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Anthocyanins in Purple−Orange Carrots (*Daucus carota* L.) Do Not Influence the Bioavailability of β-Carotene in Young Women

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Purple carrots contain anthocyanins in addition to the provitamin A carotenoids in typical orange carrots. Simultaneous consumption of these phytochemicals in carrots may affect the bioavailability of carotenoids. The bioavailability of β-carotene in humans was assessed from an acute feeding of orange (OC) and purple (PC) carrots with white (WC) as a control. Carrot smoothies were served to female subjects (n = 5, aged 21−26 years) for breakfast after 1 week on a low carotenoid diet and overnight fast. OC and PC smoothies were equalized to 10.3 mg of all-*trans* β-carotene. Plasma β-carotene was measured for 144 h following treatments. Peak plasma concentrations of OC and PC treatments did not differ. The PC treatment 0−144 h area-under-the-curve for β-carotene was 76% of the OC treatment ($P < 0.05$). However, when the first 24 h were compared, OC and PC treatments did not differ, suggesting that anthocyanins in purple carrots do not affect the absorption of β-carotene postprandially.

KEYWORDS: Anthocyanin; absorption; β-carotene; bioavailability; carrot (*Daucus carota*)

INTRODUCTION

Consumption of fruits and vegetables is associated with decreased risk of cardiovascular disease and some forms of cancer (*1*). Fruits and vegetables are rich sources of important nutrients and also complex mixtures of bioactive compounds that may work together to protect consumers from disease. Carotenoids, a large class of pigmented compounds responsible for the colors of many fruits and vegetables, are widely studied for their antioxidant and provitamin A activity. The most relevant provitamin A carotenoids in the human diet are α-carotene, β-carotene, and β-cryptoxanthin and together supply up to 80% of the dietary vitamin A in developing parts of the world (*2*). Vitamin A deficiency is a serious public health concern in developing regions for which dietary provitamin A carotenoids are a safe and important strategy to combat this problem (*3*).

Orange carrots are a popular and widely available vegetable that are rich in β-carotene and also phenolic compounds, which contribute to a majority of its in vitro antioxidant capacity (*4*). Orange carrot cultivation, reported first in the 15th and 16th centuries, is predated by yellow and purple carrot cultivation by at least five centuries (*5*). The color of purple carrots is due to anthocyanins, a family of red, blue, and purple water-soluble pigments that have been linked to a variety of health benefits including reduced risk of cardiovascular disease (*6*) and cancer (*7*). Simon et al. reported the development of a purple carrot with an orange core, i.e., B7262 (*8*), a traditionally bred cross between Turkish purple carrot and a dark orange inbred Danvers, which contains both provitamin A carotenoids and anthocyanins.

An important factor to consider in the ability of a food to provide health benefits is bioavailability of its bioactive components. Bioavailability is the fraction of a compound that is absorbed and available for utilization in physiologic functions or for storage. Anthocyanins from whole and juiced purple carrots are bioavailable (*9*, *10*). Additionally, purple carrots are high in beneficial phenolics relative to other colored carrots (*11*) and have demonstrated favorable quality and sensory attributes comparable to orange carrots (*11*, *12*). The bioavailability of β-carotene from purple−orange carrots as well as the vitamin A bioefficacy, the fraction of provitamin A carotenoids converted to vitamin A, was comparable to typical orange carrots in Mongolian gerbils (*13*, *14*). However, the relative bioavailability of β-carotene from purple−orange carrots to orange has not been tested in humans. On the basis of the relative antioxidant capacity of purple and orange carrots (*4*), this study hypothesized that α- and β-carotene may be chemically protected during digestive and absorption processes by the presence of anthocyanins, positively impacting the relative bioavailability. The objective of this study was to determine if β-carotene is as bioavailable from purple−orange carrot inbred B7262 as from typical orange carrots using plasma β-carotene response from an acute feeding in young women.

MATERIALS AND METHODS

Subjects. The study was approved by the University of Wisconsin Medical School Human Subjects Committee. Seven healthy, nonsmoking female subjects aged 21−26 years were enrolled and written informed

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Table 1. Characteristics of Subjects at Baseline[a]

characteristic	value
age (y)	22.4 ± 2.1
weight (kg)	67.1 ± 6.7
BMI (kg/m^2)	22.8 ± 3.0
percent body fat (%)	27.7 ± 5.8
total cholesterol (mg/dL)	167.8 ± 3.0
initial β-carotene (μmol/L)[b] (range)	0.40 ± 0.12 (0.15, 0.59)

[a] All values are mean ± SD; n = 5 F. [b] Fasting plasma β-carotene concentration prior to study.

Table 2. Macronutrient Content of Carrot Smoothie Treatment and Breakfast[a]

	carrot smoothie	total breakfast
energy (kcal)	230. ± 11	551 ± 11
protein (g)	8.3 ± 0.3	20.0 ± 0.3
carbohydrate (g)	43.5 ± 2.6	89.5 ± 2.7
fat (g)	2.2 ± 0.1	11.5 ± 0.1
fiber (g)	4.3 ± 0.8	6.3 ± 0.8

[a] Expressed as mean ± SD. Foods analyzed by Nutritionist Pro software (version 4.2.0, 2009; Axxya Systems, Stafford, TX).

consent was obtained. Criteria for exclusion were smoking, pregnancy, chronic disease, gastrointestinal problems, unusual food habits, and body mass index <18.5 or >28 kg/m^2. Prior to enrollment, hemoglobin concentrations were determined with a HemoCue test system (HemoCue, Inc., Lake Forest, CA) and all were within normal limits. Percent body fat at the beginning and end of the study was determined by air displacement plethysmography (BodPod v. 2.30; Life Measurement Inc., Concord, CA). Baseline characteristics of the subjects are described in **Table 1**. Total cholesterol was within normal range for all subjects. C-reactive protein was within normal limits, indicating lack of infection or chronic inflammation. The initial mean plasma β-carotene concentration was 0.40 ± 0.12 μmol/L and had a wide range (0.15−0.59 μmol/L) but was typical (*15*, *16*).

Study Design, Treatment, and Sample Collection. In an unblinded, crossover design, each subject ingested orange (OC) or purple (PC) carrot smoothies that had identical all-*trans* β-carotene content or white carrot (WC) smoothie, which was devoid of carotenoids. The three smoothies were ingested in random order, each separated by ≥2 weeks on their regular diet in order to mitigate sequence effects (*17*, *18*) and 1 week washout on a low-carotenoid, low-anthocyanin diet. Breakfast consisted of a carrot smoothie, a plain bagel, and 1 oz cream cheese (**Table 2**). The smoothie was standardized to contain 10.3 mg of all-*trans* β-carotene and equivalent total carrot content within a treatment cycle. The smoothies were made with blanched and frozen carrots microwaved on high power for 2 min, cooled for 30 min at −20 °C, and then blended with 153 g of plain low-fat yogurt, 18.9 g of granulated sugar, and 148 mL of filtered drinking water. Total carrot content of the smoothies (125 ± 0.1 g) was standardized by adjusting with white carrot during each treatment period and needed to be increased during the last two treatment periods (154 ± 0.1 and 181 ± 0.2 g, respectively) due to β-carotene degradation of the frozen carrots. Fat contributed 18.7% of kcal to the meal.

Breakfast was served to fasting subjects in the morning, and blood samples were drawn by certified phlebotomists from the antecubital vein of each subject at baseline and 1, 2, 4, 6, 8, 12, 16, 24, 32, 72, 144 h after treatment. Subjects were provided with carotenoid and anthocyanin-free lunch and dinner at 5 and 11.5 h post treatment, respectively, and snacks during the first 24 h of each treatment period. Subjects were instructed to avoid carotenoid-containing foods for 1 week prior to treatment and during the collection period. They were also instructed to avoid anthocyanin-containing foods for 3 days prior to treatment and during the collection period. Subjects were provided with lists of low carotenoid and anthocyanin foods to include in their diets. Alcohol was prohibited for 2 days prior to and the day of treatment. Vitamins and supplements were prohibited throughout the study. Compliance was monitored by diet record logs provided to subjects, collected weekly, and reviewed. Food records and the treatment breakfasts were analyzed for β-carotene content with Nutritionist Pro 4.2.0 software (2009; Axxya Systems, Stafford, TX).

Table 3. α- and β-Carotene and Anthocyanin Concentrations in Carrots and Carrot Smoothie Treatment[a]

	nmol/g		μmol/g	mg	
carrot color	carrot α-carotene	carrot β-carotene[b]	carrot anthocyanin[c]	total treatment α-carotene	total treatment β-carotene
orange	97.7 ± 9.1	178 ± 11.4	ND[d]	5.6 ± 0.12	10.3 ± 0.12
purple	58.7 ± 8.8	135 ± 22.6	1.79 ± 0.3	4.5 ± 0.18	10.3 ± 0.12
white	ND	ND	ND	0	0

[a] Means ± SD, n = 12. Frozen carrots were analyzed in triplicate prior to every treatment period. [b] All-*trans* isomer. [c] Total anthocyanin content includes 5 anthocyanins. [d] ND, not detected.

Carrots and HPLC Analyses. The purple carrots with an orange core and white carrots (*Daucus carota*) were U.S. Department of Agriculture inbred B7262 and Seed Movement White, respectively, and provided by PWS. Orange carrots were purchased from a local grocery store. Carrots were washed and cut into 2.5 cm discs, mixed for batch uniformity, blanched in boiling water for 1 min, and frozen at −20 °C until use and analysis.

Carrots were analyzed for carotenoids and anthocyanins in triplicate once during each treatment period prior to treatment day (**Table 3**) using modified published methods (*17*). Internal standard (i.e., β-apo-8′-carotenal) was added to 1 g of ground thawed carrot. Sample was ground with mortar and pestle and mixed with 2.5 g sodium sulfate. Three extractions with 10 mL of hexanes were pooled, dried under argon, redissolved in 200 μL of 50:50 (v/v) dichloromethane:methanol, and 50 μL was injected onto an HPLC equipped with a guard column and Grace Smart Reversed-Phase C_{18} 5-μm column, 3.9 mm × 300 mm (Deerfield, IL). The Waters HPLC system (Milford, MA) consisted of a 600 solvent delivery system, 717 autosampler, and 2996 photodiode array detector. HPLC mobile phases consisted of 95:5 (v/v) acetonitrile:water as solvent A and 85:10:5 (v/v/v) acetonitrile:methanol:dichloromethane as solvent B with 10 mM ammonium acetate as a modifier (*17*). At 1.5 mL/min, the gradient procedure was as follows: (1) 100% solvent A for 3 min, (2) a 12 min linear gradient to 100% solvent B, (3) a 20 min hold at 100% solvent B, and (4) a 2 min linear gradient back to 100% solvent A.

Carrot anthocyanins were analyzed by a published procedure (*4*). Fresh carrot macerate with added malvidin-3-galactoside as an internal standard was extracted with 10% formic acid in methanol five times. Extracts were centrifuged, supernatants were pooled, and sample was injected onto the same HPLC system described above (*4*). An Agilent Zorbax C_{18} (5 μm, 4.6 mm × 250 mm) with a guard column was used for gradient analysis at 1 mL/min. Solvent A was 10% formic acid in water and B was methanol: (1) B was increased from 0 to 15% in 20 min, (2) an 18 min linear increase to 20% B, (3) a 10 min increase to 30% B, and (4) a 3-min increase to 55% B. The total run time was 53 min. Cyanidin-3-glucoside was used to prepare the standard curve for quantifying the anthocyanins. No anthocyanins were detected in the orange or white carrots. Five anthocyanins were identified in the purple−orange carrots by comparing the profile to previously published work (*19*, *20*).

Biological Sample Preparation and Carotenoid Analysis. Blood samples were collected into vacutainers containing EDTA and centrifuged at 2200*g* for 20 min at 4 °C. For carotenoid analysis, aliquots of 1.2 mL of plasma were stored in cryovials under argon at −70 °C. Plasma was analyzed for carotenoids according to a published procedure (*17*) with the following modifications. Internal standard, β-apo 8′-carotenyl decanoate, was added to 500 μL of plasma. Then 600 μL of ethanol with 0.1% butylated hydroxytoluene was added to denatured proteins. Samples were extracted three times with 1 mL of hexanes, and the extracts were pooled and dried under argon. Samples were reconstituted with 100 μL of 50:50 (v/v) methanol:dichloromethane and 50 μL was injected onto the HPLC system (*17*).

Plasma Antioxidant Capacity. The antioxidant capacities of the plasma at 0, 1, 2, and 4 h were determined using the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging decolorization assay following a published procedure (*14*). Briefly, plasma was diluted 1:10 in PBS and added directly to 1 mL of ABTS radical in PBS (absorbance 0.7), and the change in absorbance was measured after 10 min. The slope of the percent inhibition of the 3 aliquots (10, 20, and

30 μL) was compared to the Trolox standard curve. Plasma antioxidant capacity was expressed as Trolox equivalent antioxidant capacity (TEAC)/L plasma.

Statistical Analysis. Using standard expressions for power analysis, an *n* of 5 women would be needed to have a high probability of detecting a difference in carotenoid concentration or area-under-the-curve (AUC) values between any two comparisons that is about twice as large as the standard deviation. Two additional women were recruited to allow for drop outs, noncompliance, or missed blood draws. The plasma concentrations of α- and β-carotene corrected for baseline was plotted against time. The AUC for time 0 through 144 h ($AUC_{0-144\ h}$) was calculated for β-carotene by trapezoidal approximation (*15*). Statistical analyses were performed with SAS software (SAS Institute Inc., Version 8.2, Cary, NC; 2001). Post hoc differences between groups were determined using Fisher's least significant difference test at ($P < 0.05$).

RESULTS AND DISCUSSION

Carrots and Treatments. The concentrations of both α- and β-carotene in the frozen carrots remained stable during the first two treatment periods but decreased 15.4% and 11.3%, respectively, in the orange carrot, and 27.6% and 30.6%, respectively, in the purple−orange carrot between the second and fourth treatment periods. The amount of carrot used to prepare the treatment smoothies was adjusted to account for these changes to keep β-carotene content consistent at 10.3 ± 0.12 mg all-*trans* β-carotene. Macronutrient content was stable for the first two treatment periods but was slightly altered in the third and fourth treatment periods due to increased carrot. Fiber content of the breakfast had the greatest variation and increased 0.8 and 1.6 g in the third and fourth periods, respectively, which corresponded to a 12 and 25% increase; carbohydrate content increased 3 and 6%, respectively. Protein and fat content increased <3% in the last two treatment periods. The effects of fiber on carotenoid bioavailability are mixed (*21*−*24*); however, the increase in fiber content of the breakfast in this study was small (0.01 and 0.02 g fiber/kg body weight, respectively) compared with amounts shown to have an effect on carotenoid absorption (0.15 g fiber/kg body weight) (*21*). Nonetheless, the addition of white carrot to tomato paste did negatively impact serum lycopene AUC and peak concentration (*24*). On the other hand, the OC and PC treatments were evenly divided over the first and second halves of the study, with 2 OC and 3 PC treatments in the first half, and 3 PC and 2 OC treatments in the second half of the study. For these reasons, the increased fiber content likely had little effect on the outcomes.

As with the carotenoids, total anthocyanin concentration in PC declined over the course of the study by 32.9% but total smoothie anthocyanin content (255 ± 12 μmol) was fairly consistent, with a range of 238 to 267 μmol over the treatment periods. Two studies of whole and juiced purple carrots in humans demonstrated that bioavailability of anthocyanins was low and that absorption saturated between dose levels of approximately 250 and 350 μmol (*9*, *10*).

Subject Compliance. Two women only completed one arm of the study. One of these women had a high plasma carotenoid concentration at baseline, and her food record indicated noncompliance. The other woman only completed WC. Their data were excluded. In the remaining five women, compliance with the low-carotenoid and anthocyanin diets was excellent, as evidenced by the negative β-carotene AUC for WC and supported by the diet records. Body weights of all the subjects remained stable throughout the study. During recruitment efforts, potential subjects found it difficult to agree to comply with the fruit and vegetable restrictions. On the basis of a power calculation, five subjects were needed and seven subjects were originally recruited assuming a loss to follow-up.

Table 4. Area Under the Curve (AUC_{0-144h}) for Plasma β-Carotene Acute Feeding Response[a]

treatment	peak concentration change[b] μmol/L (CV)[c]	μmol·h /L (CV) plasma $AUC_{0-144\ h}$[d]	μmol·h /L (CV) plasma $AUC_{0-24\ h}$
orange	0.14 ± 0.07 (48.6) a	13.7 ± 4.1 (29.5) a	4.4 ± 0.9 (20.6) a
purple	0.10 ± 0.03 (37.3) a	10.4 ± 1.6 (15.4) b	4.0 ± 0.8 (20.5) a
white	0.01 ± 0.02 (169.4) b	−2.1 ± 0.6 (31.5) c	−1.5 ± 0.4 (25.5) b

[a] Values are means ± SD, *n* = 5 females. Means followed by different letters within a column are significantly different, $P < 0.05$. [b] This table shows the mean peak concentration change for β-carotene as opposed to **Figure 1**, which shows the mean change in β-carotene concentration at each time point. Peak concentration change values did not occur at the same time for all subjects; therefore, values in this table do not match those in **Figure 1**. [c] Coefficient of variation. [d] Four blood collections were missed, and results were interpolated for calculation of AUC.

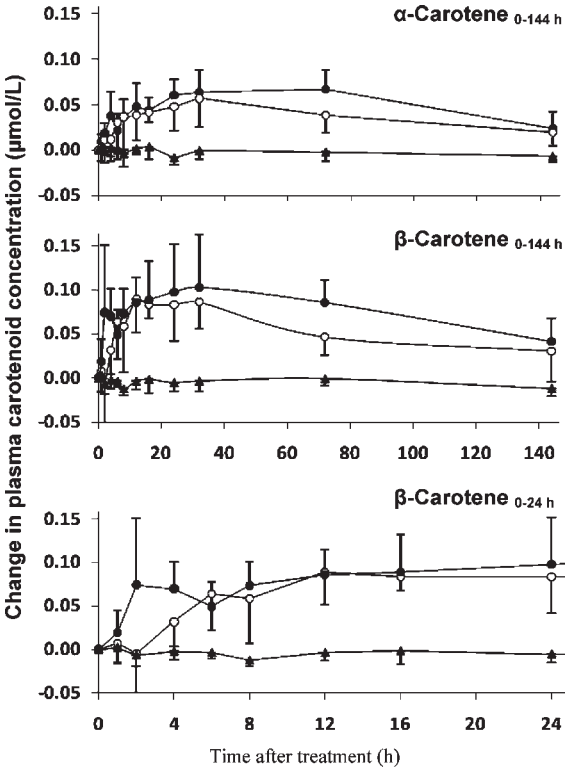

Figure 1. Mean (±SD) plasma α-carotene and β-carotene concentrations after correction for baseline concentration in 5 female adults fed acute treatment of orange (●), purple−orange (○), or white (▲) carrot smoothie.

Plasma Results. The baseline plasma β-carotene concentration at time 0 for all treatment periods (0.21 ± 0.13 μmol/L) was lower than the initial mean concentration, demonstrating adherence to the low β-carotene run-in diet. Peak β-carotene concentration changes (mean for individual subjects' peak times) are listed in **Table 4**. As expected, the WC peak β-carotene concentration change was lower than OC and PC ($P < 0.05$). The OC and PC peak concentration change did not differ and variation for both groups was moderately high, with a CV of 49% and 32% for the OC and PC groups, respectively. The magnitude of the peak β-carotene plasma response was similar to a study in which male subjects were fed nearly three times the β-carotene (29 mg) from 272 g carrots (i.e., 0.11 μmol/L) (*15*).

Composite plasma α- and β-carotene response to the carrot smoothie feeding corrected for baseline concentrations is depicted in **Figure 1**. The WC group had a negative $AUC_{0-144\ h}$ and was

different from the OC and PC groups ($P < 0.05$) (**Table 4**). The $AUC_{0-144\ h}$ of plasma β-carotene for PC showed a 24% smaller response than the OC treatment ($P < 0.05$). Because anthocyanins are rapidly absorbed and cleared, reaching a maximum concentration by 2 h or less (*9*, *10*), results of the first 24 h were evaluated to assess whether the anthocyanins may have had an effect on β-carotene uptake. The $AUC_{0-24\ h}$ of the OC and PC groups were not different, suggesting similar β-carotene absorption from the two carrot types in these female subjects. The amount of α-carotene fed was 1.1 mg higher in the OC than PC group (**Table 3**). While α- and β-carotene may compete for absorption, α-carotene AUC did not differ, which is similar to a prior study in which a 2.2 mg difference in daily α-carotene feeding did not affect AUC_{0-16d} (*18*).

As evidenced by the change in concentration curves in **Figure 1**, the OC and PC groups had a smaller early and larger late β-carotene peak. The early peak was reached at 2 h (range 2−4 h) and 6 h (range 2−12 h) for the OC and PC groups, respectively. The OC and PC late peak plasma β-carotene times were 32 h (range 2−32 h) and 12 h (range 6−32 h), respectively. This biphasic β-carotene response and range of peak times were consistent with previous work (*25*) and indicated the early appearance of β-carotene packaged in intestinally secreted chylomicra and a later increase due to hepatically derived VLDL and subsequent delipidation to LDL (*26*). The time difference of the early and late peaks between the OC and PC groups may suggest a difference in rates of absorption from these two carrots but is more likely due to intra- and interindividual variation, as indicated by the wide range of peak times and the high CV of peak values.

Both OC and PC plasma β-carotene were still elevated above baseline at 144 h, 0.04 ± 0.03 and 0.03 ± 0.03 μmol β-carotene/L, respectively, but were not different ($P < 0.05$), and mean values returned to near or below baseline by the beginning of the next treatment period. The time course of 144 h chosen for this study was based on previous observations that plasma β-carotene concentrations are at or near baseline by this time (*23*) and differences in AUC beyond this time provide little information regarding treatment differences (*27*).

No effect of treatment or time was found for plasma antioxidant activity of the baseline through 4 h time points (data not shown). These time points were chosen to mirror the steep rise in plasma anthocyanin concentration (*9*, *10*). Studies with various flavonoid-containing fruits and vegetables generally show increased plasma antioxidant activity (*28*); however, the effects may be influenced by compounds other than the flavonoids, because many flavonoids circulate at concentrations far below the extent of increase in antioxidant capacity.

The Institute of Medicine's vitamin A equivalency ratio of 12 μg of dietary β-carotene to 1 μg of retinol (*29*) was recently supported by feeding humans intrinsically deuterated carrots (*30*). On the basis of this equivalency, 1 medium purple−orange carrot (~60 g) could provide approximately 450 μg vitamin A, or 65% of the daily vitamin A requirement for a female aged 19−30 years. Additionally, this carrot could supply about 50 mg of anthocyanins, compared with 80 mg from $^1/_2$ cup blueberries and 0 for typical orange carrots, which may contribute health benefits beyond vitamin A (*14*). In addition to carotenoids and anthocyanins, carrots also contain phenolic acids such as chlorogenic acid and other compounds that can vary greatly between genotype (*11*, *31*). The matrix and the crystalline form of β-carotene in carrots (*32*, *33*) likely have a greater impact on bioavailability than other phytochemicals.

In conclusion, this is the first study to demonstrate that anthocyanins do not seem to influence the bioavailability of β-carotene from an acute feeding of purple carrots in young women, which confirmed the overall finding from a series of gerbil studies (*13*). More comprehensive studies, perhaps including stable isotope techniques or chronic feeding, should be pursued. Clearly, purple carrots are a functional food with the capacity to deliver health promoting-compounds beyond basic nutrients (*33*, *34*). Purple carrots are generally well-liked (*12*) and, if widely accepted by consumers, could provide important health benefits as part of a diet rich in fruits and vegetables.

SAFETY

Use of a fume hood for volatile organic solvents is recommended.

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