Lutein and β-carotene from lutein-containing yellow carrots are bioavailable in humans

Kirsten L Molldrem, Jialiang Li, Philipp W Simon, and Sherry A Tanumihardjo

ABSTRACT

Background: Lutein is a hydroxy-carotenoid constituting the macular pigment of the human retina. Increasing lutein intake from foods could increase the density of this pigment and decrease the risk of developing macular degeneration. Yellow carrots are a novel food source that could increase lutein consumption.

Objective: We evaluated and compared lutein uptake and clearance in humans from genetically selected lutein-containing yellow carrots fed chronically and from a lutein supplement.

Design: Four women and 5 men aged 23–28 y participated in this randomized, blinded, 3 × 3 crossover intervention. Treatments consisted of yellow carrots (YC treatment, 1.7 mg lutein/d), white carrots as a negative control (WC treatment, 0 mg lutein/d), and a lutein supplement in oil as a positive control (LS treatment, 1.7 mg lutein/d). Each treatment lasted 7 d and was followed by a 7-d washout period.

Results: Mean (± SD) peak changes in serum lutein concentration from baseline were 0.31 ± 0.08, 0.19 ± 0.08, and −0.04 ± 0.04 μmol/L for the LS, YC, and WC treatments, respectively. The areas under the curve for 0–14 d (AUC0–14d) differed significantly (P < 0.0001) between treatments. Lutein from the YC treatment was 65% as bioavailable as that from the LS treatment. The AUC0–14d for β-carotene (−0.01 ± 0.28 μmol · d/L) also showed that the YC treatment maintained peak serum β-carotene concentrations at 0.35 ± 0.30 μmol/L, whereas the LS treatment did not (AUC0–14d = −0.71 ± 0.59 μmol · d/L).

Conclusion: Lutein from this novel food source significantly increases serum lutein concentrations and does not result in the decrease in β-carotene concentrations that accompanies administration of lutein supplements. Am J Clin Nutr 2004;80:131–6.

KEY WORDS Lutein, β-carotene, bioavailability, carotenoids, carrots, supplements

INTRODUCTION

Lutein and a structural isomer, zeaxanthin, are non–provitamin A carotenoids, measurable in human serum and tissues. Both isomers are found in foods such as egg yolks, corn, and leafy green vegetables. Lutein and zeaxanthin were identified as the xanthophylls that constitute the macular pigment of the human retina (1, 2). Increased lutein intake was positively correlated with increased macular pigment density (3–5), which is inversely related to the risk of macular degeneration (6, 7). Possible mechanisms of action for these carotenoids include antioxidant protection (8) and filtering of damaging blue light by the macular pigment (9).

Because lutein and zeaxanthin could be involved in disease prevention, much needs to be learned about human consumption of these carotenoids. One complicated factor that requires better understanding is the bioavailability of lutein from food sources and supplements. Many factors influence carotenoid bioavailability, and each carotenoid can be affected differently by these factors. Lutein bioavailability studies were conducted with lutein supplements (10) or foods (11–16) containing lutein fed to human subjects. The food matrix is an important factor that influences lutein bioavailability (11). Also, the amount and type of food processing influences bioavailability. For example, the processing of spinach does not affect the bioavailability of lutein but does positively affect β-carotene bioavailability (12). Competition between carotenoids, such as lutein and β-carotene, for incorporation into chylomicrons was noted in humans consuming vegetables (13) and supplements (10). The amount of fat consumed with the lutein source is another factor that affects bioavailability, because higher fat increases the bioavailability of lutein esters (17). Decreased plasma lutein concentrations were noted when alcohol was consumed (18).

Lutein from vegetables was 5 times as bioavailable as β-carotene (16) in a study that compared human subjects fed lutein (10.7 ± 9.4 mg) and β-carotene (5.1 ± 3.2 mg) from a high-vegetable diet with those fed a low-vegetable, supplemented diet providing 12.0 ± 3.1 mg lutein and 7.2 ± 1.5 mg β-carotene. However, β-carotene bioavailability was confounded by the inability to determine bioconversion to retinol during vegetable feeding. These and other factors need to be better understood before quantitative recommendations on intake of individual carotenoids can be made.

Carrots are a familiar, widely consumed vegetable. Carrots exist naturally throughout the world in colors other than orange and contain varying concentrations of different carotenoids.
Plant-breeding techniques have been used to optimize the flavor and lutein content of yellow carrots (19, 20). The objective of this study was to determine to what extent the lutein in these specialty yellow carrots is bioavailable to humans and to compare this bioavailability with that from a supplement of lutein in oil.

SUBJECTS AND METHODS

Subjects
Nine healthy, nonsmoking students (5 men and 4 women) aged 23–28 y enrolled in this study. The subjects remained in the study for 50 d, including a 1-wk washout period before the study and between each treatment. Written informed consent was obtained from each subject. The study was approved by the University of Wisconsin Medical School Human Subjects Committee. Hemoglobin concentration was determined for each subject before beginning the study, and all concentrations were within normal limits.

Diets
Two diets were constructed for the subjects to follow: the low-lutein diet (LLD) and the low-carotenoid diet (LCD). Subjects followed the LLD during the first 4 d of each washout period. This diet eliminated foods containing significant amounts of lutein (corn products, eggs, green and yellow vegetables, some fruit), but it was not as strict as the LCD about other carotenoids; ie, tomato products were allowed during the LLD phase. For the rest of the study, the subjects were required to follow the LCD, which had all of the restrictions of the LLD but also eliminated additional foods that were high in other carotenoids. Alcohol consumption was also restricted during and 2 d before the beginning of each phase of the study. Compliance was monitored by providing subjects with sheets to record fruit and vegetable intake, which were collected weekly and reviewed.

Protocol
This study was designed as a randomized, blinded, 3 × 3 crossover with treatment groups who received yellow carrots (YC treatment), white carrots as a negative control (WC treatment), and a crossover with treatment groups who received yellow carrots (YC treatment), white carrots as a negative control (WC treatment), and a partial lunch, consisting of a carrot muffin and smoothie, 2, 5, 7, 9, 12, and 14 during each treatment. For 7 d (days 0–6), subjects were provided breakfast, including a carrot muffin and “smoothie,” and a partial lunch, consisting of a carrot muffin and soup. The YC treatment meals were made with yellow carrots, and the WC and LS treatment meals were made with white carrots. Recipes for each food item were identical for different treatment groups except for the type of carrot.

Carrots, food, and diet analysis
Carrots were provided by PWS. The yellow carrots were genetically selected to contain a higher concentration of lutein than do store-bought varieties. The white carrots were selected to be void of any carotenoids. During recipe development, much intercarrot and intracarrot variation was noted in regard to lutein content, so the entire fall 2002 harvest of yellow carrots (and white carrots) was cleaned, chopped in a food processor, and homogenized in a Hobart mixer (Troy, OH). This homogenous mixture was then divided into bags at weights appropriate for each recipe throughout the entire study and frozen until 1 d before use.

Triplicate analysis of mixed, processed carrots and foods made from the carrots was done with the use of the following extraction method: 1 mL (3.0 nmol) internal standard, β-apo-8'-carotenyl decanoate, which was synthesized in our laboratory with the use of published methods (21), was added to 1 g sample and ground with the use of a mortar and pestle; 2.5 g sodium sulfate was added to remove water and to form a paste. Carotenoids were extracted into alternating 10-mL washes of dichloromethane and acetone, which were filtered into a 100-mL volumetric flask. The filtrate (1 mL) was dried under argon, redissolved in 100 μL 50:50 (vol:vol) dichloromethane:methanol, and analyzed with the use of HPLC. Fifty microliters was injected onto a Waters Resolve C18 column (5 μm, 3.9 × 300 mm; Waters, Milford, MA) equipped with a guard column. A gradient system was developed to optimize separation of lutein and zeaxanthin. The Waters HPLC system consisted of a 600 solvent delivery system, 717 autosampler, and 996 photodiode array detector. The HPLC mobile phase consisted of 95:5 (vol:vol) acetonitrile:water with 10 mmol ammonium acetate/L and 0.1% triethylamine as solvent A and 85:10:5 (vol:vol:vol) acetonitrile:methanol:dichloroethane with 10 mmol ammonium acetate/L and 0.1% triethylamine as solvent B. At 2 mL/min, the gradient procedure was as follows: 1) 100% solvent A for 3 min, 2) a 7-min linear gradient to 100% solvent B, 3) a 15-min hold at 100% solvent B, and 4) a 2-min linear gradient back to 100% solvent A.

Analysis was confirmed by a saponification extraction method modified from Tanumihardjo and Penniston (22) in which 1 mL ethanol with 0.1% butylated hydroxytoluene was added to 0.5 g sample, and the sample was mixed by vortex for 15 s. Then, 400 μL 50:50 (wt:vol) KOH:H2O was added to the sample, and the sample was mixed by vortex for 15 s and placed in a 45 °C water bath for 60 min, with mixing by vortex every 15 min. The carotenoids in this mixture were extracted into 2 mL hexanes 3 times. The pooled organic layer was dried under argon and redissolved into 500 μL 50:50 (vol:vol) dichloromethane:methanol, and 50 μL was injected into the HPLC system just described. These 2 extraction methods gave very similar lutein concentration values. The CV for food carotenoids in our laboratory was 4% during the course of this study.

Fruit and vegetable records were analyzed for lutein and zeaxanthin content with the use of values from the US Department of Agriculture carotenoid database, because lutein and zeaxanthin are not separated in this database (23). Recipes were analyzed with the use of NUTRITIONIST FIVE software (version 2.2; First DataBank Inc, San Bruno, CA).

Macronutrients and carotenoids in carrots and foods
Raw, chopped yellow carrots had a concentration of 505 μg lutein/100 g carrots and 260 μg β-carotene/100 g carrots by HPLC analysis. All foods were cooked (ie, microwaved, baked, or simmered) before consumption. Each breakfast smoothie contained 100 g microwaved carrots and provided 0.46 mg lutein, 0.19 mg β-carotene, 895.4 kJ (214 kcal), 6 g protein, 44 g carbohydrate, 2 g fat, and 5 g fiber. Baked muffins contained 56 g carrots each. Two muffins were found to provide 0.60 mg lutein, 0.16 mg β-carotene, 1397.5 kJ (334 kcal), 6 g protein, 46 g...
carbohydrate, 14 g fat, and 4 g fiber. A serving of hot soup contained 125 g carrots and provided 0.62 mg lutein, 0.27 mg \( \beta \)-carotene, 418.4 kJ (100 kcal), 3 g protein, 14 g carbohydrate, 4 g fat, and 4 g fiber. These foods provided 1.7 mg lutein and 0.6 mg \( \beta \)-carotene/d. The zeaxanthin found in lutein-containing carrots varied from being undetectable to much <1% of the lutein concentration.

**Lutein supplement**

In the LS treatment group, subjects received 1.7 mg lutein in oil (same amount of lutein as provided from yellow carrots). The supplement was made by adding 100 mL ethyl acetate to 350 mg pure crystalline lutein (Kemin Industries, Des Moines, IA) in a round-bottom flask. This mixture was sonicated for 10 min, 230 mL canola oil was added, and the ethyl acetate was evaporated under vacuum. The lutein in oil supplement was evaluated spectrophotometrically at 445 nm. With the use of the extinction coefficient (\( E^\text{L}_{1\% cm} \) 2550, the concentration of lutein was 1304 mg/L. To provide 1.7 mg lutein, 1300 \( \mu \)L solution was needed per day. The supplement was provided in 2 doses: 650 \( \mu \)L was added to the breakfast smoothie, and 650 \( \mu \)L was added directly into the lunch muffin with a pipet. All foods were dyed with varying amounts of red and yellow food coloring (McCormick & Co, Inc, Hunt Valley, MD), and muffins not containing the lutein supplement were injected with 650 \( \mu \)L colored canola oil to maintain a blinded study.

**Blood collection and serum analysis**

During each phase of the study, morning blood samples were collected after a 10-h fast. Blood was centrifuged at 2190 \( \times \) g for 20 min at 4 °C for ≤3 mo before analysis. To analyze the serum, the internal standard \( \beta \)-apo-8’-carotenyl decanoate (0.1 nmol) was added to a sample of 200 \( \mu \)L serum. Then, 250 \( \mu \)L ethanol with 0.1% butylated hydroxytoluene was added to denature proteins. Samples were mixed by vortex for 30 s, and carotenoids were extracted 3 times with the use of 500 \( \mu \)L hexanes. The hexane layers were pooled and dried under argon. The samples were redissolved in 100 \( \mu \)L 50:50 (vol:vol) dichloromethane:methanol, and 25 \( \mu \)L was injected into the HPLC system as described earlier. A Waters Resolve C18 column (5 \( \mu \)m, 3.9 \( \times \) 300 mm) was used with a guard column packed with the same material as in the analytic column. The solvents and gradient system used were as described earlier. External standardization was performed with HPLC-purified lutein (Kemin Industries). The CV for serum lutein concentration was 2% for the same sample run several times in the same day. All samples for each subject were analyzed on the same day.

**Statistical analysis**

The serum concentration of each carotenoid corrected for the baseline value was plotted against time. The area under the curve for days 0 through 14 (AUC\(_{0-14d}\)) was calculated for lutein and \( \beta \)-carotene by trapezoidal approximation (10). Statistical analyses were performed with SAS software (version 8.2; SAS Institute Inc, Cary, NC). Initially, a mixed effect model was fitted to include fixed effect terms for order, sex, body mass index, type of carrot treatment, and the interaction between order and type. Then, the model was reduced to contain only order, type, and their interaction.

![FIGURE 1. Mean (± SD) serum lutein concentrations after correction for baseline concentration in 9 adults who were fed yellow carrots (YC treatment, 1.7 mg lutein/d, ▲), white carrots (WC treatment, 0 mg lutein/d, ●), or a lutein supplement with white carrots (LS treatment, 1.7 mg lutein/d, ■) for 7 d with a 7-d washout before the next treatment. With the use of area under the curve as a response variable in the statistical model, the effects of the treatments were significantly different from each other (P < 0.004, \( t \) test with Bonferroni correction).](image-url)
interaction of order and type. The effect of treatment type was detected and, therefore, were excluded from the model in the ment order, a more conservative estimate of relative bioavail-
lutein supplement was assigned second or third, the difference
difference between LS and YC was significant, but, when the
effects can be affected by the order of the treatment assignment. For
ment group, implying that the difference between treatment ef-

<table>
<thead>
<tr>
<th></th>
<th>LS treatment</th>
<th>WC treatment</th>
<th>YC treatment</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>2.63 ± 0.22± 0.81 ± 0.11</td>
<td>1.09 ± 0.11</td>
<td></td>
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<tr>
<td>Group 2</td>
<td>1.95 ± 0.71± 0.46 ± 0.18</td>
<td>1.55 ± 0.61±</td>
<td></td>
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<tr>
<td>Group 3</td>
<td>1.70 ± 0.29</td>
<td>0.19 ± 0.12</td>
<td>1.44 ± 0.76</td>
</tr>
</tbody>
</table>

1 All values are x ± SD; n = 4 F, 5 M. The groups differed in the order
in which the treatments were given: group 1, LS-WC-YC; group 2, YC-LS-
WC; group 3, WC-YC-LS.
2 Significant difference from WC treatment (t test with Bonferroni
correction): 2P < 0.0001, 3P < 0.0014, 4P < 0.0008, 5P = 0.011, 6P = 0.046.
3 Significant difference from LS treatment, P = 0.009 (t test with
Bonferroni correction).

DISCUSSION
The goal of this study was to determine to what extent lutein
from yellow carrots was bioavailable in humans relative to a
lutein supplement in oil. We hypothesized that the lutein from the
carrots would be bioavailable but not as bioavailable as the sup-
plement. The carotenoid profile of typical orange carrots in-
cludes α- and β-carotene. The yellow carrots used in this study
predominately contain lutein, but also β-carotene. Significant
increases in serum lutein concentrations (0.19 ± 0.08 μmol/L)
were noted in all subjects after receiving 1.7 mg lutein/d from the
YC treatment for 7 d, indicating that lutein from this novel food
source is highly bioavailable. Bioavailability was certainly
improved in this study by processing (chopping and heating) the
carrots in all of the foods served. On the basis of AUC0-14d
comparison of serum response and depletion curves, the lutein
from the yellow carrots was absorbed 65% as well as the lutein
from the supplement overall (Figure 1). This absorption varied
from 41% to 85% between individuals during treatment periods
(Table 2). A limitation of the use of AUC in this study is that we
are only able to calculate a bioavailability of lutein from carrots
relative to that from a supplement instead of absolute bioavail-
ability from the carrots. Absolute bioavailability could best be
assessed by using stable-isotope-labeled vegetables, but these
experiments are costly and technically challenging.
This percentage range, 41–85%, is greater than the bioavail-
ability reported for β-carotene from carrots (14). Subjects fed 29
mg β-carotene from carrots and an equalized capsule (30 mg, dry
gelatin beadlets) with a 6-wk washout period between treatments
had an increase in serum β-carotene concentration of 20% in the
carrot treatment compared with the supplement treatment.
Moreover, after a 6-wk chronic intake of 29 mg β-carotene from
carrots or 30-mg capsules, a serum β-carotene increase of 18%
was noted from carrots compared with supplements (15). The
present study indicates that lutein from yellow carrots is more
bioavailable than is β-carotene from orange carrots. However,
measures of β-carotene bioavailability are confounded by bio-
conversion to retinol (24).
The increase of 0.31 ± 0.08 μmol/L in serum lutein concen-
tration after the LS treatment indicates that the lutein supplement
was delivered in a very bioavailable form. After 7 d of supple-
mentation with 1.7 mg lutein, an average increase in serum lutein
concentration of 1.3-fold (range: 0.7–2.1) was noted. The in-
creased serum lutein concentration is consistent with the results
of other published studies of lutein supplementation. Be-
rendscho et al (4) gave 10 mg lutein for 12 wk (n = 8) and found
a 5-fold increase in serum lutein concentration. Granado et al (25)
also saw a 5-fold increase in serum lutein concentration after 1
mo of supplementing 9 men and 9 women with 15 mg lutein.
After 140 d of supplementation with 30 mg lutein, Landrum et al
(3) noted a 10-fold increase in serum lutein concentrations in 2
male subjects. Supplements in the present study and in the studies
just discussed were all made differently. For the present study,
we created a highly bioavailable supplement with the use of pure crystalline lutein dissolved in canola oil. Landrum et al (3) used olesoresin from marigolds in canola oil, whereas Berendschot et al (4) and Granado et al (25) used lutein diesters.

The half-life of plasma lutein was estimated to be 33–61 d (26). Serum lutein depleted at rates faster than this in our study, and the degree of decay was related to the peak lutein concentration reached after the YC or LS treatment. After 7 d (days 7–14) on the LCD and not receiving any type of supplemental lutein, a 47 ± 9% decrease in serum lutein was seen after the LS treatment, whereas a 34 ± 10% decrease in serum lutein was noted after the YC treatment. Including all data points from all 3 treatments on day 7, and with the use of the median value of 0.40 μmol/L, the mean percentage change by day 14 above and below 0.40 μmol/L was –40 ± 14% and –22 ± 17%, respectively. These values are significantly different (P = 0.005). For example, during the LS treatment, a 7-d serum lutein concentration of 0.73 μmol/L fell to 0.44 μmol/L by day 14 (7 d of withdrawal), a 40% decrease; whereas an initial serum lutein concentration of 0.20 μmol/L fell to 0.17 μmol/L in the same time period after the WC treatment, a 13% decrease. This finding indicates that there are probably many body pools of lutein as evidenced by faster depletion of serum lutein when peak concentrations are high and slower depletion when peak concentrations are low.

An intake of 1.7 mg lutein, as provided in this study, is a value that is close to an estimated mean dietary lutein intake. Rock et al (27) determined the mean dietary lutein and zeaxanthin intake to be 1.3 ± 0.9 mg/d. A 0.5-cup serving of cooked spinach provides 6.3 mg lutein and zeaxanthin, 1 cup raw spinach provides 3.6 mg, and 0.5 cup cooked corn provides 1.5 mg (23). During this study, subjects received a daily total of 337 g chopped carrots, which provides 3.6 mg lutein and zeaxanthin, 1 cup raw spinach provides 6.3 mg, 0.5 cup cooked corn provides 1.5 mg, and 2 eggs provide 7 mg of lutein. Therefore, yellow carrots have an advantage over lutein supplements in that β-carotene is also available, and serum β-carotene concentration is not decreased after consumption. This finding is similar to observations from 2 intervention studies with lycopene-containing red carrots and tomato paste that contained varying amounts of β-carotene (29). Although β-carotene concentrations were more depleted in the lycopene study than in the present study, to maintain serum β-carotene constant at a concentration of 0.35 ± 0.30 μmol/L. Therefore, yellow carrots have an advantage over lutein supplements in that β-carotene is also available, and serum β-carotene concentration is not decreased after consumption.

Although serum β-carotene remained stable, it did not increase despite daily intake of β-carotene from yellow carrots. Because our subjects went through dietary modification when they were enrolled, ie, low carotenoids, most of the ingested β-carotene was probably converted to retinol during the course of the study. The low amounts of carotenoids in our study probably did not result in competition, although others have indicated competition between lutein and β-carotene for absorption. For example, Micoczi et al (15) reported lower serum lutein concentrations in subjects receiving β-carotene supplements than in subjects not receiving the supplement. Kostic et al (10) found that, with equimolar doses of lutein and β-carotene, the serum lutein response was lower than when lutein was delivered alone. They did not see the same effect for β-carotene.

Lutein from yellow carrots is highly bioavailable. Because increasing lutein consumption could prevent macular degeneration, new dietary lutein sources would help increase intake. Egg yolks are a very bioavailable source of lutein, but they are high in cholesterol and saturated fat. Spinach is a source of lutein but is an unpopular vegetable. Carrots are a familiar and widely consumed vegetable. The introduction of “cut and peel” or “baby” carrots to the market increased consumption by 50% between 1975 and 1995 (31, 32). Carrots are a convenient source of nutrients because they are produced in the United States and have an excellent shelf life. Yellow carrots are a novel source of bioavailable lutein and, if accepted by consumers, could increase lutein consumption.

**TABLE 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lutein AUC(^2)</th>
<th>Percentage of change from baseline</th>
<th>β-Carotene AUC(^2)</th>
<th>Percentage of change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC</td>
<td>1.36 ± 0.53(^a)</td>
<td>95 ± 41</td>
<td>–0.01 ± 0.28(^a)</td>
<td>0 ± 15</td>
</tr>
<tr>
<td>LS</td>
<td>2.09 ± 0.58(^b)</td>
<td>127 ± 52</td>
<td>–0.71 ± 0.59(^ab)</td>
<td>–18 ± 15</td>
</tr>
<tr>
<td>WC</td>
<td>–0.36 ± 0.46(^a)</td>
<td>–11 ± 15</td>
<td>–0.33 ± 0.54(^ab)</td>
<td>–16 ± 12</td>
</tr>
</tbody>
</table>

\(^1\) All values are ± SD; n = 4 F, 5 M. The amounts of lutein and β-carotene given during the treatments were as follows: YC, 1.7 and 0.6 mg/d, respectively; LS, 1.7 and 0 mg/d, respectively; WC, 0 and 0 mg/d, respectively.

\(^2\) Values in this column with different superscript letters are significantly different (t test with Bonferroni correction): \(^3\)P < 0.004, \(^4\)P = 0.03.
We thank Amy Sitek for her assistance in food preparation and the participants for their commitment to the study. KLM was responsible for orchestrating the study, preparing the food, sample analysis, and manuscript preparation. JL performed the statistical analysis. PWS provided the specialty carrots and was a consultant in manuscript preparation. All work was conducted in the laboratory of SAT, who was responsible for study design, overseeing study operation, input into data analysis, and manuscript preparation and revision. None of the authors had any financial interest in the work or any conflict of interest with the sponsors of this study.

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10. Kostic D, White WS, Olson JA. Intestinal absorption, serum clearance, or any conflict of interest with the sponsors of this study.