with the same HPLC system as was used for serum and tissue analysis. Peak identification was confirmed by absorption spectra taken from the diode-array detector during HPLC analysis. The 60-g serving of spinach contained 19.0 μmol lutein/d, 0.5 μmol zeaxanthin/d, and 9.3 μmol β-carotene/d. The corn contained 0.7 μmol lutein/d and 0.5 μmol zeaxanthin/d. For comparison, a typical diet high in fruit and vegetables contains ≈4.0 μmol lutein/d and 0.5 μmol zeaxanthin/d (8) and typical β-carotene intakes of American adults are 0.9–12.1 μmol/d (9, 10). Therefore, during the dietary modification, subjects received ≈5 times as much lutein and 2 times as much zeaxanthin as in a usual healthy diet.

Schedule of measurements

At the start of the study, 2 baseline fasting blood samples and 2 measurements of MP density were obtained on 2 separate days, 1–2 wk apart. Serum was prepared from the blood samples (800 × g for 15 min at 4°C) for later analysis of carotenoids. To assess dietary carotenoid intake, the Health Habits and History Questionnaire was administered (11). The database for this questionnaire sums zeaxanthin values with lutein values; however, of the 2, lutein is the major component (12). In addition, single baseline samples of BMCs and adipose tissue were collected. At 4, 8, 12, and 15 wk of the spinach and corn feeding period, serum, BMCs, and dietary questionnaires were collected and MP densities were measured. Adipose tissue biopsies were performed at 4, 8, and 15 wk. Two months after the end of the spinach and corn feeding period, serum, BMCs, adipose tissue, and dietary questionnaires were collected and MP was measured for poststudy comparisons.

Study 2: cross-sectional relations among anthropometric measurements, macular pigment density, and serum, tissue, and dietary lutein concentrations

Subjects

Cross-sectional relations were obtained for healthy adults who were recruited from the general population (13 women and 8 men aged 33–83 y). Ten of the women were postmenopausal; one of these was receiving estrogen replacement therapy. All subjects were nonsmoking. Two blood samples and 2 measurements of MP density were obtained within a 2-wk period. BMC and adipose tissue samples were obtained once and percentage body fat was measured once. Two women did not participate in the adipose tissue sampling because of aversion to the needle biopsy procedure. Dietary carotenoid intake was assessed by using the Health Habits and History Questionnaire (11).

Buccal mucosa cells and adipose tissue collection

BMCs were collected by the method of Peng et al (13). Briefly, after rinsing their mouths vigorously with drinking water, the subjects were asked to brush the insides of their cheeks with a soft toothbrush for ≈1 min on each side. After the brushing, they rinsed their mouths with 30 mL distilled water and deposited the rinsing solution into a 50-mL vial. The toothbrush was then washed with 20 mL water, which was deposited into the vial. BMCs were centrifuged at 800 × g for 10 min at 4°C and the supernate discarded. To the pellet was added 10 mL of phosphate-buffered saline. After mixing in a Deluxe Mixer (Scientific Products, McGraw Park, IL), the sample was centrifuged at 800 × g for 5 min at 4°C and the supernate removed. To the pellet was added 0.85 mL cold phosphate-buffered saline.

Adipose tissue samples were collected by using needle biopsies as described by Beynen and Katan (14). Subcutaneous adipose tissue was obtained from the lateral buttock by using a 15-gauge needle attached to an evacuated tube containing 1 mL saline solution. The container was placed immediately on ice. All serum and tissue samples were protected from light. Samples were stored at −70°C until analysis for carotenoids.

Serum and tissue analysis for carotenoids

Serum extraction

Serum was prepared for extraction by using a 150-μL sample and 1 mL 0.9% saline solution. Echinonone, in ethanol, was added as an internal standard. The mixture was extracted by using 2 mL CHCl₃:CH₂OH (2:1, by vol). The mixture was mixed in a Deluxe Mixer and then centrifuged at 800 × g for 15 min at 4°C. The CHCl₃ layer was removed and evaporated to dryness under nitrogen. The mixture was extracted again by using 3 mL hexane. The mixture was again mixed and centrifuged as described above. 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 in a Deluxe Mixer, and sonicated for 30 s. A 50-μL portion was used for HPLC analysis (8).
Buccal mucosa cell extraction

BMCs were extracted for carotenoids according to the method of Peng et al (13). Briefly, frozen cells were allowed to thaw at room temperature for a few minutes. After being mixed in a Deluxe Mixer, a 10-μL sample was taken for protein determination by using bicinchoninic acid (15). To 1.0 mL cells, 1–2 μg butylated hydroxytoluene (BHT) crystal and 200 μL 1% protease solution (prolase E from Streptomyces griseus; Sigma Chemical Company, St Louis) were added and the tubes were incubated at 37°C for 45 min. After the incubation, the samples were treated with 400 μL 1% sodium dodecyl sulfate in ethanol containing 0.1% BHT (wt:vol:wt) and then extracted by using 3 mL ether:hexane (2:1, by vol). Echinonone, in ethanol, was added as an internal standard. The mixture was mixed in a Deluxe Mixer and then centrifuged at 800 × g for 15 min at 4°C. The upper layer was removed. The ether:hexane extraction was repeated and combined with the first extraction and evaporated to dryness under nitrogen. The residue was redissolved in 150 μL ethanol, mixed in a Deluxe Mixer, and sonicated for 30 s. A 50-μL sample was used for HPLC analysis. BMC concentrations of carotenoids are expressed as pmol/mg protein.

Adipose tissue extraction

Carotenoids were extracted from ~30 mg adipose tissue. Samples were lyophilized (20 h at -20°C, <100 lb/sq inch). To the sample was added 100 μL 12% pyrogallol in ethanol, 200 μL 30% KOH, and 1 mL ethanol. The mixture was mixed in a Deluxe Mixer and incubated at 37°C for 2 h. After incubation, the sample was cooled down to room temperature and 1 mL water was added; the mixture was then mixed in a Deluxe Mixer. Echinonone in ethanol (100 μL) was added as an internal standard. The mixture was extracted by using 3 mL ether:hexane (2:1, by vol). Echinonone, in ethanol, was added as an internal standard. The mixture was mixed and then centrifuged at 800 × g at 4°C for 5 min. The upper layer was then removed. The extraction with ether:hexane was repeated and the upper layers combined. To the extract was added 1 mL water. The mixture was mixed in a Deluxe Mixer and 1 mL ethanol was added to make the solution clear. The mixture was centrifuged at 800 × g for 5 min. The water layer (lower layer) was removed and discarded. Another 1 mL water was added and removed as described above. The extract was evaporated to dryness under nitrogen. The residue from adipose tissue was redissolved in 100 μL ethanol, mixed, and sonicated for 30 s. A 50-μL sample was used for HPLC analysis. Adipose tissue concentrations of carotenoids are expressed as pmol/mg dry wt.

Chemicals

HPLC-grade methanol and water were purchased from JT Baker Chemical Co, Phillipsburg, NJ. Methyl-tert-butyl ether, carotenoids used for HPLC standard curves, and ammonium acetate were purchased from Sigma Chemical Co. Echinonone was purchased from Roche, Inc, Nutley, NJ. Solvents were acetate were purchased from Sigma Chemical Co. Echinenone in ethanol, mixed in a Deluxe Mixer, and sonicated for 30 s. A 50-μL sample was used for HPLC analysis. BMC concentrations of carotenoids are expressed as pmol/mg protein.

HPLC analysis

The HPLC system consisted of a series 410 LC pump (Perkin-Elmer, Norwalk, CT), a Waters 717 plus autosampler (Millipore, Milford, MA), a C30 carotenoid column (3 μm, 150 × 4.6 mm; YMC, Wilmington, NC), HPLC column temperature controllers (model 7950 column heater and chiller; Jones Chromatography, Lakewood, CO), a Waters 994 programmable photodiode-array detector, and a Waters 840 digital 350 data station. The HPLC column was maintained at 16°C. The HPLC mobile phase was methanol:methyl-tert-butyl ether:water (83:15:2, by vol, with 1.5% ammonium acetate in water, solvent A) and methanol:methyl-tert-butyl ether:water (8:90:2, by vol, with 1% ammonium acetate in water, solvent B). The gradient procedure, at a flow rate of 1 mL/min at 16°C, was as follows. The procedure began at 100% solvent A before going to 93% solvent A and 7% solvent B over a 1-min linear gradient. This was followed by a 3-min hold at 93% solvent A, followed by a 17-min linear gradient to 45% solvent A and a 1-min hold at 45% solvent A, then an 11-min linear gradient to 95% solvent B, a 4-min hold at 95% solvent B, and finally a 2-min gradient back to 100% solvent A. The system was held at 100% solvent A for 10 min for equilibration back to initial conditions. By using this method, lutein, zeaxanthin, cryptoxanthin, α-carotene, 13-cis-β-carotene, all-trans-β-carotene, and 9-cis-β-carotene are adequately separated. Also, 4 geometric isomers of lycopene (15-cis, 13-cis, 9-cis, and all-trans lycopenes) are separated.

Carotenoids were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. Concentrations were corrected for extraction and handling losses by monitoring the recovery of the internal standards. The lower limit of detection is 0.2 pmol for carotenoids.

Measurement of density of macular pigment

MP density (for a 1° stimulus) was measured by flicker photometry as the difference between sensitivity to 460 and 550 nm lights in the fovea compared with the parafovea. This percuttal test determines the sum of lutein and zeaxanthin optical densities in the retina at the border of the stimulus, which corresponds to a retinal location =150 μm from the center of the fovea. MP density declines approximately exponentially with distance from the center of the fovea (16), so a measurement at this single location provides an index of the amount of MP in the retina. Our measurement scale (density units) for MP is directly related to the actual chemical concentrations of the pigments in the retina (17). For a full description of the apparatus and procedure, readers are referred to Hammond et al (16); for background information about this psychophysical method, readers are referred to Snodderly and Hammond (18).

Body fat determination and anthropometric measurements

Body fat was determined by using hydrodensitometry. The weight of each subject was determined by using a Sauter Scale (model K120; Denshore Scale, Holbrook, MA) while the subject was seated and submerged in water. A weight belt was placed around the subject’s waist to ensure a positive underwater weight. Body fat and lean tissue were calculated by using densities of 1.100 and 0.900 g/L for fat-free and fat tissue, respectively. Percentage body fat was calculated by applying Siri’s equation (19) and fat-free mass (FFM; in kg) was calculated by subtraction of fat mass from total body weight (19). Anthropometric measurements that were considered were body mass index (BMI; in kg/m²), percentage body fat, amount of body fat (in kg), percentage FFM, and FFM (in kg).

Statistics

Results are expressed as means ±SEs. The averages of the 2 baseline measurements were used as the baseline values for serum lutein and zeaxanthin and for MP density. In the feeding
study, significant differences from baseline were measured by using analysis of variance (ANOVA) with repeated measurements at the 95% confidence level (STATVIEW 5.0, 1998; SAS Institute, Inc, Cary, NC). We controlled for cumulated type I error by planning the comparisons that were made, minimizing the number of comparisons, and considering each comparison as independent. However, we also considered the effects of multiple testing and used the Bonferroni test when the result of the F test was significant (STATVIEW). Student’s unpaired t test was used to compare differences between sexes. Spearman correlations were used to examine cross-sectional relations (20). Analysis of covariance (ANCOVA) was used to measure differences in cross-sectional relations between women and men (21). Differences for which P < 0.05 were regarded as significant.

RESULTS

Study 1: serum and tissue response to dietary lutein and zeaxanthin

Serum

Lutein concentrations in serum significantly increased 2-fold from baseline values after 4 wk of spinach and corn feeding and remained significantly higher than baseline values throughout the 15-wk supplementation period. When the Bonferroni correction was applied for multiple testing, however, the P value of 0.05 did not meet the critical value of P < 0.01 for significance. Two months after consumption of the spinach and corn was discontinued, serum concentrations of lutein decreased to baseline values (Table 1).

Sixth serum zeaxanthin concentrations were about one-sixth the concentrations of baseline serum lutein concentrations (58 ± 12 compared with 370 ± 53 nmol/L, respectively). Serum zeaxanthin concentrations remained low but were significantly greater (P < 0.003) at 4 wk of supplementation (74 ± 14 nmol/L) than at baseline (58 ± 12 nmol/L). For the remainder of the study, serum zeaxanthin concentrations were not significantly different from baseline concentrations. Despite daily consumption of ≥9.3 μmol β-carotene contained in the spinach, the mean serum concentrations of β-carotene did not change significantly throughout the entire study (1.17 ± 0.20 μmol/L at baseline and 1.14 ± 0.21 μmol/L at 15 wk).

Buccal mucosa cells

Lutein concentrations in BMCs significantly increased after 4 wk of spinach and corn feeding (P < 0.010, Table 1). Lutein concentrations remained significantly higher than at baseline at 8 and 12 wk of supplementation (P < 0.010 and 0.001, respectively). At 15 wk the mean concentration was also above baseline, but the difference was not significant because of the large variation. Mean concentrations of zeaxanthin and β-carotene in BMCs did not change throughout the study.

Adipose tissue

For lutein in adipose tissue, the 4-wk mean value was low (Table 1) but not significantly below baseline because of the variability in the data and the small number of subjects (n = 7). However, the lutein concentration in adipose tissue significantly increased from baseline after 8 wk of spinach and corn feeding (P < 0.012) and remained elevated at 15 wk (P < 0.035, Table 1). However, when the Bonferroni correction was applied for multiple testing, the P value of 0.035 did not meet the critical value of P < 0.01 for significance. At 2 mo poststudy, the mean adipose tissue concentrations of lutein was not significantly different from baseline concentrations (Table 1). The changes in adipose tissue concentrations of zeaxanthin were small and not significant; however, the temporal pattern was similar to the pattern of changes in concentrations of lutein (data not shown). Adipose tissue concentrations of β-carotene did not change significantly with spinach and corn feeding.

Dietary intakes

Analysis of the dietary records indicated that the subjects maintained their usual carotenoid intakes from their self-selected diets throughout the study.

Macular pigment

Daily consumption of spinach and corn resulted in a significant increase in MP density from baseline at 4 wk (P < 0.05, Table 1). Although the mean MP density was not significantly different from baseline at 8 wk, at all other times (12 and 15 wk, 2 mo postfeeding) it was significantly elevated (P < 0.05, Table 1). Unlike the other tissues (BMCs and adipose), the concentration of lutein plus zeaxanthin in the retina indicated by MP density remained elevated after serum lutein concentrations declined. However, these changes were not considered significant in light of multiple testing.

The pattern of adipose tissue lutein concentrations was inverse to the pattern of MP density. That is, compared with baseline, there was a decrease in adipose tissue lutein concentration at 4 wk, followed by a significant increase at 8 wk. This is in contrast with the changes observed for MP density in which there was a significant increase at 4 wk followed by a decrease to a near-baseline value at 8 wk. These contrasting patterns suggest a

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**TABLE 1**

Serum and tissue concentrations of lutein during dietary modification with spinach (60 g/d) and corn (150 g/d).

<table>
<thead>
<tr>
<th></th>
<th>0 wk</th>
<th>4 wk</th>
<th>8 wk</th>
<th>12 wk</th>
<th>15 wk</th>
<th>2 mo poststudy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (μmol/L)</td>
<td>0.37 ± 0.05</td>
<td>0.72 ± 0.11</td>
<td>0.63 ± 0.11</td>
<td>0.62 ± 0.11</td>
<td>0.67 ± 0.11</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>Buccal mucosa cells (pmol/mg protein)</td>
<td>4.45 ± 0.86</td>
<td>7.78 ± 0.77</td>
<td>7.17 ± 0.74</td>
<td>10.30 ± 1.85</td>
<td>5.95 ± 1.73</td>
<td>4.56 ± 1.16</td>
</tr>
<tr>
<td>Adipose tissue (μmol/mg dry wt)</td>
<td>0.23 ± 0.07</td>
<td>0.07 ± 0.04</td>
<td>0.47 ± 0.08</td>
<td>—</td>
<td>0.38 ± 0.11^t</td>
<td>0.09 ± 0.11</td>
</tr>
<tr>
<td>MP density^t^</td>
<td>0.399 ± 0.045</td>
<td>0.506 ± 0.065</td>
<td>0.423 ± 0.052</td>
<td>0.469 ± 0.056^t</td>
<td>0.469 ± 0.059^t</td>
<td>0.476 ± 0.059^t</td>
</tr>
</tbody>
</table>

*xt ± SE; n = 7. MP, macular pigment.

Significantly different from 0 wk (ANOVA with repeated measures and Bonferroni and Dunn tests): ^2P < 0.001, ^3P < 0.010, ^4P < 0.050. (When a correction for multiple testing is applied, differences for which P < 0.01 are significant.)

A measure of the sum of lutein and zeaxanthin in the retina.

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*Note:* This page is a continuation of the previous page from the document.
role of adipose tissue in the MP response to diet, perhaps indicating a competition between adipose and retina for the serum carotenoids. Although there were only 4 women and 3 men in the study, the temporal patterns were similar in women and men.

**Study 2: cross-sectional relations among anthropometric measurements, macular pigment density, and serum, tissue, and dietary lutein concentrations**

For the larger study population (n = 21) that was used to evaluate cross-sectional relations, the most striking results emerged from comparing the data for men and women. The personal characteristics of the men and women were fairly well matched. There were no significant sex differences in age, BMI, or amount of body fat (Table 2). However, as would be expected (22), the women tended to have a higher percentage body fat than did the men (P < 0.065) and the men had a significantly higher FFM than did the women (Table 2).

The serum and BMC lutein concentrations and MP density were not significantly different between the women and the men (Table 3), but the lutein concentration in adipose tissue was 4–5 times higher in women (Table 3). This difference was not due to diet because dietary intakes of lutein were significantly less for women than for men (4.8 ± 0.9 compared with 11.1 ± 3.0 μmol/d, respectively), whereas intakes of dietary fat were not significantly different (69 ± 16 and 79 ± 13 g/d, respectively). For all subjects, there was a significant negative correlation of BMC lutein concentration with age and with percentage body fat and a significant positive correlation of serum lutein concentration with BMC lutein concentration and with adipose tissue lutein (Table 4). Despite the similarity in mean serum lutein concentrations, cross-sectional relations among the variables measured were different for women and men. BMC lutein was significantly and positively correlated with serum lutein in women (r = 0.712) and not related in men. BMI was positively correlated with adipose tissue lutein in men (r = 0.877) but not in women (Table 4).

The most striking sex difference (P < 0.05) was found for the relation between MP density and adipose tissue lutein. MP density was negatively correlated with adipose tissue lutein in women (r = –0.546, P < 0.035) but positively correlated with adipose tissue in men (r = 0.797, P < 0.032) (Table 4). Furthermore, these opposite trends were significant for both sexes. This relational difference may be related to the large average difference between the sexes. In both women and men, dietary lutein intake was not significantly correlated with any other variable measured.

**DISCUSSION**

**Effects of modifying carotenoid intake**

In most studies in which a biological response to carotenoid intake was measured, the serum response to large oral doses of carotenoid supplements was examined (23–25). Many factors may influence the serum response to a single oral carotenoid dose, such as gastric emptying, efficiency of absorption, release into and clearance from the circulation, tissue uptake, and release from body stores (23). The influence of such factors on serum response may differ for purified supplements or for foods. Because the results of epidemiologic studies indicate that intake of carotenoid-rich foods may be protective against AMD (1), certain cancers, and other diseases (26, 27), it is important to increase our understanding of the effects of intake of carotenoid-rich foods on serum and tissue carotenoid concentrations.

**TABLE 2**

Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 13)</th>
<th>Men (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>63 ± 4</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 1.3</td>
<td>25.4 ± 2.1</td>
</tr>
<tr>
<td>Percentage body fat (%)²</td>
<td>40 ± 4²</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Body fat (kg)²</td>
<td>26.2 ± 3.6</td>
<td>25.6 ± 6.3</td>
</tr>
<tr>
<td>Fat-free mass (kg)²</td>
<td>37.7 ± 1.0²</td>
<td>59.3 ± 2.5</td>
</tr>
</tbody>
</table>

¹ x ± SE.
²n = 6 women, 7 men.
³Significantly different from men: ¹P < 0.065, ²P < 0.0001.

Lutein and zeaxanthin appear to have a special role because they are concentrated in the retina, forming the MP. Intake of the MP carotenoids has been shown to be inversely related to the risk of advanced AMD (28). As mentioned previously, spinach and corn were chosen because these foods have high amounts of lutein and zeaxanthin, respectively, and are common in the US diet. Furthermore, frequent consumption of spinach, in particular, is associated with a reduced risk of advanced AMD (28).

Our study showed that concentrations of lutein and zeaxanthin in serum and tissues can be increased by increasing dietary lutein and zeaxanthin supplied from spinach and corn. Although the dietary modification based on a single daily serving of spinach and corn was modest, analysis of the dietary records indicated that the intake of lutein and zeaxanthin increased ≈7-fold. These observations may be important in making recommendations for food selections that could decrease the risk of AMD.

**Changes in serum and tissue lutein concentrations**

In our analyses, we emphasized lutein rather than zeaxanthin because of the lower concentration of zeaxanthin in the dietary intervention and in serum and nonretinal tissues. Serum lutein concentrations increased significantly with the intake of spinach and corn containing 19.7 μmol lutein/d. Increases in serum concentrations of zeaxanthin, however, were modest; there was a significant increase from baseline at 4 wk only. The small increase in serum zeaxanthin concentrations may indicate a lower bioavailability of this carotenoid but is more likely due to low amounts of zeaxanthin in the food (1.1 μmol/d). The mean peak serum concentrations per mg carotenoid in the food were comparable for lutein and zeaxanthin (20 and 24 nmol/L/μmol carotenoid, respectively). For both lutein and zeaxanthin, serum

**TABLE 3**

Cross-sectional serum and tissue concentrations of lutein and macular pigment (MP) density in healthy adults

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 13)</th>
<th>Men (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (μmol/L)</td>
<td>0.32 ± 0.04</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>Buccal mucosa cells (pmol/mg protein)</td>
<td>1.94 ± 0.72</td>
<td>2.27 ± 0.56</td>
</tr>
<tr>
<td>Adipose tissue (μmol/mg dry wt)</td>
<td>0.36 ± 1.0²</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>MP density⁴</td>
<td>0.483 ± 0.004</td>
<td>0.406 ± 0.072</td>
</tr>
</tbody>
</table>

¹x ± SE.
ⁿ = 11.
³Significantly different from men, P < 0.04.
⁴A measure of lutein and zeaxanthin in the retina.
concentrations decreased to baseline values 2 mo after consumption of spinach and corn was discontinued, indicating that serum concentrations of these carotenoids are responsive to increases and decreases in dietary intake.

The BMC response to spinach and corn consumption was similar to the serum response. Lutein concentrations increased significantly throughout the spinach and corn feeding and then decreased to baseline concentrations after consumption of spinach and corn was discontinued. This temporal pattern differed from that of adipose tissue, which showed a delayed increase in lutein concentration, and also differed from the pattern for retina, ie, retention of a high MP density after the intervention was discontinued. There were no significant changes in zeaxanthin concentrations in BMCs. Again, this is most likely because of the low amounts of zeaxanthin in the diet and in serum.

Adipose tissue lutein concentrations during ingestion of a high-lutein diet showed a delayed increase compared with concentrations in serum, BMCs, and MP. At 4 wk, 6 of the 7 subjects had unexpectedly low adipose tissue lutein concentrations during the initial period of increased dietary intake. This observation is difficult to explain. If the apparent decrease was real, it may have been due to dynamic interactions with body compartments, which may include the retina, that were triggered by our intervention. Larger studies need to be conducted to confirm this observation. Adipose tissue lutein concentrations increased significantly from baseline after a longer period of spinach and corn intake (8 wk). Adipose tissue lutein concentrations returned to a low value 2 mo after consumption of spinach and corn was discontinued, indicating that lutein is a dynamic component of adipose tissue, responding to changes in dietary intake.

Spinach, rather than corn, is likely responsible for the changes in lutein concentrations in serum and tissues. For the one subject who consumed spinach only, the patterns of increase in serum and tissue concentrations of lutein were similar to the group average. In addition, we previously studied 2 subjects who were fed corn only (data not shown). There were only slight changes in their serum lutein (11%, −6%), but one subject had an increased serum zeaxanthin concentration as a result of corn consumption (4).

There was no significant change from baseline in the mean concentration of β-carotene in serum or tissue in response to the dietary intervention, despite an intake of ≥9.3 mmol β-carotene/d. This result was unexpected given that lutein from the same food source resulted in a significant increase in serum and tissue lutein concentrations. The phenomenon of high responders and low responders to oral supplementation with β-carotene was observed previously (23). Our data may indicate that a high responder to lutein may not be a high responder to β-carotene. There also may be an interaction between carotenoids such that lutein interferes with the absorption of β-carotene, as was suggested from feeding experiments with carotenoid mixtures (24). This may be a difficult issue to resolve because experiments with purified supplements (25) showed that some individuals show inhibition of the serum response to β-carotene by lutein, whereas others show facilitation.

From our experimental design it is difficult to rule out the possibility of interactions in the other direction—namely, that β-carotene affected the absorption of lutein. However, in a study comparing a single oral dose of pure lutein with an equal dose of lutein from spinach in healthy men, we showed that the serum lutein response is similar for the 2 carotenoid sources (unpublished observations). This suggests that the β-carotene in spinach does not inhibit the absorption of lutein from spinach.

Another explanation for the difference in the serum and tissue responses to lutein and β-carotene from spinach may relate to the difference between baseline concentrations (lutein concentrations being one-fourth to one-third those of β-carotene concentrations) and amounts ingested (lutein concentrations in spinach being twice those of β-carotene in spinach). Given these conditions, it would be easier to measure a change in serum and tissue concentrations from lutein in spinach than from β-carotene in spinach.

Cross-sectional relations and sex differences

Influence of adipose tissue

The observation of a possible inverse relation between MP and adipose tissue lutein during dietary modification is intriguing. These preliminary data suggest that there may be a tissue interaction or competition for lutein between adipose tissue and the retina. To further evaluate possible interactions among tissues, we examined cross-sectional relations among anthropometric measurements, MP density, and serum, tissue, and dietary

| TABLE 4 | Spearman correlations among anthropometric measures, serum and tissue lutein concentrations, and macular pigment (MP) density in 13 healthy women and 8 healthy men |
|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                        | BMI                   | Percentage body fat   | Serum                 | Buccal mucosa cell    | Adipose tissue        | MP density  |
| Age (y)                | NS                    | NS                    | NS                    | −0.522^2              | NS                    | NS          |
| BMI (kg/m^2)           |                        |                       |                       | −0.620^2              |                       | NS          |
| Percentage body fat (%)|                        |                       |                       |                       |                       | NS          |
| Lutein                 |                        |                        |                       |                       |                       | NS          |
| Serum (g/L)            |                        |                       |                       | 0.569^4,5             | 0.495^6              | NS          |
| Buccal mucosa cells (ng/mg protein) |                       |                       |                       |                       |                       | NS          |
| Adipose tissue (μg/mg dry wt) |                       |                       |                       |                       |                       | NS^7        |

^1 A measure of lutein and zeaxanthin in the retina.
^2 P < 0.020.
^3 Men: r = 0.877, P < 0.009.
^4 P < 0.011.
^5 Women: r = 0.712, P < 0.006.
^6 P < 0.036.
^7 Women: r = −0.546, P < 0.035; men: r = 0.797, P < 0.032.
lutein concentrations (study 2). A negative correlation between percentage body fat and BMC lutein concentrations was observed, which supports the idea of competition for lutein among tissues. That is, a higher amount of body fat provides a larger “sink” for lutein, which makes less lutein available for other tissues. The fact that we did not find other relations among anthropometric measurements and tissue and dietary carotenoid concentrations may be related to the small number of subjects studied and possible differences in lutein metabolism between men and women. The different cross-sectional relations among variables measured between women and men suggest a sex difference in lutein metabolism.

The idea of sex differences in carotenoid metabolism is consistent with our observation of higher lutein concentrations in the adipose tissue of women than of men, despite similar serum concentrations of lutein and, more surprisingly, a significantly lower dietary lutein intake by women. Additionally, there was a significant negative correlation of MP with adipose tissue lutein concentrations in women but a significant positive correlation in men. The opposite relation for the 2 sexes may contribute to the sex differences in MP density that were reported previously (29). That is, if adipose tissue competes for carotenoids more effectively in women than in men, MP density and risk of advanced AMD in women would be expected to decrease and increase, respectively (1).

In our study population, it was difficult to determine whether a sex difference in carotenoid metabolism was due to differences in hormonal status because 9 of the 13 women were postmenopausal and not taking estrogen therapy. Also, the sex difference may not have been due to the amount or percentage of body fat. Although women had a higher percentage of body fat than men did, men and women had a higher FFDM than did women, the percentage of body fat and FFDM were not related to serum or tissue lutein concentrations or MP density, with the exception of a negative relation between percentage body fat and BMC lutein. It is likely that the sex differences in adipose tissue relations were influenced by differences in the distribution of body fat (22). Our preliminary data showed that lutein concentrations differ among body fat sites (abdomen, buttocks, and thigh) (EJ Johnson, ALA Ferreira, S Paiva, C Castaneda, and RM Russell, unpublished observations, 2000). Future work needs to examine the role of the distribution of body fat in the biological response to dietary carotenoids.

Our study provided preliminary data to suggest that lutein and zeaxanthin are dynamic components of tissues and that the metabolism of lutein may differ between women and men. However, the study was limited by the small number of subjects studied. Further studies, with a larger number of subjects, are needed to confirm these preliminary observations.

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