Biofortified Carrot Intake Enhances Liver Antioxidant Capacity and Vitamin A Status in Mongolian Gerbils¹,²

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Abstract

Biofortification efforts have increased concentrations of bioactive compounds in carrots. We measured the antioxidant potential and vitamin A bioefficacy of 4 biofortified carrot varieties [purple/orange, purple/orange/red, orange/red, and orange] in Mongolian gerbils (n = 73). Following a 4-wk vitamin A depletion period and baseline kill (n = 7), freeze-dried carrot powders were mixed into purified feeds and fed to 6 groups (n = 11/group) for 4 wk. White carrot-fed control and vitamin A-supplemented groups were used to calculate carrot provitamin A bioefficacy. Antioxidant capacities of carrot powders, sera, and livers were determined using the 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay and carotenoid and retinol concentrations were determined by HPLC. Antioxidant capacity of liver extracts from the 4 colored carrot-fed groups [10.1 ± 1.2 μmol Trolox equivalent antioxidant capacity (TEAC)/g] was significantly higher than the white carrot-fed control group (9.3 ± 0.9 μmol TEAC/g) and vitamin A-supplemented group (8.8 ± 1.4 μmol TEAC/g, P < 0.05). Liver retinol stores in the colored carrot-fed groups (0.62 ± 0.13 to 0.67 ± 0.08 μmol retinol/liver) did not differ and were higher than the white carrot-fed control group (0.32 ± 0.08 μmol retinol/g, P < 0.0001). Serum antioxidant capacity and retinol did not differ among treatment groups. Liver antioxidant capacity and vitamin A stores were higher in gerbils fed colored carrots than in those fed white carrots. Antioxidant activity is one of several proposed mechanisms by which plant foods, like biofortified carrots, may provide additional health benefits beyond maintenance of vitamin A status. J. Nutr. 138: 1692–1698, 2008.

Introduction

Dietary provitamin A carotenoids constitute a vital source of vitamin A for much of the world’s population. Using the Mongolian gerbil (Meriones unguiculatus) model of provitamin A carotenoid bioconversion to vitamin A (1), we have demonstrated consistently that modest intake of provitamin A carotenoids from plant sources alone can maintain adequate vitamin A status (2–5). Moreover, dietary β-carotene and β-cryptoxanthin vitamin A bioefficacies have compared favorably to purified carotenoid supplements in oil (2,5).

In areas at risk of vitamin A deficiency, improvement in vitamin A status through dietary provitamin A carotenoid intake may be more advantageous than periodic supplementation with preformed vitamin A (6). Dietary provitamin A carotenoids pose no danger of toxicity, because efficiency of bioconversion to vitamin A is largely dependent on vitamin A status (7), among other factors (8). Conversely, excessive intake of preformed vitamin A can result in hypervitaminosis A (9) and vitamin A supplementation can have adverse effects (10), especially if excessive amounts are administered erroneously (11). Furthermore, plant sources of provitamin A carotenoids typically contain additional bioactive compounds with putative health benefits. Some of the best characterized and most widely studied of these (i.e. carotenoids and polyphenols) are present in carrots of various colors (12). In addition to the provitamin A carotenoids and phenolic compounds found in typical orange carrots, purple carrot varieties contain anthocyanins, whereas red varieties contain lycopene and concentrations of these phytochemicals have increased dramatically in carrots as a result of biofortification efforts to enhance nutritional quality (13).

Biofortification involves selective breeding of food crops to increase concentrations of bioavailable micronutrients, such as provitamin A carotenoids, zinc, and iron (14). With growing interest in plant food bioactivity, efforts have expanded to include additional phytochemicals. As a result, several carrot varieties have been developed with greater concentrations of provitamin A carotenoids, nonprovitamin A carotenoids (i.e. lycopene and lutein), and polyphenolic compounds (i.e. anthocyanins and phenolic acids) (15). In addition to meeting vitamin A requirements, carrot provitamin A carotenoids have demonstr...
Lipophilic antioxidant capacity, hydrophilic antioxidant capacity, anthocyanins, lycopene, b systems.

...distribution of dietary antioxidants to the liver antioxidant defense extracts provides a more comprehensive picture of the contribution of dietary antioxidants stored in liver to account for dietary antioxidants such as provitamin A, lycopene and anthocyanin-free white carrot powder was added to all feeds to normalize for total antioxidant capacities of the colored carrots were much higher than the white carrot, but were similar to each other. Concentrations of lycopene in OR and anthocyanins in PO were both higher than that of the newly developed POR. The POR carrot uniquely contained all of the compounds of interest to this study at biologically relevant concentrations.

**Table 1** Characteristics of freeze-dried carrot powders and feeds fed to Mongolian gerbils for 4 wk

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>White</th>
<th>PO</th>
<th>POR</th>
<th>OR</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-Carotene, nmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>0.7 ± 0.2</td>
<td>444 ± 43</td>
<td>103 ± 2</td>
<td>56 ± 14</td>
<td>583 ± 379</td>
</tr>
<tr>
<td>Feed</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>0.7 ± 0.06</td>
<td>0.9 ± 0.058</td>
<td>2.8 ± 0.06</td>
</tr>
<tr>
<td>b-Carotene, nmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Carrot</td>
<td>1.0 ± 0.08</td>
<td>1238 ± 118</td>
<td>510 ± 21</td>
<td>551 ± 126</td>
<td>1268 ± 231</td>
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<tr>
<td>Feed</td>
<td>ND</td>
<td>3.7 ± 0.06</td>
<td>5.3 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>3.5 ± 0.08</td>
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<tr>
<td>Lycopene, nmol/g</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>0.3 ± 0.1</td>
<td>31 ± 4</td>
<td>569 ± 42</td>
<td>1433 ± 372</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Feed</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anthocyanins, μmol C3GE/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Carrot</td>
<td>ND</td>
<td>15.1 ± 1.3</td>
<td>3.0 ± 1.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Feed</td>
<td>ND</td>
<td>0.11 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Hydrophilic antioxidant capacity, μmol TEAC/g</td>
<td></td>
<td></td>
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<tr>
<td>Carrot</td>
<td>4.20 ± 0.92</td>
<td>47.0 ± 196</td>
<td>22.2 ± 0.97</td>
<td>7.80 ± 0.27</td>
<td>10.2 ± 0.84</td>
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<tr>
<td>Feed</td>
<td>0.18 ± 0.037</td>
<td>0.29 ± 0.017</td>
<td>0.24 ± 0.011</td>
<td>0.20 ± 0.026</td>
<td>0.21 ± 0.083</td>
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<tr>
<td>Lipophilic antioxidant capacity, μmol TEAC/g</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Carrot</td>
<td>0.16 ± 0.020</td>
<td>3.5 ± 0.034</td>
<td>4.0 ± 0.50</td>
<td>4.4 ± 0.18</td>
<td>3.5 ± 0.13</td>
</tr>
<tr>
<td>Feed</td>
<td>0.05 ± 0.001</td>
<td>0.07 ± 0.002</td>
<td>0.09 ± 0.002</td>
<td>0.08 ± 0.001</td>
<td>0.07 ± 0.001</td>
</tr>
<tr>
<td>Total phenolics, mg GAE/g</td>
<td>4.6 ± 0.2</td>
<td>12.0 ± 0.3</td>
<td>5.9 ± 0.2</td>
<td>4.9 ± 0.1</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>Feed</td>
<td>0.35 ± 0.035</td>
<td>0.45 ± 0.052</td>
<td>0.43 ± 0.032</td>
<td>0.37 ± 0.034</td>
<td>0.36 ± 0.039</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 3. ND, not detected.
2 For the 4-wk treatment period, PO, POR, OR, and O feeds contained their respective carrot powders in the following proportions (g/kg feed): 3.4, 8.7, 6.0, and 3.2, respectively. The OR feed also contained O carrot powder (1.2 g/kg) to maintain similar provitamin A concentrations among all 4 diets and similar lycopene concentrations in the OR and POR diets. To achieve similar total anthocyanin concentrations in the PO and POR feeds, a purple/white carrot powder (234 nmol β-carotene/g, 64 nmol a-carotene/g, and 28.5 μmol cyanidin-3-O-galactoside equivalents/g) was added to the PO and POR feeds at 2.4, and 3.6 g/kg feed, respectively. After similar concentrations of provitamin A, lycopene and anthocyanins were obtained in treatment feeds and carotenoids and anthocyanin-free white carrot powder was added to all feeds to normalize for total antioxidant capacities of the colored carrots were much higher than the white carrot, but were similar to each other. Concentrations of lycopene in OR and anthocyanins in PO were both higher than that of the newly developed POR carrot. The POR carrot uniquely contained all of the compounds of interest to this study at biologically relevant concentrations.

**Materials and Methods**

**Carrot powders.** Carrots were grown from October 23, 2006 until harvest on March 6, 2007 at the University of California Desert Research and Extension Center between El Centro and Holtville, CA. Carrots were harvested in May 2006 and shipped to Wisconsin for evaluation. Carrots were cut into chips, freeze-dried, and ground into fine powders. O and PO carrot powders contained greater a- and β-carotene concentrations than did the OR and POR carrot powders and purple varieties (POR and PO) demonstrated greater phenolic concentration and hydrophilic antioxidant capacities (Table 1). Lipophilic antioxidant capacities of the colored carrots were much higher than the white carrot, but were similar to each other. Concentrations of lycopene in OR and anthocyanins in PO were both higher than that of the newly developed POR carrot. The POR carrot uniquely contained all of the compounds of interest to this study at biologically relevant concentrations.

**Phenolic concentration and antioxidant capacity of carrot powders.** Total phenolic concentrations of carrot powder and diets were determined using the Folin-Ciocalteau method (25), with gallic acid rather than ferulic acid as the external standard. Lipophilic and hydrophilic antioxidant capacities of carrot powders were determined using the 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay (26). This in vitro assay involves the generation of a relatively stable free radical that loses color after scavenging electrons from antioxidants in a sample. The color change, monitored by the change in absorbance at 734 nm after a specified time, is proportional to the antioxidant concentration. ABTS, potassium persulfate, and Trolox were purchased from Sigma-Aldrich. The ABTS radical was generated by incubating ABTS (38.4 mg) with potassium persulfate (6.0 mg) in water (10 mL) for 12 h in the dark at room temperature followed by dilution in ethanol to an absorbance of 0.70 at 734 nm. Lipophilic antioxidants were extracted from freeze-dried carrot powder (0.5 g) with 2 mL hexane:dichloromethane (75:25, v/v) repeatedly until 10 mL, and the extract was saved. The remaining organic solvent was removed from the carrot powder by evaporation under argon and hydrophilic antioxidants were extracted using 2 mL acetone:water:acetic acid (70:29.5:0.5, v/v/v) repeatedly until 10 mL. One milliliter of ABTS free radical solution was mixed with aliquots of carrot extracts (200, 300, and 400 μL for lipophilic extracts; 50, 100, and 150 μL for hydrophilic extracts). After 10 min at room temperature, the absorbance was determined at 734 nm. The inhibition percentage of the absorbance of ABTS solution was calculated using the following equation:

\[
\text{Inhibition} = \frac{\text{Abs}_{0} - \text{Abs}_{10\min}}{\text{Abs}_{0}} \times 100.
\]

\[
\text{Abs}_{0} = \text{Abs}_{0} \times 100.
\]

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percent was plotted against the volume of extract added to obtain a linear regression line. The slope of the regression line was compared with that of a standard Trolox solution to calculate antioxidant capacity of carrot powders, expressed as Trolox equivalent antioxidant capacity (TEAC)/g carrot powder. Among the assortment of antioxidant capacity assessment methods, only the ABTS and oxygen radical absorbance capacity assays are able to determine both lipophilic and hydrophilic antioxidant capacities of foods (27). We chose ABTS over oxygen radical absorbance capacity, because it has been successfully applied in the determination of tissue and serum antioxidant capacity (23,24), which was an aim of this investigation.

**Carotenoid composition of carrot powders and diets.** For carotenoid analyses, β-apo-8’-carotenal was added as an internal standard to carrot powder (0.1 g) or feed (0.6 g) to determine extraction efficiency (94 ± 4 and 92 ± 2%, respectively) and carotenoids were extracted 3 times with hexane (3 mL) until the residue was colorless. Extracts were combined and washed with water (1 mL). The organic phase was dried under argon, redissolved in dichloroethane:methanol (200 μL, 50:50, v/v), and injected (25 μL) into a Waters HPLC system (Waters) (28). HPLC purified a-carotene, β-carotene, and lycopene standards and absorption spectra were used for peak identification. Chromatograms were generated at 450 and 470 nm.

**Anthocyanin concentration of carrot powders and diets.** For anthocyanin analyses of purple carrot varieties, malvidin-3-O-galactoside chloride (Indofine Chemical) was added as an internal standard to carrot powder (0.03 g) or carrot feed (1.2 g). Anthocyanins were extracted repeatedly with methanol:formic acid (90:10, v/v) and transferred to a 10-ml plastic flask until full volume. A 700-μL aliquot was dried under argon, redissolved in 100 μL methanol:formic acid (v/v), and injected (50 μL) onto the HPLC system, which consisted of a Waters guard column and an Agilent Zorbax C18 column (5 μm, 4.6 × 250 mm) heated at 30°C, 1525 binary HPLC pump, 717 autosampler, and 2996 photodiode array detector. The mobile phases were water:formic acid (90:10, v/v) and methanol (solvent B). Samples were analyzed at 1 mL/min using a gradient procedure starting at 95% A, followed by: 1) a 20-min linear gradient to 80% A; 2) 8-min hold; 3) 1 min linear gradient to 65% A; 4) 7 min linear gradient to 45% A; and 5) 1 min reverse gradient back to initial conditions. Anthocyanins were identified by matching HPLC peaks with carrot anthocyanins identified by MS (29). Cyanidin-3-O-glucoside chloride (ChromaDex) was used as an external standard to quantify carrot anthocyanin concentrations.

**Preparation of vitamin A supplement.** Oil doses of vitamin A were prepared by dissolving retinyl acetate (Sigma) directly into cottonseed oil or redissolved in dichloroethane:methanol (200 μL, 50:50, v/v), and injected (25 μL) into a Waters HPLC system (Waters) (28). HPLC purified a-carotene, β-carotene, and lycopene standards and absorption spectra were used for peak identification. Chromatograms were generated at 450 and 470 nm.

**Animals and procedures.** Male 40-d-old Mongolian gerbils (n = 73) were obtained from Charles River Laboratories. Gerbils were individually housed in plastic cages and consumed feed ad libitum during the starvation period. Gerbils were weighed daily and monitored for health until all were thriving, at which time they were weighed every 2 d. Gerbils were killed by exsanguination while under isofluorane anesthesia. Blood was centrifuged at 2200 × g; 15 min in BD Vacutainer Gel and Clot Activator tubes (Becton Dickinson) for serum isolation. Livers were excised and stored at −80°C until analyses. All animal handling procedures were approved by the College of Agriculture and Life Sciences Animal Care and Use Committee of the University of Wisconsin-Madison.

**Experimental design and diets.** A carotenoid and vitamin A-free purified basal feed (Table 2) was fed to all gerbils for 4 wk to deplete liver retinol stores. Depletion was followed by a baseline kill (n = 7) to establish pretreatment vitamin, carotenoid, and antioxidant statuses. During the treatment phase, freeze-dried carrot powder (O, OR, PO, POR) was added to the basal feed to achieve a provitamin A concentration of 6 nmol β-carotene equivalents/g feed (Table 1) and fed to gerbils (n = 11/group) for 4 wk. Control and vitamin A supplement groups (n = 11/group) were fed the basal feed with added carotenoid and anthocyanin-free white carrot powder and supplemented with cottonseed oil or vitamin A in oil, respectively. Vitamin A supplements were given as oral doses of retinyl acetate twice daily to match the previous day’s provitamin A carotenoid intake from the colored carrot-fed groups, based on a theoretical 100% vitamin A bioefficacy. All feeds were equalized for total vitamin A content (2.5%) with the addition of carotenoid-free white carrot powder. Feeds were analyzed for carotenoid and anthocyanin concentrations every 5 d and adjusted when necessary to maintain an equal intake of β-carotene equivalents among O, OR, PO, and POR groups, lycopene between POR and OR, and anthocyanin equivalents between POR and PO (Table 1). Total phenolic concentration and hydrophilic antioxidant capacities were higher in PO and POR carrot feeds due to anthocyanins. Lipophilic antioxidant capacities of the 4 colored carrot diets were higher than the white carrot feeds fed to control and vitamin A-supplement groups due to much greater concentrations of carotenoids. Vitamin E concentrations in carrots were nominal relative to the basal diet; therefore, treatment feeds contained the same concentration of α-tocopherol (Table 2).

**Serum and liver antioxidant capacity analyses.** The ABTS radical scavenging decolorization assay (26) was used to determine antioxidant capacity of gerbil serum (24,30) and livers (23,24). Serum was diluted 1:10 in PBS and added directly to 1 mL ABTS radical in PBS (absorbance 0.7), and the change in absorbance was measured after 10 min. Inhibition percent of the 3 aliquots (10, 20, and 30 μL) was graphed and the slope was compared with a standard curve generated with Trolox dissolved in PBS. Serum antioxidant capacity was expressed as TEAC/L serum.

For the liver antioxidant capacity determinations, aliquots (200, 300, and 400 μL) of the dichloromethane extracts from the carotenoid and vitamin A analyses were added to 1 mL ABTS radical in ethanol immediately following extraction and the change in absorbance was determined after 10 min. Following the lipophilic extraction with dichloromethane, hydrophilic antioxidants were extracted from liver samples with 50 mL acetone: water:acetic acid (70:29.5:0.5, v/v/v). Aliquots (50, 100, and 150 μL) of the aqueous extract were added to 1 mL ABTS radical in ethanol (absorbance 0.7), and the change in absorbance (inhibition percent) was measured after 10 min. Liver lipophilic and hydrophilic antioxidant capacities were

| TABLE 2 Composition of purified basal feed provided to Mongolian gerbils during a study to evaluate antioxidant and vitamin A effects of a variety of carrots 1 |
|----------------------|------------------|
| g/kg feed            |
| Vitamin-free casein  |
| l-Cystine            | 3                |
| Sucrose              | 360              |
| Malodextrin          | 120              |
| Corn starch          | 150              |
| Cottonseed oil       | 60               |
| Cellulose            | 60               |
| Mineral mix 2         | 35               |
| CaHPO4               | 2                |
| MgO                  | 1.75             |
| Vitamin mix 3         | 5                |
| Cholecalcifer         | 0.0044           |
| dl-α-Tocopherol acetate | 0.242         |
| Choline bitartrate    | 2.5              |

1 Purified diet provided by Harlan Teklad was fed to all gerbils with no added carrot powder during the 4-wk vitamin A-depletion phase and with added carrot powder (25 g/kg feed) during the following 4-wk treatment period.

2 Mineral Mix (AIN-93G-MX/2).

3 Vitamin Mix provided the following (mg/kg feed): biotin, 0.4; calcium panthenolate, 66.1; folic acid, 2; inositol, 110.1; menadione, 49.6; niacin, 99.1; paminobenzoic acid, 11.0; pyridoxine-HCl, 22; riboflavin, 22; thiamin-HCl, 22; vitamin B-12 (0.1% in mannitol), 29.7; ascorbic acid (97.5%), 1016.6.
determined by comparing the inhibition percent slope from the 3 aliquots to a standard curve generated with Trolox dissolved in ethanol and expressed as TEAC/g liver.

**Serum and liver retinol, α-tocopherol, and carotenoid analyses.** All samples were analyzed under gold fluorescent lights to prevent carotenoid photooxidation and isomerization. Published procedures were used for vitamin A and carotenoid analyses of serum and tissues (4). Retinyl butyrate was used as an internal standard to determine extraction efficiency in serum and livers and externally for quantification of retinol and retinyl esters. Dried serum and liver extracts were reconstituted in methanol:dichloroethane (100 μL, 50:50, v:v) and injected (50 μL) into the HPLC (28). Scanning from 210 to 550 nm on the photodiode-array HPLC allowed for the simultaneous quantification of α-tocopherol (31), vitamin A, and carotenoids by generating chromatograms at 295, 325, and 450 nm, respectively.

**Statistical analyses.** Values are means ± SD. Data were analyzed using Statistical Analysis System software (SAS Institute, version 8.2, 2001). Outcomes of interest (i.e. gerbil weights; feed and carotenoid intakes; serum and liver vitamin A; carotenoid concentrations; and antioxidant capacities) were evaluated using ANOVA at α < 0.05. Post hoc differences among treatment groups were determined using Fisher’s least significant difference test at α < 0.05. Contrasts compared antioxidant capacities of serum and liver extracts from the combined colored carrot groups against that of the white carrot group or the vitamin A-supplemented group.

**Results**

**Gerbil weights and intakes.** Gerbil body weights did not differ among groups, ranging from 67.4 ± 10.4 g in the baseline group to 74.3 ± 7.5 g in the O group. Liver weights did not differ, ranging from 2.6 ± 0.2 g in the control group to 2.9 ± 0.6 g in the baseline group. Daily carrot-feed intake did not differ among groups and ranged from 4.9 ± 0.3 g in the OR group to 5.2 ± 0.7 g in the PO group. Total intake of β-carotene equivalents over the 4-wk treatment period did not differ among groups fed colored carrots and ranged from 810 ± 54 nmol for the OR group to 857 ± 121 nmol for the PO group. The vitamin A-supplemented group received 1680 nmol retinyl acetate over the 4-wk treatment period. Total lycopene intake was similar in POR (1268 ± 48 nmol) and OR groups (1298 ± 80 nmol) as was total anthocyanin intake in POR (15.3 ± 0.6 μmol cyanidin-3-O-glucoside equivalents) and PO groups (15.0 ± 2.0 μmol cyanidin-3-O-glucoside equivalents).

**Serum and liver antioxidant capacities.** Serum antioxidant capacity was significantly lower in the baseline group than in all treatment groups, which did not differ (Fig. 1). Due to the difference in ages at kill, we cannot conclude that this difference was due solely to carrot intake. Total liver antioxidant capacity of the 4 colored carrot-fed groups (10.1 ± 1.2 μmol TEAC/g) was higher than that of both the white carrot-fed control (9.3 ± 0.9 μmol TEAC/g) and vitamin A-supplemented groups (8.8 ± 1.4 μmol TEAC/g) (P < 0.05). The combined colored carrot-fed groups differed from the control (P = 0.033) and vitamin A-supplemented (P = 0.0015) groups. Liver antioxidant capacities of the individual colored carrot-fed groups were each significantly higher than the vitamin A-supplemented group, but only POR was significantly higher than control (Fig. 1).

Relationships between groups were well conserved when total liver antioxidant capacity was broken down into its lipophilic and hydrophilic components, with the exception of the baseline group ranking, which differed by extract. The antioxidant capacity of the baseline hydrophilic liver extracts (5.34 ± 0.53 μmol TEAC/g) was lower than every other group (O, 8.09 ± 0.94; OR, 8.08 ± 0.73; PO, 8.01 ± 0.15; POR, 8.33 ± 1.4; control, 7.37 ± 0.90; vitamin A supplement, 6.97 ± 1.41 μmol TEAC/g) (P < 0.05). The baseline lipophilic antioxidant capacity (1.92 ± 0.13 μmol TEAC/g) did not differ from O, OR, PO, or control groups but was higher than the vitamin A-supplement group (1.86 ± 0.09 μmol TEAC/g) (P < 0.05).

**Serum and liver retinol, α-tocopherol, and carotenoid concentrations.** Serum retinol concentrations did not differ among groups (P = 0.2), but liver total retinol concentrations were higher in the colored carrot-fed groups than in the control group (P < 0.0001) (Fig. 2). All feeds except PO improved vitamin A status above baseline levels. Liver total retinol was higher in the vitamin A-supplemented group than in all other groups (P < 0.0001).

Liver α-tocopherol content (Fig. 3A) and serum concentration generally were higher in the baseline group than in the other groups, which may partially explain why the antioxidant capacity of the baseline lipophilic liver extracts, unlike the hydrophilic liver extracts, was not lower than that of the carrot-fed treatment groups. Among the treatment groups, liver α-tocopherol in the colored carrot-fed groups combined (1.73 ± 0.64 μmol/liver) was higher than the vitamin A supplement group (1.27 ± 0.39 μmol/liver) (P = 0.028) but did not differ from the control group (1.44 ± 0.34 μmol/liver).

In contrast to humans, gerbils do not circulate appreciable amounts of carotenoids in serum when dietary intake is low.
(2,5,31), as was the case in the current study; thus, carotenoids were not detected in serum. Liver lycopene contents did not differ between the OR (16.6 ± 5.4 nmol/liver) and POR (14.9 ± 3.6 nmol/liver) groups and β-carotene contents did not differ among the 4 colored carrot-fed groups (1.1 ± 0.02 nmol/liver). The liver α-carotene content was higher in the O (0.47 ± 0.15 nmol/liver) and PO groups (0.44 ± 0.11 nmol/liver) than in the OR (0.16 ± 0.04 nmol/liver) and POR groups (0.11 ± 0.03 nmol/liver) (P < 0.0001).

**Carrot provitamin A carotenoid conversion factors.** Vitamin A bioefficacy (i.e. provitamin A conversion factors) was calculated by dividing the difference between the vitamin A-supplemented and control group liver retinol values by the liver retinol values of the colored carrot groups (5). Conversion factors are presented as β-carotene equivalent intake (µg) necessary to yield 1 µg retinol. Conversion was slightly more efficient in the O and OR groups (both 2.7 µg β-carotene equivalents:1 µg retinol) than in the PO and POR groups (3.1:1 and 2.8:1), but groups did not differ.

**Discussion**

Plant food sources of provitamin A carotenoids contain bioactive compounds that may provide additional health benefits beyond the maintenance of adequate vitamin A status. Biofortification of carrots has resulted in increased concentrations of bioactive compounds, namely carotenoids and polyphenols. The current study aimed to determine the influence of bioactive compounds in biofortified carrots on antioxidant capacity of liver and serum and vitamin A bioefficacy of provitamin A carotenoids. Antioxidant capacity of serum did not differ among the treatment groups, whereas antioxidant capacities of liver extracts were greater in gerbils fed colored carrots compared with gerbils fed white carrots, especially those supplemented with vitamin A. This supports the notion that vitamin A is not an in vivo antioxidant. Carrots of all colors, except white, supported vitamin A status equally well.

Human studies often report no significant gains in antioxidant capacity of serum or reductions in circulating markers of oxidative damage after interventions involving intake of dietary antioxidants (32,33). Antioxidant feeding interventions in animals have enhanced serum antioxidant capacity in some studies (30,34) but not in others (24,31). Specific dietary antioxidants fed and the duration of feeding largely determine whether serum antioxidant capacity will be enhanced. Our relatively short study duration may be responsible for the lack of improvement in serum antioxidant capacity observed. Serum retinol did not differ by group, despite significant differences in liver retinol stores. Perhaps serum antioxidant capacity, like serum retinol, is not a sensitive indicator of what is truly occurring at the cellular level in tissues (35).

Animal studies allow direct determination of cellular antioxidant defense capability, because tissues are accessible. Gao et al. (24) observed that liver, but not serum, antioxidant capacity of rats fed rutin or baicalin (both dietary flavonoids) for...
3 wk was greater compared with control-fed rats. We also observed greater liver, but not serum, antioxidant capacities among gerbils fed colored carrots for 4 wk. Liver antioxidant capacities in gerbils fed white carrots and supplemented with oil or vitamin A were lower, suggesting that the bioactive compounds in the colored carrots, such as \( \alpha \)-carotene, \( \beta \)-carotene, lycopene, and anthocyanins, may have enhanced liver antioxidant capacity either by acting directly as antioxidants or indirectly by sparing \( \alpha \)-tocopherol, which was higher in gerbils fed colored carrots than in those fed white carrots and supplemented with vitamin A. The liver is among the most metabolically active organs, accounting for \( \sim 20\% \) of the total oxygen consumed by the body despite constituting only 2–5% of the body weight (36). As a prolific generator of reactive oxygen species, the liver may benefit from additional antioxidative support from food to complement endogenous defenses such as superoxide dismutase, glutathione peroxidase, and catalase enzymes. Future investigations should determine antioxidant enzyme activity in response to interventions involving dietary antioxidant intake or supplementation to confirm enhanced antioxidant capacity in vivo.

The presence of lycopene did not influence efficiency of provitamin A carotenoid conversion to vitamin A. Negative postprandial interactions between lycopene and \( \beta \)-carotene have been observed during micellization and chylomicron assembly in human and in vitro studies (37–39). We have observed that lycopene bioavailability is greater from tomato paste than from OR carrots when equal amounts were fed to humans (40) and gerbils (4). With the gerbil model, we were able to assess vitamin A bioefficacy, accounting for all of the individual steps influencing provitamin A carotenoid bioconversion to vitamin A (bioaccessibility and micellization, bioavailability, chylomicron assembly, and bioconversion). In the current study, intake of the O and OR carrots yielded similar vitamin A bioefficacies.

This result, in conjunction with previous data showing that lycopene is less bioavailable from OR carrots than from tomato paste with low \( \beta \)-carotene, suggests that lycopene bioavailability is more negatively affected than \( \beta \)-carotene bioefficacy when the 2 carotenoids interact.

Our observations in gerbils are supported mechanistically by investigation of the regulation of the \( \beta \)-carotene cleavage enzyme, 15, 15'-carotene monoxygenase 1 (CMO1) responsible for the conversion of \( \beta \)-carotene to vitamin A. Recently, a molecular mechanism was elucidated to describe how CMO1 responds to vitamin A status by metabolically limiting the amount of intact \( \beta \)-carotene that can be absorbed from the diet (41). The metabolic fate of lycopene does not appear to be as tightly regulated, although this is currently under investigation (42). Additionally, lycopene feeding does not alter the abundance of CMO1 in rat intestine (21), the predominant site of \( \beta \)-carotene conversion to vitamin A. Therefore, \( \beta \)-carotene may have a competitive advantage over lycopene when vitamin A status is low, as is the case in our vitamin A-depleted gerbil model. Furthermore, lycopene in raw carrots exists primarily in the all-trans configuration (4), which is considerably less bioavailable than cis forms (43), providing an even greater potential advantage for \( \beta \)-carotene over lycopene.

The presence of anthocyanins did not influence efficiency of provitamin A carotenoid conversion to vitamin A. Dietary flavonoids have been shown to decrease expression of CMO1 in vitro (44), which could potentially reduce \( \beta \)-carotene bioconversion to vitamin A in vivo. Previously, we found that carrots with anthocyanins demonstrated similar vitamin A bioefficacy as carrots with no anthocyanins in vitamin A-sufficient gerbils fed high carotenoid concentrations (3). In the present study, we fed vitamin A-depleted gerbil feeds with lower carotenoid and greater anthocyanin concentrations to further test anthocyanin and carotenoid interaction. Based on the current and previous studies, there is no substantial influence of anthocyanin intake on vitamin A bioefficacy of provitamin A carotenoids from carrots in vivo.

The study of human nutrition has traditionally adopted a reductionist point of view and in no area of research is this more apparent than nutritional epidemiology, where individual dietary compounds are routinely associated with specific health outcomes. Yet, when whole foods are compared with their individual components (e.g. tomato vs. lycopene or carrot vs. \( \alpha \)- and \( \beta \)-carotene), intake of the whole food is often associated with equal or greater reduction in disease risk than individual compounds (45,46). The enhancement of liver antioxidant capacity observed in gerbils consuming biofortified carrots was likely due to the combined bioactivities of multiple compounds rather than the individual activities of carotenoids, anthocyanins, or phenolic acids, illustrating the synergistic benefits associated with intake of whole foods (6,47). The enhanced liver antioxidant capacity in gerbils fed white carrots (control) relative to baseline may suggest a role of compounds other than pigments in whole foods, but POR carrots, with the most diverse and plentiful pigments, had the highest antioxidant capacity. Designer vegetables, such as the POR carrot used in this study, package and deliver multiple bioactive compounds. Whole food-based strategies utilizing functional foods like biofortified carrots are growing in popularity (48), because they have the potential to prevent chronic disease in addition to micronutrient deficiencies and should, therefore, be promoted in both developing and developed countries.

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