Serum α- and β-Carotene Concentrations Qualitatively Respond to Sustained Carrot Feeding

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α-Carotene is a predominant source of vitamin A in developing countries. Genetically selected "high carotene" carrots could have an impact on the vitamin A and antioxidant status of people if widely adopted. A 3 × 3 crossover study in humans (n = 10) evaluated the difference in uptake and clearance of α- and β-carotene from carrots genetically selected and traditionally bred to have high, typical, or no carotene. Subjects were fed white (0 mg α- and β-carotene/day), orange (1.8 mg α-carotene and 2.6 mg β-carotene/day), or dark-orange (4 mg α-carotene and 7 mg β-carotene/day) carrots in muffins for 11 d, with a 10-d washout phase between treatments. Serum carotenoid and retinol concentrations were measured by HPLC. C-reactive protein (CRP), an indicator of underlying inflammation or infection which may lower serum retinol, was measured at the beginning of each period. A significant treatment effect occurred for serum α- and β-carotene concentrations (P < 0.001), and a trend towards a negative effect of subjects' BMI on concentrations (P = 0.08). A significant treatment by sequence interaction was observed (P = 0.038), which was attributable to a difference in serum α- and β-carotene concentrations between carrot treatments in the first period. Serum retinol remained stable for the first 20 d of the intervention and then decreased (P = 0.02). CRP was not elevated in any subject. High carotene carrots provide more provitamin A carotenoids than the typical store-bought variety, without a change in flavor. The availability of high carotene carrots could readily increase consumption of β-carotene and potentially impact the vitamin A status of those individuals who are deficient or at risk of depletion. Exp Biol Med 234:1280–1286, 2009

Key words: α-carotene; β-carotene; bioavailability; carrots; serum retinol

Introduction

Diets rich in fruits and vegetables are recommended to reduce the risk of cardiovascular disease and some forms of cancer (1). Carotenoids, which are responsible for the color of many fruits and vegetables, are widely studied phytochemicals due to their antioxidant and provitamin A activities. Over 600 carotenoids exist in nature and about 50 of these have provitamin A activity. The most relevant provitamin A carotenoids for humans are α-carotene, β-carotene, and β-cryptoxanthin (2), of which β-carotene provides the majority of vitamin A to Africa and Asia (3). Several factors influence the bioavailability of carotenoids from food, thereby decreasing overall bioefficacy (4–8).

Bioavailability of β-carotene, which is defined as the fraction of carotenoid that is absorbed and available for utilization in physiologic functions or for storage, is assumed to be low when comparing β-carotene in food to β-carotene supplements (3–8). Processing and cooking enhance β-carotene bioavailability from raw and processed carrots (7). However, by feeding a single meal of cooked carrots to a group of schoolchildren in Guatemala, an appreciable change in serum β-carotene concentrations was not uniform (9). Thus, there is a need for more detailed information about the bioavailability and nutritional fate of provitamin A carotenoids from orange carrots. Chronic dark-orange carrot feeding to vitamin A–adequate gerbils resulted in more than a 100% increase in β-carotene and...
only a 10% increase in vitamin A liver reserves over typical orange carrots (10). The estimated bioconversion factors based on β-carotene alone were 9–11 μg β-carotene to 1 μg retinol for typical orange carrots and ~23 μg β-carotene to 1 μg retinol for the dark-orange carrots (11). However, when red specialty carrots were fed to vitamin A–depleted gerbils, conversion factors were more favorable, i.e., 3.5 μg β-carotene to 1 μg retinol (12). Compiling data from several gerbil studies revealed a linear relationship of vitamin A liver reserves to the bioconversion factor (2), suggesting that the vitamin A status of the host is a driving factor in the bioconversion of provitamin A carotenoids to retinol.

This study evaluated the serum response of α- and β-carotene from carrots genetically selected and traditionally bred to have high α- and β-carotene compared with typical orange and white carrots in chronic feeding trials to young, well-nourished adults. The goal was to determine if an increase in β-carotene content within the same amount of food would result in an increase in β-carotene in the serum after an 11-d intervention. Given that β-carotene is the primary source of vitamin A in many developing countries (13), these nutritionally enhanced “high carotene” carrots might impact vitamin A status if widely adopted (7, 9, 14).

Subjects and Methods

Subjects. Ten subjects (n = 10), 5 females and 5 males, were recruited from university staff and students via email and posters. Sample size was based on prior studies with biofortified carrots (15, 16) and standard expressions of sample size calculations where a sample size of 5 would appear similar to the dark-orange carrot muffins. Muffins for fat (33%), carbohydrate, protein, fiber, and carrot content. Subjects were blinded by adding red and yellow food coloring to the orange and white carrot muffins to appear similar to the dark-orange carrot muffins. Muffins were made in advance and frozen until use. All food preparation was performed in the research kitchen in the Department of Nutritional Sciences at UW-Madison.

Serum Preparation and Analysis for Carotenoids. All serum preparation and analyses were conducted under gold fluorescent lighting to protect carotenoids from isomerization and photodegradation, and the methods are published (15). Prepared serum was analyzed on a Waters Resolve C18 5 μm column, 3.9 × 300 mm. The mobile phase was modified (21) and consisted of 85:10:5 acetonitrile:methanol:dichloroethane (v:v:v), with 10 mM ammonium acetate. The flow rate was 2 mL/min and the run time was 40 min. Serum concentrations of lycopene, α-
Table 3. Area Under the Curve (AUC_{0–16d}) for \(\alpha\)- and \(\beta\)-Carotene for Each Muffin Type Fed to Adults\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>(\alpha)-Carotene,(^b) (\mu\text{mol-d/L})</th>
<th>(\beta)-Carotene,(^b) (\mu\text{mol-d/L})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark-orange carrot</td>
<td>2.76 (\pm) 1.25(^b)</td>
<td>2.32 (\pm) 1.52(^b)</td>
</tr>
<tr>
<td>Orange carrot</td>
<td>2.48 (\pm) 1.05(^b)</td>
<td>1.55 (\pm) 1.18(^b)</td>
</tr>
<tr>
<td>White carrot</td>
<td>–0.52 (\pm) 0.70(^c)</td>
<td>–0.53 (\pm) 1.16(^c)</td>
</tr>
</tbody>
</table>

\(^a\) Mean \(\pm\) SD; \(n=5\) F, 5M. Values in the same column with different superscript letters are significantly different.

\(^b\) Significantly different from the white carrot muffin: \(P \leq 0.006\).

\(^c\) Significantly different from the white carrot muffin: \(P < 0.001\).

carotene, and \(\beta\)-carotene were corrected for internal standard recovery. Extraction efficiency ranged from 81 to 105%.

Serum Retinol and C-Reactive Protein Analysis. All serum samples were separately analyzed for retinol concentration; 100 \(\mu\)L serum was mixed with 40 \(\mu\)L retinyl acetate solution as internal standard; 100 \(\mu\)L ethanol was added and mixed by vortex. Retinol was extracted 3 times with 250 \(\mu\)L hexanes. Hexanes were evaporated with argon and the sample was resuspended in 50 \(\mu\)L 75:25 methanol:dichloroethane (v:v); 25 \(\mu\)L was injected into the HPLC system described above. The column was a Waters Symmetry\(^\circ\) C18 3.5 \(\mu\)m 4.6 \(\times\) 75 mm column with a mobile phase of 95:5 methanol:water (v:v) at a flow rate of 0.7 mL/ min. Millenium\(^{32}\) software provided peak areas for retinol, which were corrected for internal standard recovery. Concentrations of retinol were determined, and means and coefficients of variation (CV) were calculated for each subject in each treatment arm.

C-reactive protein (CRP) concentrations were determined on the first day of each treatment period using an enzyme-linked immunosorbent assay for human CRP (Diagnostic Automation, Inc.; Calabasas, CA).

Statistical Analysis. The concentrations of \(\alpha\)- and \(\beta\)-carotene were plotted vs. time and the area under the curves (AUC) were calculated by trapezoidal approximation. A split-plot ANOVA was conducted with fixed-effect terms for sequence, carrot, and interaction, and a random effects term for subjects. A repeated-measures analysis of variance was also conducted for serum retinol. Treatment (carrot muffin type), sequence (order of muffins), gender, BMI, and age effects were determined. \(P < 0.05\) was considered significant.

Results

All 10 subjects completed the study. Compliance with the low carotenoid diet was good, as evidenced by the negative AUC for the white carrot treatment arm and supported by the food diaries.

Means \(\pm\) SD for \(\alpha\)- and \(\beta\)-carotene AUC are listed in Table 3. Figure 1 depicts the serum response to carotenoid feeding in \(\mu\)mol/L for \(\alpha\)- and \(\beta\)-carotene corrected for baseline concentrations. Similar to other studies of this type, the serum response to carotenoid feeding was highly variable between subjects (6, 22). The high \(\beta\)-carotene muffin treatment (7 mg/d) resulted in the largest increase in serum concentration for \(\beta\)-carotene, with a 127% increase above baseline by d 11 of the treatment period. The \(\beta\)-carotene concentration was still elevated by d 15, and did not return to baseline by the beginning of the next treatment period. Treatment with the low \(\beta\)-carotene muffins (2.6 mg/d) resulted in an 85% increase in serum \(\beta\)-carotene above baseline on d 11 of the treatment, almost 50% less than the high \(\beta\)-carotene muffin. As expected, the white carrot muffin, which was devoid of carotenoids, resulted in a 26% decrease by d 11.

Results of the ANOVA for \(\beta\)-carotene showed an effect of treatment (\(P < 0.001\)). The white carrot muffin resulted in a lower serum \(\beta\)-carotene concentration compared with the low \(\beta\)-carotene muffin (\(P = 0.006\)) and the high \(\beta\)-carotene muffin (\(P < 0.001\)), but the orange carrot muffins did not differ. An effect of sequence of treatments (\(P = 0.038\)) was observed, indicating that the order in which subjects ate the muffins affected the serum response. In addition, a trend towards a negative effect of BMI existed (\(P = 0.08\)).

Serum \(\alpha\)-carotene concentrations increased dramatically for both carrot feeding periods and did not differ (\(P = 0.8\)). Treatment with the orange carrots (1.8 mg \(\alpha\)-carotene) resulted in a 285% increase above baseline (0.092 \(\mu\)mol/L at baseline to 0.354 \(\mu\)mol/L at d 11), and treatment with dark-orange carrots (4 mg \(\alpha\)-carotene) resulted in a 210% increase from baseline (0.139 \(\mu\)mol/L at baseline to 0.431 \(\mu\)mol/L at d 11). White carrots resulted in a 35% decrease in serum \(\alpha\)-carotene concentration, and the decrease was lower than in the orange carrot groups (\(P < 0.001\)). \(\alpha\)-Carotene concentrations were affected by treatment (\(P < 0.001\)) and sequence of feeding (\(P = 0.024\)). The \(\alpha\)-carotene response was lower for those individuals with larger BMIs.

To account for the carryover effect of the high \(\beta\)-carotene muffin, the first 20-d period was evaluated alone. When only this treatment period is analyzed, there is a significant treatment effect (\(P = 0.01\)) with regards to \(\beta\)-carotene, and the low and high \(\beta\)-carotene carrot treatments differed (\(P = 0.01\)). \(\alpha\)-Carotene serum concentrations were different between all treatment groups (\(P = 0.004\)).

Serum retinol concentration and CV for each treatment type and each 20-d treatment period are listed in Table 4. Treatment, sequence, BMI, gender, and age did not affect serum retinol concentration. However, there was a significant treatment by sequence interaction (\(P = 0.02\)). This indicates that the highest retinol concentration occurred during the first 20 d of the study, regardless of the treatment type. After accounting for this effect, there was no remaining treatment effect.

Serum CRP concentrations for all participants on the first day of each treatment period were normal (<2.5 mg/L). Values were 0.58 \(\pm\) 0.40, 0.75 \(\pm\) 0.42, and 0.91 \(\pm\) 0.69 mg/
L for periods 1, 2, and 3, respectively. CRP was not related to serum retinol concentrations.

Discussion

This crossover study sought to determine the relative bioavailability of \( \alpha \)- and \( \beta \)-carotene from dark-orange and typical orange carrots with chronic feeding. Numerically, these results indicate an increased serum response with the dark-orange carrot, and statistically, this occurred in the first treatment period. Carrot muffins with 2.6 or 7 mg/d \( \beta \)-carotene were fed once a day for 11 d and blood was sampled in the fasting state. The difference in the fasting serum was not large enough to induce a differential response between orange carrot groups, given the length of feeding.

**Table 4.** Mean Serum Retinol Concentration (\( \mu \text{mol/L} \)) as a Function of Muffin Type or Treatment Period in Adults

<table>
<thead>
<tr>
<th>Muffin Type</th>
<th>Serum Retinol, ( \mu \text{mol/L} )</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark-orange carrot</td>
<td>1.76 ± 0.14</td>
<td>8</td>
</tr>
<tr>
<td>White carrot</td>
<td>1.63 ± 0.10</td>
<td>6</td>
</tr>
<tr>
<td>Orange carrot</td>
<td>1.66 ± 0.10</td>
<td>6</td>
</tr>
<tr>
<td>Treatment period 1*</td>
<td>2.07 ± 0.16(^b)</td>
<td>8</td>
</tr>
<tr>
<td>Treatment period 2</td>
<td>1.33 ± 0.10(^c)</td>
<td>7</td>
</tr>
<tr>
<td>Treatment period 3</td>
<td>1.64 ± 0.06(^c)</td>
<td>4</td>
</tr>
</tbody>
</table>

* Significant treatment by sequence interaction (\( P = 0.02 \)).
and the sustained low-carotenoid diet. Serum carotenoid concentrations have limited utility in reflecting tissue storage in both animals (10, 23) and humans (6). However, this does not negate the usefulness of fasting serum carotenoids as a dietary biomarker of fruit and vegetable intake. During the orange carrot feeding, which was once/d, the fasting serum reflected an increase in α- and β-carotene intake. α-Carotene is very specific to carrot intake (24) because it is not widely distributed in other fruits and vegetables.

Qualitatively, changes in serum α- and β-carotene concentrations reflected dietary intake of orange carrots (6). Quantitatively, fasting serum concentrations did not reflect the difference in α- and β-carotene content of the carrots when all of the treatment periods are combined. Tissue concentrations in animal models reflect quantitative differences in carotenoid intake (10, 23). Assessing differences in fruit and vegetable intake based on current recommendations (1) may require more sensitive indicators of tissue storage. Changes in natural abundance of $^{13}$C in serum retinol reflected an increase in vegetable intake with dietary change when the vegetables contained provitamin A carotenoids and may act as a biomarker (25).

Analysis of the dark-orange and orange carrots showed α- to β-carotene ratios of 1.2:2 and 2:3, respectively, with the dark-orange carrot having twice as much α-carotene as the orange carrot (26). α- and β-Carotene may be competing for absorption, because the fasting serum responses for α-carotene were the same for both carrot types. The larger amount of β-carotene present in the gut during treatment with the high β-carotene muffin may have interfered with α-carotene absorption. The lower β-carotene concentration in the regular carrot did not appreciably interfere with α-carotene absorption. Thus, relatively more α-carotene was absorbed during the low β-carotene carrot treatment.

The 10-d washout period did not bring β-carotene serum concentrations back to baseline after feeding the dark-orange carrot muffin, but was sufficient for the typical orange carrot muffin. The sequence placed subjects on the white carrot muffin after the dark-orange carrot muffin. The white carrot muffin resulted in the expected decrease in serum carotenoid concentration, but the slope was affected by this carryover effect. This effect was largest for those individuals with higher β-carotene responses from the dark-orange carrot. Future crossover-type studies should allow subjects to resume their normal dietary patterns for a period of time before the onset of the next treatment. Groups were not equalized at baseline for race, gender, age, or BMI, because of the crossover design.

As with other carotenoid bioavailability studies, a large individual variation in the serum response existed. When evaluating the individual responses to the orange carrots, the rankings of the individual subjects’ AUC were similar. In fact, the highest responder had AUC of 5.20 and 4.18 μmol/dL and the lowest responder had AUC of 0.577 and 0.089 μmol/dL for the high and low β-carotene muffins, respectively. The phenomenon of low and high responders to β-carotene supplementation is well documented in the literature (27). Further studies should determine if this is due to differences in absorption, altered chylomicron metabolism (27), or differences in the ability to cleave β-carotene to vitamin A.

β-Carotene can be cleaved in the intestine to form 2 molecules of retinal, which can then be reduced to form retinol. However, retinol is homeostatically controlled, and therefore, unless an individual is vitamin A deficient, one would expect that administration of β-carotene would not affect serum retinol concentrations. Serum retinol concentrations decreased after the first period. These subjects were on a low carotenoid diet 1 week prior to and throughout the study. In addition, subjects were not allowed to consume multivitamins. Therefore, it is possible that for some habitual multivitamin users, their dietary vitamin A sources were limited during the intervention, causing a shift in the serum retinol set-point. The loss of the multivitamin as a continuous source of preformed vitamin A may have exacerbated the decrease in serum retinol over the first 20 days. Data in piglets (28) showed a decrease in serum retinol concentration when vitamin A sources were withdrawn, even though liver reserves were the same or higher. The decrease in serum retinol may have been a regulatory change to the shift in dietary sources of retinol to reduce the retinol utilization rate (29, 30) due to lower vitamin A intake. To rule out an effect of inflammation or infection on depressed serum retinol concentrations, CRP concentrations were determined at the beginning of each period, and all were normal, i.e., <10 mg/L (31).

Applying a bioconversion factor of 12 μg β-carotene to 1 μg retinol from foods (32), 220 and 600 μg retinol were fed from the low and high β-carotene muffins, respectively. Current US Recommended Dietary Allowances are 700 and 900 μg retinol for women and men (32), respectively. The muffins provided a significant source of dietary retinol for our subjects due to the sustained low carotenoid diet. This may have led to a more muted response in the β-carotene concentration curve, as more was converted to retinol during the latter treatment periods. Furthermore, a study in Nepalese women showed that β-carotene sources of vitamin A, i.e., carrots and spinach, resulted in a lower serum retinol concentration but a similar improvement in pupillary threshold measurements than liver or vitamin A supplements (33). Perhaps serum retinol concentrations are lower in groups that rely on β-carotene for vitamin A.

Epidemiological studies have associated β-carotene with a decreased incidence of coronary heart disease and some types of cancer (34). Carrots are a familiar and popular vegetable providing a significant source of provitamin A. β-Carotene and α-carotene provide vitamin A (2, 35). As a vegetable, carrots are inexpensive, have a long shelf-life, and a mild flavor (26, 36). These high carotene carrots can provide more provitamin A per carrot than the typical store-bought varieties, without a change in flavor. If these
biofortified carrots were widely available in regions where orange carrots are grown (37), vitamin A status may be impacted. This needs to be tested in a population that has inadequate vitamin A stores. In individuals who have a normal vitamin A status, high carotene carrots may enhance tissue concentrations of α- and β-carotene. Animal models have shown improved bioconversion with lower liver vitamin A reserves (2) and enhanced antioxidant status with carrot feeding (38). Furthermore, orange carrot extract prevented in vitro degradation of cholesterol with heating (39). Thus, the epidemiological evidence of decreased risk of disease may be due to the dual role that β-carotene plays in health.

35. Tanumihardjo SA, Howe JA. Twice the amount of α-carotene isolated from carrots is as effective as β-carotene in maintaining the vitamin A status of Mongolian gerbils. J Nutr 135:2622–2626, 2005.


