Spinach or carrots can supply significant amounts of vitamin A as assessed by feeding with intrinsically deuterated vegetables1–4

Guangwen Tang, Jian Qin, Gregory G Dolnikowski, Robert M Russell, and Michael A Grusak

ABSTRACT

Background: The vitamin A value of spinach and carrots needs to be measured directly.

Objective: The objective was to determine the vitamin A value of intrinsically labeled dietary spinach and carrots in humans.

Design: Spinach and carrots were intrinsically labeled by growing these plants in 25 atom% \(2\text{H}_2\text{O}\) nutrient solution. Growth in this medium yielded a range of trans \(\beta\)-carotene (\(\beta\)-carotene) isoto-pomers with a peak enrichment at molecular mass plus 10 mass units. Seven men with a mean (±SD) age of 59.0 ± 6.3 y and a body mass index (in kg/m²) of 25.7 ± 1.5 consumed puréed spinach (300 g, 20.8 \(\mu\)mol \(\beta\)-carotene equivalents) or carrots (100 g, 19.2 \(\mu\)mol \(\beta\)-carotene equivalents) with a standardized liquid diet (no extra fiber) in random order 4 mo apart. Seven women with a mean (±SD) age of 55.5 ± 6.3 y and a body mass index of 26.4 ± 4.2 consumed puréed spinach only (300 g, 20.0 \(\mu\)mol \(\beta\)-carotene equivalents). A reference dose of \(\left[{\text{\textsuperscript{13}}\text{C}}\right]\text{retinyl acetate (8.9 \(\mu\)mol) in oil was given to each subject 1 wk after each vegetable dose. Blood samples were collected over 35 d.}

Results: Areas under the curve for total labeled serum \(\beta\)-carotene responses were 42.4 ± 8.5 \(\mu\)mol · d per \(\mu\)mol spinach \(\beta\)-carotene and 119.8 ± 23.0 \(\mu\)mol · d per \(\mu\)mol carrot \(\beta\)-carotene (\(P < 0.01\)). Compared with the \(\left[{\text{\textsuperscript{13}}\text{C}}\right]\text{retinyl acetate reference dose, spinach \(\beta\)-carotene conversion to retinol was 20.9 ± 9.0 to 1 (range: 10.0–46.5 to 1) and carrot \(\beta\)-carotene conversion to retinol was 14.8 ± 6.5 to 1 (range: 7.7–24.5 to 1) by weight.}

Conclusions: Spinach and carrots can provide a significant amount of vitamin A even though the amount is not as great as previously proposed. Food matrices greatly affect the bioavailability of plant carotenoids, their efficiency of conversion to vitamin A, or both. Am J Clin Nutr 2005;82:821–8.

KEY WORDS Vegetables, stable isotope, hydroponics, spinach, carrots, mass spectrometry

INTRODUCTION

Vitamin A is essential for the promotion of general growth, the maintenance of visual function, the regulation of differentiation in epithelial tissues, and embryonic development (1). Vitamin A deficiency can cause visual malfunctions such as night blindness and xerophthalmia (2) and reduces immune responsiveness (3), which results in an increased incidence or severity of respiratory infections, gastrointestinal infections (4), and measles (5). It is estimated that \(\approx250\) million preschool children, mostly in developing countries, have some level of vitamin A deficiency (6).

In contrast, excessive intake of retinol may result in teratogenesis (7), liver damage (8), and possibly increased bone loss in the elderly (9).

Vitamin A can be obtained from food, either as preformed vitamin A in animal products or as provitamin A carotenoids, mainly as \(\beta\)-carotene in plant products. In some countries, such as China (10), provitamin A carotenoids in vegetables and fruit supply most of the daily vitamin A intake, even when based on a conversion factor of \(\beta\)-carotene to retinol of 12 to 1 by weight (11). Indeed, food-based interventions to increase the availability of provitamin A–rich foods and their consumption have been advocated as realistic and sustainable strategies to overcome vitamin A deficiency globally (1).

However, the metabolism in humans of carotenoids contained in various food matrices has not been well studied. To understand the biological characteristics of \(\beta\)-carotene and other carotenoids from food sources, it is essential to investigate their characteristics of absorption and disposal kinetics and the efficiency of their conversion to vitamin A in healthy persons after the consumption of carotenoid-rich foods.

Currently, per the US National Academy of Science, the retinol equivalence of carotenoids in food is 12 \(\mu\)g all-trans \(\beta\)-carotene, or 24 \(\mu\)g of other provitamin A carotenoids, equivalent to 1 \(\mu\)g retinol (11). The vitamin A value of a food has traditionally been based on the amounts of preformed vitamin A and provitamin A carotenoids contained in that food. However, major factors that affect the bioavailability of food carotenoids and the bioconversion of food carotenoids to vitamin A in humans are the food matrix, the method of food preparation, and the

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2 Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

3 This material is based on work supported by the US Department of Agriculture under Cooperative Agreements 581950-9-001, 58-6250-1-001, and 51000-065 and by a grant from USDA-CSREES-NRI (99-35200-7564).

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fat content of a meal. Although factors such as the food preparation method, the fat content of a meal, or the relative bioavailability of carotenoids from mixed vegetables and fruit have been investigated (12, 13), the bioavailability of carotenoids and their conversion to vitamin A from single vegetable sources has not been extensively studied because of the limited availability of isotopically labeled foods that can be fed to humans (14). This is unfortunate because estimates from some previous studies suggest that the conversion of β-carotene to retinol is as high as 26 to 1 (13). To achieve an accurate assessment of carotenoid bioavailability from an individual plant food and its subsequent vitamin A value, one can use foods in which the carotenoids have been endogenously or intrinsically labeled with a low-abundance stable isotope. This method allows presentation of the carotenoids in their normal cellular compartments, and the isotopic tag enables identification of serum carotenoids (or derived retinol and other metabolites), which come from the specific food being tested. Determinations of the efficiency of absorption of plant provitamin A carotenoids and their conversion to vitamin A are needed to make sound recommendations on which plant foods can provide vitamin A to humans (15).

This article reports the findings of a study that used intrinsically labeled vegetable carotenoids, in conjunction with an isotope reference method, to define the vitamin A equivalence of dietary spinach and carrots in healthy adults.

SUBJECTS AND METHODS

Subjects

Seven male and 7 female subjects were recruited from the general public in the Boston, MA, area. The mean (±SD) age of the subjects was 57.1 ± 6.5 y (range: 45-72 y); the mean (±SD) BMI (in kg/m²) was 26.1 ± 3.0 (range: 20.0-30.2). The subjects were healthy nonsmoking adults who had not taken vitamin supplements in the previous month. For the 2 wk before a residence period in a metabolic unit, the subjects were instructed to eat their normal diet but to not consume vitamin supplements or foods containing large amounts of β-carotene or vitamin A; a list of such foods was provided to the subjects by the study dietitian. The subjects were instructed to not consume carrots, dark-green leafy vegetables, pumpkins, sweet potatoes, and liver products. Each subject was required to fill out 3-d food records for the assigned days during each week of the 2-wk preparatory phase. The dietitian reviewed the food records on admission for compliance. Informed consent was obtained from all subjects under the guidelines established by the Institutional Review Board of the Tufts–New England Medical Center.

Study design

The participants were housed in the Metabolic Research Unit of the US Department of Agriculture (USDA) Human Nutrition Research Center on Aging at Tufts University for a 14-d resident stay and were free-living from day 15 to day 36 of the study. The study design was as outlined below.

On day 1, frozen and processed deuterated vegetables were provided to the subjects after they had fasted overnight; spinach or carrots was provided in random order to men, but spinach only was provided to women. The vegetables were heated in a microwave oven for 2 min before being given to each of the subjects with a 480-kcal liquid formula breakfast (no added fiber) that contained 25% of energy as fat. Five hours after the breakfast, the subjects consumed the same amount of liquid diet for lunch. In the evening, 10 h after the breakfast, the subjects were provided a dinner containing 31 g fat and 35 g protein with a total energy content of 880 kcal (containing only 7 μg β-carotene). Seven days after the labeled vegetable dose was provided, the subjects consumed a capsule containing 3.0 mg [13C8]retinyl acetate (8915 nmol) as a reference dose in 170-mg corn oil with a liquid formula breakfast identical to the one taken with the labeled vegetable dose on day 1. To avoid absorption competition between the vegetable dose and the reference dose, the 2 doses were provided 1 wk apart. For the first 14 d of the study, the subjects consumed a 2-d rotation diet containing 100 μg vitamin A/d and 25 μg β-carotene/d at the Nutrition Center, but from day 15 to day 36 they were free-living. Blood samples (10 mL for each time point) were collected from all subjects at 1.5, 2, 3, 4, 5, 6, 7, 9, 11, and 13 h on day 1 and day 8 of the study, and fasting blood samples were collected in the morning 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21, and 35 d after the labeled supplement was provided. Blood samples (no additive) were protected from light, kept at room temperature for 0.5 h after being collected, and then centrifuged with Sure-sep II (Organon Teknika Corp, Durham, NC) at 4 °C and 800 × g for 15 min. Serum was stored at −70 °C until processed.

During the free-living phase of the study (days 15–35), the subjects were given a list of fruit and vegetables to avoid and a list of fruit and vegetables with low amounts of β-carotene, which were allowed in their diet. In addition, the subjects were counseled to not consume multivitamins, minerals, nutritional supplements, fortified cereals, and fish liver oil. The amounts of carotenoids and vitamin A used to create all food-instruction sheets were derived from the USDA and the Nutrition Coordinating Center Carotenoid Database for US Foods 1998. Nutrient calculations were performed by using the Nutrition Data System for Research software version 4.02, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN. A research dietitian followed each subject weekly by phone to check for compliance.

Male subjects returned to the Nutrition Coordinating Center for the second phase of the study, ≥4 mo after the first dose of the labeled vegetable was provided. The procedures followed for the second dose of vegetables were the same as those used for the first vegetable dose.

Supplements

Labeled compounds

[13C8]Retinyl acetate (8, 9, 10, 12, 13, 14, 19, 20-[13C8]-retinyl acetate) was purchased from Cambridge Isotope Laboratory (Andover, MA). The purity of [13C8]retinyl acetate was >99%. The isotopic profile was as follows: 0.0% [12C8]retinyl acetate, 0.5% [11C8], 3.9% [13C7], 77.3% [13C8], 16.6% [13C9], and 1.6% [13C10]retinyl acetate, for which the 13C8 and 13C10 were derived from the addition of natural abundance 13C and 13C2.

Preparation of the deuterated vegetables

Spinach (cultivar Melody) and carrots (cultivar Danvers) were grown hydroponically at the USDA/Agricultural Research Service Children’s Nutrition Research Center in Houston, TX,
which used a nutrient solution enriched with 25 atom% deuterium oxide as previously described (16). At harvest, all edible leaves and roots were packaged and shipped overnight on ice to the USDA Agricultiral Research Service Human Nutrition Research Center on Aging at Tufts University in Boston, MA. The vegetables were weighed, chopped, and steamed in thin layers (2–3 spinach leaves or 1–2 slices of carrots) for 5 min (spinach) or 10 min (carrots). The steamed vegetables were soaked with tap water (∼200 g in 1 L water) for 2 min. Afterward, the vegetables were drained, puréed, portioned, sealed in plastic containers, and kept at −70 °C until analyzed or used for human feeding studies.

**Vegetable and serum sample analysis**

The extraction of the carotenoids from the vegetables and serum and their analyses were performed as previously described (17–20). Liquid chromatography–atmospheric pressure chemical ionization mass spectrometry was used to measure the percentage enrichment of labeled β-carotene and α-carotene in the vegetable doses and serum (19). When the chromatographic separation is performed by liquid chromatography–mass spectrometry, it is possible to monitor the extracted ion chromatograms (EIC) of the mass-to-charge ratio (m/z) regions 537–539 and 541–557. The m/z region 537–539 is assigned as the predominant natural abundance isotopomer of β-carotene, whereas the region of m/z 541–557 was the labeled β-carotene with its different degrees of deuterium. The enrichment of α-carotene in the carrot dose or serum sample collected after the carrot dose was determined similarly, i.e., by monitoring the EIC of the m/z regions 537–539 and 541–557.

Before the consumption of deuterated vegetables, the EIC of serum samples showed only the endogenous, unlabeled β-carotene with m/z 537–539. The EIC of m/z 541–557, assigned as deuterated β-carotene, showed a distinct peak in the serum extract collected 5 h after the supplementation with labeled spinach or carrot.

**Gas chromatography–electron capture negative chemical ionization mass spectrometry**

To determine the percentage serum enrichment of labeled retinol from the labeled vegetable dose, a 200-μL serum sample (or up to 400 μL for poor responders or at later time points) was extracted following the same procedures without saponification (17). The extract was injected into an HPLC equipped with a C18 column (Perkin-Elmer Inc. Norwalk, CT) (21). The retinol collected from the HPLC system was dried under nitrogen, and the residue was derivatized with N,N,N-trimethylsilyl)trifluoroacetamide containing 10% trimethylchlorosilane (Pierce Chemical, Rockford, IL) before undergoing gas chromatography–electron capture negative chemical ionization mass spectrometry analysis. The percentage enrichment of [2H] retinol derived from vegetable [13C8]β-carotene was calculated by integrating the peak areas under the reconstructed mass chromatogram of the negative ions at m/z 271 (1H2), 272 (H2 + 13C2H2), and 273 (13C2H2) and dividing by the total area response of labeled and unlabeled retinol ions. The percentage enrichment of [13C8]retinol derived from the reference dose (13C8-retinyl acetate) was calculated by integrating the peak area under the reconstructed mass chromatogram of the negative ions at m/z 274 (13C8), 275 (13C7), 276 (13C6), 277 (13C5), and 278 (13C4) and dividing by the total area response of labeled and unlabeled retinol fragment ions. The linearity of the gas chromatography–mass spectrometry response and the detection limit of the gas chromatography–electron capture negative chemical ionization mass spectrometry were previously described (19, 21). The analysis showed that the enrichments of all samples analyzed until 35 d after administration of the vegetable dose were above the detection limit of 0.005%. The percentage enrichments measured by gas chromatography–mass spectrometry and the concentration of retinol in the serum were used to calculate the concentration of labeled retinol in the circulation.

**Areas under the curve of labeled retinol or β-carotene in serum**

Total serum responses to the [3H]β-carotene and [13C8]retinyl acetate doses were determined by multiplying the total serum volume (0.0435 L of kg body wt) by the concentration of [3H]β-carotene, [3H] retinol, and [13C8]retinol in the circulation (determined for each time point of serum sampling by adding all enrichment masses). Areas under the curve (AUC) for serum labeled retinol or β-carotene (in nmol · d) after the [3H]β-carotene and [13C8]retinyl acetate doses were calculated by using the curves of total serum responses (in nmol · y axis) versus time (in d; x axis) via Integral-Curve of Kaleidagraph (Synergy Software, Reading, PA). Because of the 7-d delay in the administration of the reference vitamin A dose, the AUCs were calculated for 21 d after each labeled tracer.

**Vitamin A equivalence calculations**

The AUC of serum [3H]retinol (from the labeled spinach and carrots) was compared with the AUC of the vitamin A reference dose (8915 nmol [13C8]retinol). The amount of 3H retinol was calculated as follows:

\[
[3H]\text{Retinol formed from the } \beta\text{-carotene dose (nmol)} = (\text{AUC of [3H]retinol/AUC of [13C8]retinol}) \times 8915
\]

**Conversion factor calculations**

The amount of a given oral dose of vegetable β-carotene (∼20 μmol, or 11 mg; Table 1) compared with the amount of vitamin A derived from the β-carotene dose was defined as the β-carotene to vitamin A conversion factor. Thus, the conversion

| TABLE 1 |  
| --- | --- |
| **Provitamin A carotenoid content in labeled spinach and carrots that were consumed by the male subjects** |  
| Spinach | Carrots |
| Trans β-Carotene | 18.8 ± 1.9 | 10.3 ± 0.2 |
| 9-cis β-Carotene | 2.6 ± 0.2 | 0.2 ± 0.2 |
| 13-cis β-Carotene | 0.6 ± 0.2 | 0.7 ± 0.1 |
| α-Carotene | 0.7 ± 0.2 | 16.8 ± 0.1 |
| Total Trans β-carotene equivalence | 20.8 ± 2.4 | 19.2 ± 0.4 |

1 All values are x ± SD.
2 Calculated assuming that 9-cis and 13-cis β-carotene and α-carotene have half the vitamin A activity of trans β-carotene.
factor of vegetable β-carotene (calculated β-carotene from all-trans β-carotene plus 50% of all other provitamin A carotenoids; Table 1) to vitamin A was determined as follows:

Conversion factor of β-carotene to retinol (by wt) =

\[
\text{β-carotene in vegetable dose (nmol) \times 536/} \]

\[
\text{[^2H]retinol formed from the labeled β-carotene dose (nmol) \times 286} \quad (2)
\]

Statistical analysis

The paired \( t \) test was used to assess statistical differences between vegetable sources of β-carotene. In addition, results of AUCs, equivalents, and conversion factors were analyzed with an independent-samples \( t \) test to determine the difference between sexes. SYSTAT 10.0 (SPSS Inc, Chicago, IL) was used for the statistical calculations. A difference was considered to be statistically significant at \( P < 0.05 \).

RESULTS

Our analysis showed that the distribution of the β-carotene isotopomers in the labeled plants was a symmetrical normal curve (Figure 1) after correction of the right skew, which was the result of the natural \(^{13}\)C enrichment (22). This finding is consistent with that of a previous study (23), which showed that β-carotene from deuterated spinach and carrots is randomly labeled throughout the molecule. The maximum isotopomer abundance of both spinach (top panel) and carrot (bottom panel) β-carotene was at \( m/z \) 547 in the positive ionization analysis, which is equal to the masses of unlabeled natural β-carotene \((M_0)\), plus \( H^+ \), plus 10 mass units, ie, \( M_0 + H^+ + 10 = 547 \).
There were no ions of unlabeled β-carotene (Mₙ) at m/z 537 in the labeled vegetables.

The provitamin A carotenoids in labeled spinach and carrots are in the form of all-trans, 9-cis, and 13-cis β-carotene and α-carotene. Assuming that 9-cis and 13-cis β-carotene and α-carotene have half the vitamin A activity of β-carotene, the total trans β-carotene (equivalence) contents in the labeled doses administered to the 7 men were 20.8 μmol (11.2 mg) in a 300-g dose of spinach and 19.2 μmol (10.4 mg) in a 100-g dose of carrots as presented in Table 1. The labeled spinach dose to 7 women contained ≈20 μmol (11 mg) total trans β-carotene (equivalence).

There were no differences observed between sex groups with respect to age, BMI, and the fasting serum concentrations of retinol, carotenoids, or tocopherols at the start of the study (Table 2). Compliance with the dietary instructions showed that the daily intake of carotenoids was <1 mg, and the intake of preformed vitamin A was ≈0.35 mg.

Serum responses to the reference doses of [13C₈]retinyl acetate given to the men during each of the 2 vegetable experiments 4 mo apart varied from 0.9% to 14%; the mean variation was 1.4%.

After the spinach or carrot dose, 2H retinol formed from the labeled β-carotene in the dose was detected as early as 3 h after administration of the dose, but the highest concentration of 2H retinol was reached 13 h after the labeled vegetable dose. A representative serum response curve from subject number 7 is shown in Figure 2. In the men, the AUC for serum labeled retinol (calculated through 21 d after each vegetable dose) showed that 1 μmol carrot β-carotene (trans β-carotene equivalents) provided 32 ± 16 (x ± SD) nmol retinol · d and 1 μmol spinach β-carotene provided 24 ± 15 nmol retinol · d. For the women, 1 μmol spinach β-carotene provided 18 ± 6 nmol retinol · d. The

### TABLE 2
Subject characteristics and fasting serum concentrations of retinol, carotenoids, and tocopherols at the beginning of the carrot (men) or spinach (men and women) study

<table>
<thead>
<tr>
<th>Carrot phase</th>
<th>Spinach phase</th>
<th>Women, Spinach phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>1.8 ± 0.4 (1.4–2.6)</td>
<td>1.80 ± 0.26 (1.37–2.21)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.27 ± 0.35 (0.08–1.05)</td>
<td>0.37 ± 0.19 (0.07–0.87)</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.15 ± 0.07 (0.07–0.28)</td>
<td>0.14 ± 0.04 (0.08–0.17)</td>
</tr>
<tr>
<td>Cryptoxanthin</td>
<td>0.16 ± 0.14 (0.05–0.14)</td>
<td>0.17 ± 0.12 (0.08–0.32)</td>
</tr>
<tr>
<td>Total lycopene</td>
<td>0.20 ± 0.12 (0.04–0.33)</td>
<td>0.15 ± 0.09 (0.05–0.52)</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>29.99 ± 13.3 (10.90–49.70)</td>
<td>31.99 ± 9.59 (18.09–41.63)</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>6.21 ± 3.11 (3.00–11.49)</td>
<td>7.83 ± 6.10 (2.12–17.05)</td>
</tr>
</tbody>
</table>

1 All values are x ± SD; range in parentheses.
2 Age: 58.9 ± 6.8 y (range: 51.3–71.0 y); BMI (in kg/m²): 25.7 ± 1.5 (range: 22.9–27.3).
3 Age: 55.4 ± 4.6 y (range: 50.5–62.3 y); BMI: 26.4 ± 7.2 (range: 20.0–30.2).
responses between men and women (n = 7 per group) after a dose of spinach β-carotene were not significantly different. However, this finding may have been due to the limited number of subjects (n = 7 per group), which might have had insufficient power to identify a significant difference between men and women.

On the basis of serum responses to the reference dose, the calculated vitamin A equivalence showed that ≈0.061 mg retinol was formed from 1 mg spinach β-carotene, and 0.080 mg retinol was formed from 1 mg carrot β-carotene; thus, the retinol equivalence was significantly better for carrot β-carotene than for spinach β-carotene (P < 0.03, paired t test) (Table 3). The derived conversion efficiency for carrot β-carotene to retinol (men) was 14.8 to 1 and that of spinach β-carotene to retinol (men and women) was 20.9 to 1.

The AUC for labeled β-carotene serum concentrations showed that the serum response to molar spinach β-carotene (42.4 ± 8.5 nmol · d per μmol β-carotene) was significantly less than that of both carrot β-carotene (119.8 ± 23.0 nmol · d per μmol carrot β-carotene; P < 0.01, paired t test) and carrot α-carotene (68.7 ± 10.7 nmol · d per μmol carrot α-carotene; P = 0.05, paired t test) as presented in Table 4. In addition, after the carrot dose, the molar serum β-carotene response was significantly higher than that of α-carotene in the same dose (P < 0.02, paired t test) (Table 4).

**DISCUSSION**

As a plant source of provitamin A, β-carotene serves as an alternative to preformed vitamin A intake for a large number of the world’s population. However, the efficiency of carotenoids, especially those contained in green vegetables and carrots, in providing vitamin A nutrition for humans has heretofore not been directly quantified. On the basis of the design of intervention studies by de Pee et al (24) and Bulux et al (25), who used the measurement of changes in serum retinol concentration as the indicator, dark-green leafy vegetables and carrots had no effect on serum retinol concentrations. The failure to observe the effect has been discussed in several publications (26, 27). The current investigation used dietary spinach and carrots that were intrinsically labeled with deuterium as well as labeled vitamin A (as a reference dose) in healthy adult subjects to directly measure the blood response to spinach and carrot provitamin A carotenoids and their bioconversion to retinol. It has been reported that the

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Spinach β-carotene</th>
<th>Carrot β-carotene</th>
<th>Spinach β-carotene</th>
<th>Carrot β-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.092</td>
<td>0.090</td>
<td>10.98</td>
<td>11.29</td>
</tr>
<tr>
<td>2</td>
<td>0.071</td>
<td>0.110</td>
<td>14.37</td>
<td>9.23</td>
</tr>
<tr>
<td>3</td>
<td>0.102</td>
<td>0.132</td>
<td>9.98</td>
<td>7.70</td>
</tr>
<tr>
<td>4</td>
<td>0.022</td>
<td>0.045</td>
<td>46.51</td>
<td>22.36</td>
</tr>
<tr>
<td>5</td>
<td>0.036</td>
<td>0.041</td>
<td>28.06</td>
<td>24.81</td>
</tr>
<tr>
<td>6</td>
<td>0.063</td>
<td>0.066</td>
<td>16.03</td>
<td>15.41</td>
</tr>
<tr>
<td>7</td>
<td>0.040</td>
<td>0.078</td>
<td>25.31</td>
<td>13.10</td>
</tr>
<tr>
<td>Average</td>
<td>0.061 ± 0.033</td>
<td>0.080 ± 0.033</td>
<td>21.61 ± 12.96</td>
<td>14.84 ± 6.51</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Spinach β-carotene</th>
<th>Carrot β-carotene</th>
<th>Spinach β-carotene</th>
<th>Carrot β-carotene</th>
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<tbody>
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<td>1</td>
<td>0.056</td>
<td>—</td>
<td>18.01</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>0.051</td>
<td>—</td>
<td>19.72</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>0.043</td>
<td>—</td>
<td>23.22</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>0.063</td>
<td>—</td>
<td>15.92</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>0.046</td>
<td>—</td>
<td>21.96</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>0.047</td>
<td>—</td>
<td>21.25</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>0.055</td>
<td>—</td>
<td>21.02</td>
<td>—</td>
</tr>
<tr>
<td>Average</td>
<td>0.050 ± 0.007</td>
<td>—</td>
<td>20.16 ± 2.49</td>
<td>—</td>
</tr>
<tr>
<td>Average of all subjects</td>
<td>0.055 ± 0.021</td>
<td>—</td>
<td>20.88 ± 9.00</td>
<td>—</td>
</tr>
</tbody>
</table>

1 No significant differences were observed between men and women when the results were evaluated by using an unpaired t test.
2 ± SD (all such values).
3 Significantly different from carrot β-carotene, P < 0.03 (paired t test, unadjusted).

### Table 3

Calculated vitamin A equivalences and conversion efficiencies of spinach or carrot [3H]β-carotene to vitamin A by using an isotope reference dose of labeled vitamin A.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Spinach β-carotene</th>
<th>Carrot β-carotene</th>
<th>Spinach β-carotene</th>
<th>Carrot β-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.056</td>
<td>—</td>
<td>18.01</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>0.051</td>
<td>—</td>
<td>19.72</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>0.043</td>
<td>—</td>
<td>23.22</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>0.063</td>
<td>—</td>
<td>15.92</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>0.046</td>
<td>—</td>
<td>21.96</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>0.047</td>
<td>—</td>
<td>21.25</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>0.055</td>
<td>—</td>
<td>21.02</td>
<td>—</td>
</tr>
<tr>
<td>Average</td>
<td>0.050 ± 0.007</td>
<td>—</td>
<td>20.16 ± 2.49</td>
<td>—</td>
</tr>
<tr>
<td>Average of all subjects</td>
<td>0.055 ± 0.021</td>
<td>—</td>
<td>20.88 ± 9.00</td>
<td>—</td>
</tr>
</tbody>
</table>

### Table 4

Area under the serum [3H]β-carotene and [3H]α-carotene response curves over 21 d after the consumption of spinach (containing 18.8 μmol trans β-carotene) and carrot (containing 10.3 μmol trans β-carotene and 16.8 μmol α-carotene) 4 mo apart in random order by 7 men.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Spinach β-carotene</th>
<th>β-Carotene</th>
<th>α-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>63</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>147</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>82</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>101</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>216</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>55</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>175</td>
<td>99</td>
</tr>
<tr>
<td>3 ± SEM</td>
<td>42.4 ± 8.5</td>
<td>119.8 ± 23.0</td>
<td>68.7 ± 10.7</td>
</tr>
</tbody>
</table>

1 Significantly different from carrot β-carotene, P < 0.01 (paired t test).
2 Significantly different from α-carotene, P < 0.02 (paired t test).
application of triacylglycerol-rich lipoprotein response curves to
measure absorption of carotenoids from carrots and spinach con-
taining 15 mg carotenoids was not successful because the re-
 sponses were hardly measurable (28). However, the use of stable-
 isotope-labeled dietary spinach and carrots and the good sensi-
tivity of mass spectrometry technologies (21) enabled the
current evaluation. Recently, a report showed that 2 mo of supple-
mentation with vegetables containing 750 μg retinol equival-
technological advances permitted comparisons of retinol derived from
spinach and carrots to vitamin A status might have improved (ie, become more effi-
cient) conversion of provitamin A carotenoids to vitamin A. Further research
is needed, using advanced isotopic technologies, to assess caro-
tenoid availability and vitamin A conversion in all of these pop-
ulation groups. However, a modification of current procedures
would be desirable to reduce the number of blood samples
needed.

Carrots contain both β-carotene and α-carotene. The blood
response to each mole of carrot β-carotene was significantly
higher (nearly doubled), relative to equal moles of carrot
α-carotene. Rao and Rao (39) reported that β-carotene was absorbed
nearly twice as well as was α-carotene from carrots after analyzing
feces to estimate the extent of intestinal absorption of carrot caro-
tenoids. Although the results from their analysis of β-carotene and
α-carotene in feces represented the outcome of not only intestinal
absorption but also of possible catabolism by intestinal microorganisms, their assessment that β-carotene is more bioavailable than α-carotene seems justified. Our results support the notion that β-carotene is twice as bioavailable as α-carotene.

Although vegetable β-carotene is a safe form of provitamin A and can provide substantial amounts of vitamin A to humans, the use of plant provitamin A as a sustainable and effective strategy for combating vitamin A deficiency in various parts of the world, from an array of plant sources, needs continued scientific and quantitative evaluation.

We thank the Metabolic Research Unit of the Jean Mayer USDA Human Nutrition Research Center on Aging for recruiting the subjects and for performing the human study procedures.

GT designed the study, supervised the data collection, analyzed the data, and wrote the manuscript. JQ collected and analyzed the samples. GGD supervised the mass spectrometric analysis and revised the manuscript. RMR supervised the human study as the study physician and revised the manuscript. MAG designed the use of labeled vegetables in humans, produced the labeled hydropnic vegetables, and revised the manuscript. No financial benefit was obtained from this research study.

REFERENCES


