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

SHERRY A. TANUMIHARDJO*,[1,2] MICAH A. HORVITZ*,[2] MANDY PORTER DOSTI,* AND PHILIPP W. SIMON†

*Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706; and †USDA Agricultural Research Service, Vegetable Crops Research Unit, Department of Horticulture, University of Wisconsin-Madison, Madison, Wisconsin 53706

β-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/d), orange (1.8 mg α-carotene and 2.6 mg β-carotene/d), or dark-orange (4 mg α-carotene and 7 mg β-carotene/d) 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

This research was supported by USDA-IFAFS grant number 2000–4258, American Cancer Society IRG-580114402, Hatch-Wisconsin Agricultural Experiment Station WIS04533, the UW-Madison Graduate School, and the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2003-35200-05377.

[1] To whom correspondence should be addressed at 1415 Linden Drive, University of Wisconsin-Madison, Madison, WI 53706. E-mail: sherry@nutrisci.wisc.edu

[2] These two authors contributed equally to this work and should be considered co-first authors.

Received March 13, 2009.
Accepted June 23, 2009.

DOI: 10.3181/0903-RM-106
1535-3702/09/23411-1280$15.00

Table 1. Baseline Characteristics for All Subjects (n = 5F, 5M)

Characteristic	Mean ± SD
Body mass index (kg/m^2)	23.0 ± 2.7
Age (y)	27.5 ± 4.4
Baseline α-carotene (μmol/L)	0.200 ± 0.009
Baseline β-carotene (μmol/L)	0.128 ± 0.032

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 allow a mean difference between any two comparisons that is about twice as large as the SD. The mean age was 27.5 ± 4.4 y and the body mass index (BMI) was 23.0 ± 2.7 kg/m^2 (Table 1). Subjects were healthy, non-smoking adults who were free of metabolic diseases, such as diabetes mellitus or thyroid problems. Written informed consent was obtained from all subjects, and they voluntarily agreed to the conditions of the study, which included abstinence from alcohol use before and during treatment periods. The Human Subjects' Research Institutional Review Boards at the University of Wisconsin (UW)-Madison College of Agriculture and Life Sciences and the UW-Hospital approved the protocol and informed consent form.

Experimental Design. Subjects were placed on a low carotenoid diet 1 week prior to the study and during the 3 treatment periods. A list of foods to eliminate was provided to each subject and was determined using the USDA carotenoid database (17). Exclusion criteria included any fruit or vegetable with more than 300 μg β-carotene/100 g. To ensure that the test food, i.e., muffins, was consumed, subjects reported to the research kitchen between 07.00 and 09.00 throughout the study to eat a breakfast that was provided for them. Breakfast consisted of muffins (2/d), yogurt (regular, not fat-free), fruit, and coffee, tea, or juice. In addition, subjects were asked to keep a daily fruit and vegetable diary to monitor outside carotenoid intake.

Table 2. Sequence of Carrot Type for Each Treatment Group Where Dark-Orange and Orange Refer to Relative High and Low Carotenoid Concentration and White Is Carotenoid-Free[a]

	Treatment 1	Treatment 2	Treatment 3
Group 1	Dark-orange	White	Orange
Group 2	White	Orange	Dark-orange
Group 3	Orange	Dark-orange	White

[a] n = 3, 4, and 3 for Groups 1, 2, and 3, respectively.

Beginning on d 0 of the intervention, subjects were randomized into 3 groups: a low carotene carrot muffin group which had 0.9 mg α-carotene and 1.3 mg β-carotene/muffin; a high carotene carrot muffin group, which had 2 mg α-carotene and 3.5 mg β-carotene/muffin; and a white carrot muffin control group, which was devoid of carotenoids. Subjects were fed 2 muffins per d for 11 d (d 0–10), with a 10-d washout period before the start of the next treatment period (d 11–20). Fasting blood samples were taken on d 0, 1, 3, 5, 7, 9, 11, 13, and 15. Subjects crossed over into the next treatment group in the order listed in Table 2.

Carrots and Muffins. Carrots were genetically selected to have high (dark-orange), typical (orange), or no carotenoids (18–20). The carrot muffins were equalized 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 HPLC: 600 solvent delivery system, 717 autosampler, and 996 photodiode array detector. The column was a Waters Resolve™ C_{18} 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 α- and β-Carotene for Each Muffin Type Fed to Adults[a]

	α-Carotene,* μmol•d/L	β-Carotene,** μmol•d/L
Dark-orange carrot	2.76 ± 1.25[b]	2.32 ± 1.52[b]
Orange carrot	2.48 ± 1.05[b]	1.55 ± 1.18[b]
White carrot	–0.52 ± 0.70[c]	–0.53 ± 1.16[c]

[a] Mean ± SD; $n = 5F, 5M$. Values in the same column with different superscript letters are significantly different.
* Significantly different from the white carrot muffin: $P \leq 0.006$.
** Significantly different from the white carrot muffin: $P < 0.001$.

carotene, and β-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 μL serum was mixed with 40 μL retinyl acetate solution as internal standard; 100 μL ethanol was added and mixed by vortex. Retinol was extracted 3 times with 250 μL hexanes. Hexanes were evaporated with argon and the sample was resuspended in 50 μL 75:25 methanol:dichloroethane (v:v); 25 μL was injected into the HPLC system described above. The column was a Waters Symmetry® C_{18} 3.5 μm 4.6 × 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 α- and β-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 ± SD for α- and β-carotene AUC are listed in Table 3. Figure 1 depicts the serum response to carotenoid feeding in μmol/L for α- and β-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 β-carotene muffin treatment (7 mg/d) resulted in the largest increase in serum concentration for β-carotene, with a 127% increase above baseline by d 11 of the treatment period. The β-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 β-carotene muffins (2.6 mg/d) resulted in an 85% increase in serum β-carotene above baseline on d 11 of the treatment, almost 50% less than the high β-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 β-carotene showed an effect of treatment ($P < 0.001$). The white carrot muffin resulted in a lower serum β-carotene concentration compared with the low β-carotene muffin ($P = 0.006$) and the high β-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 α-carotene concentrations increased dramatically for both carrot feeding periods and did not differ ($P = 0.8$). Treatment with the orange carrots (1.8 mg α-carotene) resulted in a 285% increase above baseline (0.092 μmol/L at baseline to 0.354 μmol/L at d 11), and treatment with dark-orange carrots (4 mg α-carotene) resulted in a 210% increase from baseline (0.139 μmol/L at baseline to 0.431 μmol/L at d 11). White carrots resulted in a 35% decrease in serum α-carotene concentration, and the decrease was lower than in the orange carrot groups ($P < 0.001$). α-Carotene concentrations were affected by treatment ($P < 0.001$) and sequence of feeding ($P = 0.024$). The α-carotene response was lower for those individuals with larger BMIs.

To account for the carryover effect of the high β-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 β-carotene, and the low and high β-carotene carrot treatments differed ($P = 0.01$). α-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 ± 0.40, 0.75 ± 0.42, and 0.91 ± 0.69 mg/

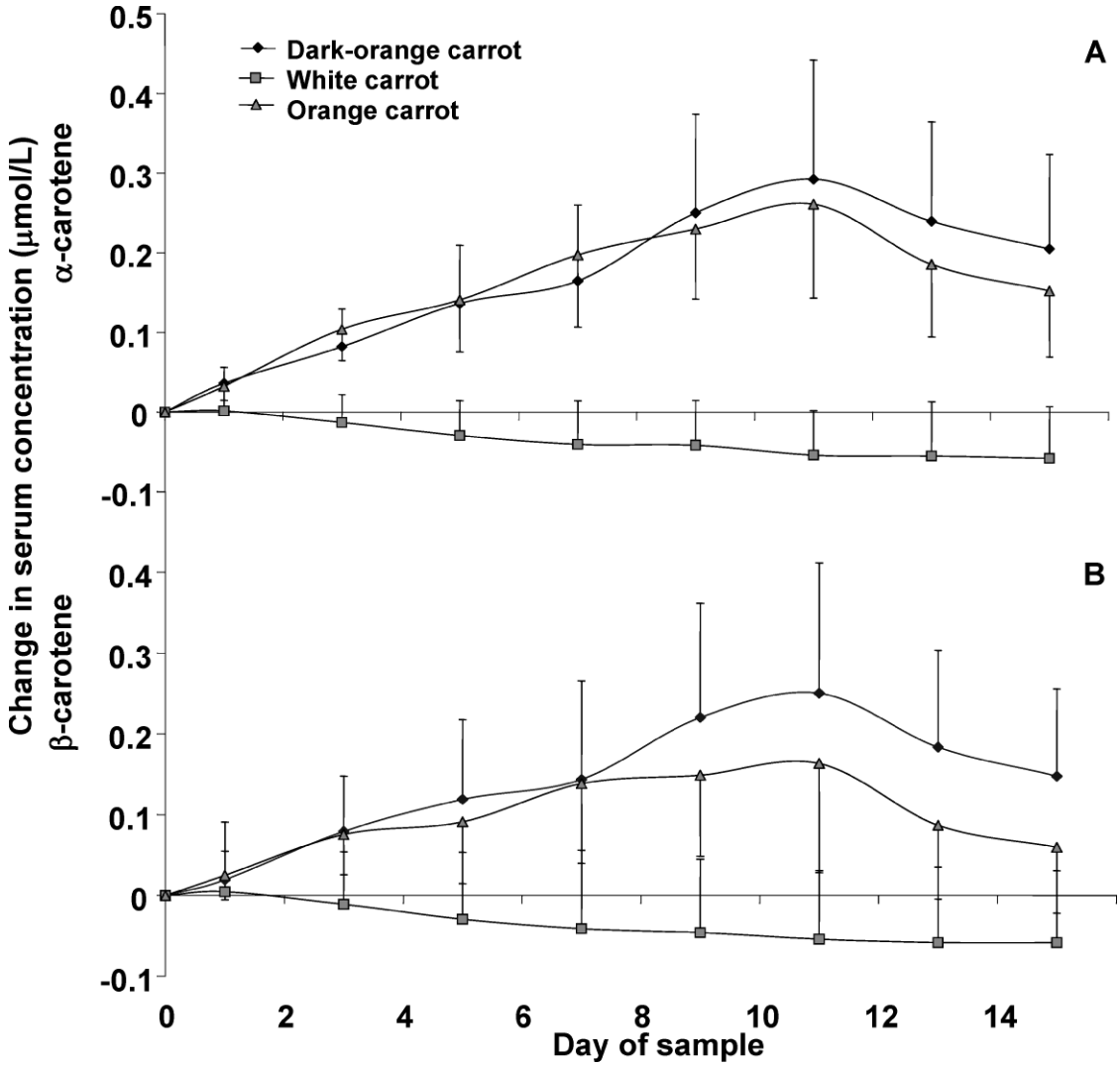

Figure 1. Serum α-carotene (A) and β-carotene (B) concentrations corrected for baseline concentration for each treatment type in human adults ($n = 10$). (♦) Indicates the high β-carotene carrot treatment; (▲) indicates treatment with low β-carotene carrots; and (■) indicates treatment with carotenoid-free white carrots. A significant effect of treatment ($P < 0.001$) and a significant effect of sequence ($P = 0.024$) for α-carotene. The white carrot muffin response was significantly different from the low and high β-carotene muffin response. However, there was no significant difference between treatments with the low and high β-carotene carrot muffin. Statistical analysis indicated a significant effect of treatment and a significant effect of sequence ($P = 0.038$) for β-carotene. The white carrot muffin resulted in a lower serum β-carotene concentration compared to the low β-carotene ($P = 0.006$) and high β-carotene treatments ($P < 0.001$). The high β-carotene muffin was not different from the low β-carotene muffin, except in the first treatment period.

Table 4. Mean Serum Retinol Concentration (μmol/L) as a Function of Muffin Type or Treatment Period in Adults[a]

	Serum retinol, μmol/L	CV %
Dark-orange carrot	1.76 ± 0.14	8
White carrot	1.63 ± 0.10	6
Orange carrot	1.66 ± 0.10	6
Treatment period 1*	2.07 ± 0.16[b]	8
Treatment period 2	1.33 ± 0.10[c]	7
Treatment period 3	1.64 ± 0.06[c]	4

[a] Mean ± SD; $n = 5$F, 5M. Values for treatment period with different superscript letters are significantly different.
* Significant treatment by sequence interaction ($P = 0.02$).

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 α- and β-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 β-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

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·d/L and the lowest responder had AUC of 0.577 and 0.089 μmol·d/L 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 intakes. 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.

1. US Department of Health and Human Services, US Department of Agriculture. Dietary Guidelines for Americans, 6th ed. Washington, DC: US Government Printing Office, 2005.
2. Tanumihardjo SA. Food-based approaches for ensuring adequate vitamin A nutrition. Comp Rev Food Sci Food Saf 7:373–381, 2008.
3. De Pee S, West CE, Muhilal, Karyadi D, Hautvast JG. Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 346:75–81, 1995.
4. Zhou JR, Gugger ET, Erdman JW Jr. The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of β- and α-carotene in the ferret model. J Am Coll Nutr 15:84–91, 1996.
5. Tanumihardjo SA. Factors influencing the conversion of carotenoids to retinol: bioavailability to bioconversion to bioefficacy. Int J Vitam Nutr Res 72:40–45, 2002.
6. Faulks RM, Southon S. Challenges to understanding and measuring carotenoid bioavailability. Biochim Biophys Acta 1740:95–100, 2005.
7. Rock CL, Lovalvo JL, Emenhiser C, Ruffin MT, Flatt SW, Schwartz SJ. Bioavailability of β-carotene is lower in raw than in processed carrots and spinach in women. J Nutr 128:913–916, 1998.
8. De Pee S, West CE. Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. Eur J Clin Nutr 50: S38–S53, 1996.
9. Bulux J, Serrano JQD, Perez R, Rivera C, Solomons NW. The plasma β-carotene response to a single meal of carrots in Guatemalan schoolchildren. Int J Food Sci Nutr 49:173–179, 1998.
10. Porter Dosti M, Mills JP, Simon PW, Tanumihardjo SA. Bioavailability of β-carotene (βC) from purple carrots is the same as typical orange carrots while high-βC carrots increase βC stores in Mongolian gerbils (*Meriones unguiculatus*). Br J Nutr 96:258–267, 2006.
11. Simon PW, Tanumihardjo SA, Clevidence BA, Novotny JA. Role of color and pigments in breeding, genetics, and nutritional improvement of carrots. In: Culver CA, Wrolstad RE, Eds. Color Quality of Fresh and Processed Foods. ACS Symposium Series 983. Washington, DC: ACS Books, pp151–165, 2008.
12. Mills JP, Simon PW, Tanumihardjo SA. β-Carotene from red carrot maintains vitamin A status, but lycopene bioavailability is lower relative to tomato paste in Mongolian gerbils. J Nutr 137:1395–1400, 2007.
13. Ruel MT. Can food-based vitamin A strategies help reduce vitamin A and iron deficiencies? International Food Policy Research Institute, Washington, DC, 2001.
14. Duitsman PK, Cook LR, Tanumihardjo SA, Olson JA. Vitamin A inadequacy in socioeconomically disadvantaged pregnant Iowan women as assessed by the modified relative dose response (MRDR) test. Nutr Res 15:1263–1276, 1995.
15. Horvitz MA, Simon PW, Tanumihardjo SA. Lycopene and β-carotene are bioavailable from lycopene "red" carrots in humans. Eur J Clin Nutr 58:803–811, 2004.
16. Molldrem KL, Li J, Simon PW, Tanumihardjo SA. Lutein and β-carotene from lutein-containing yellow carrots are bioavailable in humans. Am J Clin Nutr 80:131–136, 2004.
17. US Department of Agriculture. USDA-NCI carotenoid database. Available at: http://www.nal.usda.gov/fnic/foodcomp/Data/Carot/file1.dat. Accessed October 2008.
18. Simon PW. Plant pigments for color and nutrition. HortScience 32:12–13, 1997.
19. Simon PW, Rubatzky VE, Bassett MJ, Strandberg JO, White JM. B7262 purple carrot inbred. HortScience 32:146–147, 1997.
20. Simon PW, Wolff XY, Peterson CE, Kammerlohr DS, Rubatzky VE, Strandberg JO, Bassett MJ, White JM. High carotene mass carrot population. HortScience 24:174–175, 1989.
21. Kostic D, White WS, Olson JA. Intestinal absorption, serum clearance, and interactions between lutein and β-carotene when administered to human adults in separate or combined oral doses. Am J Clin Nutr 62: 604–610, 1995.
22. Brown ED, Rose A, Craft N, Seidel KE, Smith JC Jr. Concentrations of carotenoids, retinol, and tocopherol in plasma, in response to ingestion of a meal. Clin Chem 35:310–312, 1989.
23. Howe JA, Tanumihardjo SA. Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. J Nutr 136:2562–2567, 2006.
24. Yang Z, Zhang Z, Penniston KL, Binkley N, Tanumihardjo SA. Serum carotenoid concentrations in postmenopausal women from the United States with and without osteoporosis. Int J Vit Nutr Res 78:105–111, 2008.
25. Howe JA, Valentine AR, Hull AK, Tanumihardjo SA. ^{13}C natural abundance in serum retinol acts as a biomarker for increases in dietary provitamin A. Exp Biol Med 234:140–147, 2009.
26. Surles RL, Weng N, Simon PW, Tanumihardjo SA. Carotenoid profiles and consumer sensory evaluation of specialty carrots (*Daucus carota*, L.) of various colors. J Agric Food Chem 52:3417–3421, 2004.
27. Borel P, Grolier P, Mekki N, Boirie Y, Rochette Y, Le Roy B, Alexandre-Gouahau, Lairon D, Azais-Braesco V. Low and high responders to pharmacological doses of β-carotene: proportion in the population, mechanisms involved and consequences on β-carotene metabolism. J Lipid Res 39:2250–2260, 1998.
28. Valentine AR, Tanumihardjo SA. One-time vitamin A supplementation of lactating sows enhances hepatic retinol in their offspring independent of dose size. Am J Clin Nutr 81:427–433, 2005.
29. Kelley SK, Green MH. Plasma retinol is a major determinant of vitamin A utilization in rats. J Nutr 128:1767–1773, 1998.
30. Davis CR, Jing H, Howe JA, Rocheford T, Tanumihardjo SA. β-Cryptoxanthin from supplements or carotenoid enhanced maize maintains liver vitamin A in Mongolian gerbils (*Meriones unguiculatus*) better than or equal to β-carotene supplements. Br J Nutr 100:786–793, 2008.
31. Stephenson CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 72:1170–1178, 2000.
32. Institute of Medicine Food and Nutrition Board. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, pp325–382, 2000.
33. Haskell MJ, Pandey P, Graham JM, Peerson JM, Shrestha RK, Brown KH. Recovery from impaired dark adaptation in nightblind pregnant Nepali women who receive small daily doses of vitamin A as amaranth leaves, carrots, goat liver, vitamin A–fortified rice, or retinyl palmitate. Am J Clin Nutr 81:461–471, 2005.
34. Poppel GV. Epidemiological evidence for β-carotene in prevention of cancer and cardiovascular disease. Eur J Clin Nutr 50:S57–S61, 1996.

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.
36. Koca N, Karadeniz F. Changes of bioactive compounds and antioxidant activity during cold storage of carrots. Int J Food Sci Tech 43: 2019–2025, 2008.
37. Tanumihardjo SA, Bouis H, Hotz C, Meenakshi JV, McClafferty B. Biofortification of staple crops: an emerging strategy to combat hidden hunger. Comp Rev Food Sci Food Saf 7:329–334, 2008.
38. Mills JP, Simon PW, Tanumihardjo SA. Biofortified carrot intake enhances liver antioxidant capacity and vitamin A status in Mongolian gerbils. J Nutr 138:1692–1698, 2008.
39. Sun T, Simon PW, Tanumihardjo SA. Antioxidant phytochemicals and antioxidant capacity of biofortified carrots (*Daucus carota* L.) of various colors. J Agric Food Chem 57:4142–4147, 2009.