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Plasma and Urine Responses Are Lower for Acylated vs Nonacylated Anthocyanins from Raw and Cooked Purple Carrots

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The bioavailability of acylated vs nonacylated anthocyanins and the effect of cooking and dose on the comparative bioavailability were investigated in a clinical feeding study using purple carrots as the anthocyanin source. Treatments were purple carrots as follows: 250 g raw (463 μmol of anthocyanins: 400 μmol acylated, 63 μmol nonacylated), 250 g cooked (357 μmol of anthocyanins: 308.5 μmol acylated, 48.5 μmol nonacylated), and 500 g cooked (714 μmol of anthocyanins: 617 μmol acylated, 97 μmol nonacylated). Four of the five carrot anthocyanins were found intact in plasma by 30 min after carrot consumption and peaked between 1.5 and 2.5 h. Acylation of anthocyanins resulted in an 11−14-fold decrease in anthocyanin recovery in urine and an 8−10-fold decrease in anthocyanin recovery in plasma. Cooking increased the recovery of nonacylated anthocyanins but not acylated anthocyanins. Large dose size significantly reduced recovery of both acylated and nonacylated anthocyanins, suggesting saturation of absorption mechanisms.

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

INTRODUCTION

Anthocyanins are red, blue, and purple water soluble pigments found in fruits, vegetables, and ornamental plants. They consist of a flavonoid backbone bound to sugar residues, mostly glucose, galactose, rhamnose, and arabinose. The six common anthocyanidin backbones are cyanidin, malvidin, delphinidin, peonidin, petunidin, and pelargonidin. These backbones can be glycosylated and form linkages with aromatic acids, aliphatic acids, and methyl ester derivatives (*1*). In the United States, anthocyanin consumption is estimated at about 215 mg per day during summer months and about 180 mg/day during winter months (*2*). Those who regularly consume red wines may have significantly higher intakes (*3*).

Promising research has shown that anthocyanins may serve an important role in promoting health by reducing the risk of atherosclerosis (*4*, *5*) and cancer (*6*, *7*), ameliorating inflammation (*8*, *9*), and acting as antioxidants (*4*, *5*, *10*). They also have the potential to act as natural red, blue, and purple colorants that can be used in foods and beverages, but their use is limited by their poor stability (*11*, *12*). Acylated anthocyanins, such as those found in purple carrot, purple sweet potato, red radish, and red cabbage, have a greater stability than nonacylated anthocyanins and therefore may be an alternative source of natural colorants (*13*−*16*). Acylated anthocyanins have been shown to reduce oxidative stress in vivo (*17*).

Because of their potential health-promoting effects and their potential as natural food colorants, anthocyanin bioavailability has become an important issue. A few studies have investigated the bioavailability of anthocyanins from food sources such as red wine, red grape juice, elderberry, black currant, red berry fruit, and blueberry (*18*−*25*). Previously published anthocyanin bioavailability studies have been conducted primarily with nonacylated anthocyanins, and while a few studies have shown that acylated anthocyanins can be absorbed by the intestinal tract (*19*, *26*−*28*), there is a paucity of information about the effect of acylation on anthocyanin bioavailability. In addition, the effects of cooking and dose response have not been evaluated. This study was conducted to investigate the comparative bioavailability of acylated and nonacylated anthocyanins using purple carrot as an anthocyanin source, as well as whether cooking and/or dose size influences absorption.

MATERIALS AND METHODS

Chemicals. Ethyl acetate and high-performance liquid chromatography (HPLC) grade water and methanol were obtained from Fisher Scientific. Formic acid and trifluoroacetic acid (TFA) were obtained from Sigma Chemical. Cyanidin-3-galactoside (Cy-3-gal) and malvidin-3-galactoside (Mv-3-gal) were purchased from Indofine Chemical Co. (Somerville, NJ).

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Subjects. Twelve healthy, nonsmoking volunteers (6 M, 6 F) from the Beltsville, MD, area participated in this study. All procedures were approved by the Johns Hopkins University Bloomberg School of Public Health Committee on Human Research, and subjects gave written informed consent prior to participation. Subjects were on average 43 years old, 72 kg, and had a mean body mass index of 24.

Study Design, Sample Collection, and Diet. Three carrot treatments were administered to subjects in a crossover experimental design. For this design, all subjects received each of the treatments, with the treatment periods being separated by 3 week breaks. Subjects were randomly assigned to one of three groups ($n = 4$/group), with each group receiving a different treatment order. The purple carrot treatments were as follows: 250 g of sticks served raw, 250 g of sticks served microwave-cooked, and 500 g of sticks served microwave-cooked. Carrots were served to fasting subjects in the morning with 6 g of fat as dressing (soy and canola oil) for raw carrots or butter for cooked carrots. Blood was collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 h. Urine samples were collected at baseline and every 2 h until 16 h. From 16 to 24 h, urine was pooled. Subjects were provided an anthocyanin-free diet during the collection period and for 2 days prior to treatment. During the treatment day, a snack, lunch, and dinner were provided at 2, 4, and 10 h after carrot consumption, respectively. Caffeine consumption was prohibited on the dose day and for 1 day prior to dose. Vitamins and supplements were prohibited throughout the study.

Purple Carrots. The purple carrots (*Daucus carota*) were U.S. Department of Agriculture inbred B7262 (*24*). Carrots were washed, cut into sticks with the orange core discarded, and mixed for batch uniformity. Carrot treatments were weighed prior to cooking. Cooked treatments were prepared by adding 1 tablespoon (T) or 3 T of water followed by microwave cooking (Sharp 900 W microwave ovens) while covered for 12 or 20 min for the 250 and 500 g doses, respectively. For each treatment period, samples of cooked and raw carrots were frozen at −80 °C until extraction and analysis.

Carrot Extraction. Sixty grams of carrots was ground in a Waring blender with liquid nitrogen to form a powder. Duplicate 3 g samples of powdered carrot were quickly weighed into a test tube before adding 10 mL of 10% methanolic formic acid. Samples were vortex-mixed for 1 min and sonicated for 10 min prior to centrifugation at 2500*g* for 10 min. The supernatant was decanted to a collection vial, and samples were extracted three more times with 10 mL of 10% methanolic formic acid. The combined extract was diluted 10× with methanol:10% aqueous formic acid (1:9) prior to injecting onto the liquid chromatograph mass spectrometer (LC-MS).

Biological Sample Preparation. Blood samples were collected into vacutainers containing EDTA and centrifuged at 2560*g* for 10 min. Plasma aliquots of 2.2 mL were stored in cryo-vials containing 1.3 mL of 0.44 M aqueous TFA. Urine was weighed, and 10 mL aliquots were stored in vials containing 2 mL of 0.44 M TFA. All samples were stored at −80 °C until analysis.

Prior to HPLC quantification, samples were thawed, Mv-3-gal was added as an internal standard, and samples were subjected to solid phase extraction (SPE) to isolate and concentrate anthocyanins. The following procedure is a modification from Giusti et al. (*25*). SPE C-18 sep-pak cartridges (Waters Assoc., Milford, MA) were conditioned with 5 mL of methanol and then 5 mL of 0.44 M aqueous TFA prior to adding sample. After the sample was added, water soluble compounds were eluted with 5 mL of 0.44 M aqueous TFA, followed by elution of polar nonanthocyanin phenolics with 5 mL of ethyl acetate before removing and collecting anthocyanins with 5 mL of 0.44 M methanolic TFA. Samples were dried under nitrogen and then reconstituted in methanol: 10% aqueous formic acid (1:9).

LC-MS Conditions. The LC-MS consisted of an Agilent HP series 1100 G1946A MSD with an electrospray (ESI) source connected to a G1315A diode array detector (DAD), temperature-controlled column compartment (30 °C), autosampler, automatic solvent degasser, binary pump, Zorbax SB-C18 column (5 μm, 250 mm × 4.6 mm), and guard cartridge (Agilent Technol., Wilmington, DE). The MSD source was positive ESI with the spray chamber gas temperature set at 300 °C, the nebulizer pressure set at 60 psi, the VCap set at 3000 V, and the drying gas (nitrogen) set at 12 L/min. Selected ion monitoring was used to identify individual anthocyanins (**Table 1**) in the carrot, plasma, and urine samples (*26*) and to look for any cyanidin aglycone and anthocyanin glucuronide or sulfate. The DAD was set to collect signals at 530 nm. The limit of detection for Cy-3-gal (external standard for quantification) was 0.025 nmol/mL for a 50 μL injection. Data were collected using Agilent Chemstation software and quantified from the DAD.

Table 1. Carrot Cyanidin Derivatives with Retention Times and Molecular Masses[a]

compound	abbreviation	RT	MW
Cy-3-(2″-xylose-6-glucose-galactoside)	Cy3XGG	10.8	743
Cy-3-(2″-xylose-galactoside)	Cy3XG	11.9	581
Cy-3-(2″-xylose-6″-sinapoyl-glucose-galactoside)	Cy3XSGG	12.4	949
Cy-3-(2″-xylose-6″-feruloyl-glucose-galactoside	Cy3XFGG	12.9	919
Cy-3-(2″-xylose-6″-(4-coumuroyl)glucose-galactoside)	Cy3XCGG	13.2	889

[a] Reproduced from ref *29*. RT is retention time (min) for the chromatographic procedure described in the Materials and Methods section. MW is molecular weight.

The solvent system consisted of 10% aqueous formic acid (solvent A) and methanol (solvent B). The gradient was linear from 5 to 55% solvent B over 20 min, then 100% solvent B for 5 min to flush the column, and back to 5% solvent B for 10 min to reequilibrate the column. The flow rate was 1 mL/min, and the injection volume was 50 μL.

Calculations and Statistics. Mv-3-gal was used as an internal standard to account for sample loss during extraction, which was on average 25%. Molar concentrations of individual anthocyanins were calculated using an external standard curve of Cy-3-gal and are expressed as Cy-3-gal equivalents. Gram concentrations of individual anthocyanins were then calculated using the molecular weight of the individual anthocyanins.

The area under the plasma concentration time curve (AUC) was calculated by the Trapezoidal Method using Microsoft Excel 2000 v. 9. Peak plasma mass was calculated by multiplying plasma concentration by estimated plasma volume (45 mL plasma/kg body weight). The percent recovery was calculated by dividing the peak plasma mass or 24 h urinary excretion by the mass of anthocyanin in the dose. The data were tested for normality (using the Kolmogorov−Smirov test) and equal variance (using the Levene Median test), then a one-way repeated measures analysis of variance was used to compare plasma and urine responses among treatments, with $p < 0.05$ considered significant. The Holm−Sidak method (*29*) was used for pairwise multiple comparisons. A post-hoc power analysis showed that the study design provided power to detect differences of 15% or greater for plasma AUC, 20% or greater for peak plasma concentration, and 25% or greater for urinary accumulation. Statistical analysis was performed using the SigmaStat Software Package (version 3.0, SPSS Inc.).

RESULTS

HPLC profiles of purple carrot extract showed five peaks with retention times as follows: (A) 10.8, (B) 11.9, (C) 12.4, (D) 12.9, and (E) 13.2 min. Mass spectrometry was used to verify identification. These peaks were previously identified by Gläbgen and co-workers (*30*) using spectroscopy and NMR and are defined with their abbreviations in **Table 1**. These same profiles (plus the internal standard Mv-3-gal at 15.4 min and minus the Cy3XCGG) were also found in plasma and urine. Baseline samples of plasma and urine contained no anthocyanin other than the internal standard, Mv-3-gal.

Analysis of treatments showed the following total anthocyanin contents (**Table 2**): (1) 463 μmol (416 mg) in 250 g of raw carrots, (2) 357 μmol (321 mg) in 250 g of cooked carrots, and (3) 714 μmol (643 mg) in 500 g of cooked carrots. Acylated anthocyanins comprised 86% of total anthcyanins for each treatment, and nonacylated comprised 14%. The microwave-cooked carrots showed a trend toward lower anthocyanin

Table 2. Acylated and Nonacylated Anthocyanin Content of Treatments[a]

	μmol per treatment (% of total)		
treatment	acylated	nonacylated	total
250 g raw	400.3 ± 75.2 am (86.5)	62.6 ± 25.6 an (13.5)	462.9 ± 75.2 ab
250 g cooked	308.5 ± 65.9 am (86.3)	48.5 ± 8.5 an (13.7)	357.1 ± 72.1 a
500 g cooked	617.0 ± 131.8 bm (86.3)	97.0 ± 17.0 bn (13.7)	714.2 ± 144.3 b

[a] Values are expressed as means ± SD. Superscripts a, b, and c show treatment differences (comparison down columns) with $p < 0.05$. Superscripts m and n show differences between acylated and nonacylated content for a given treatment (comparison across rows) with $p < 0.05$.

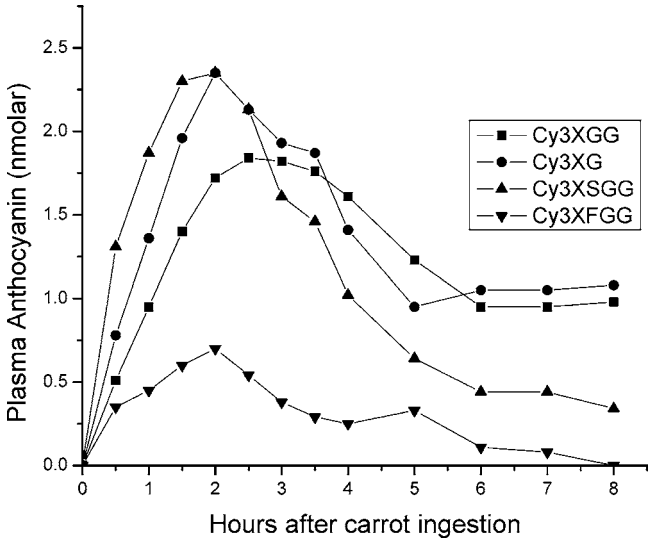

Figure 1. Plasma anthocyanin absorption of a representative subject after consumption of 250 g of raw purple carrot.

contents than raw carrots (23% decrease in total anthocyanin content and a 23% decrease in both acylated and nonacylated anthocyanins alike), although this reduction did not reach statistical significance.

A sample time course of plasma anthocyanin response is shown in **Figure 1**. Anthocyanins appeared in plasma by 30 min after dosing, steadily rose until peaking at an average of 2 h, then slowly decreased but were still detectable at 8 h after consumption. A sample time course for accumulation of anthocyanins in urine is shown in **Figure 2**. By the first urine collection at 2 h after dosing, anthocyanins were detected in urine, with the greatest rate of excretion occurring 4 h after dosing. Anthocyanins were still detectable in the 16−24 h collection.

One volunteer was found to have extraordinarily high uptake and excretion of anthocyanins after receiving the 250 g dose of raw carrots. For this case, AUC and urinary excretion fell more than 3 standard deviations above the mean, and these data were therefore excluded from the data analyses. This subject reported having stomach flulike symptoms 3 days prior to consuming the carrot dose, and this may have affected anthocyanin absorption.

Plasma anthocyanin responses (represented by peak plasma concentration, percent recovered at peak, and AUC) for the three treatments are shown in **Table 3**. Neither cooking nor dose size affected peak total anthocyanin plasma concentration or AUC. However, cooking did increase the recovery of nonacylated anthocyanins at the peak plasma concentration for nonacylated anthocyanins while having no affect on acylated anthocyanins (**Table 4**). Percent recoveries at peak plasma concentrations were

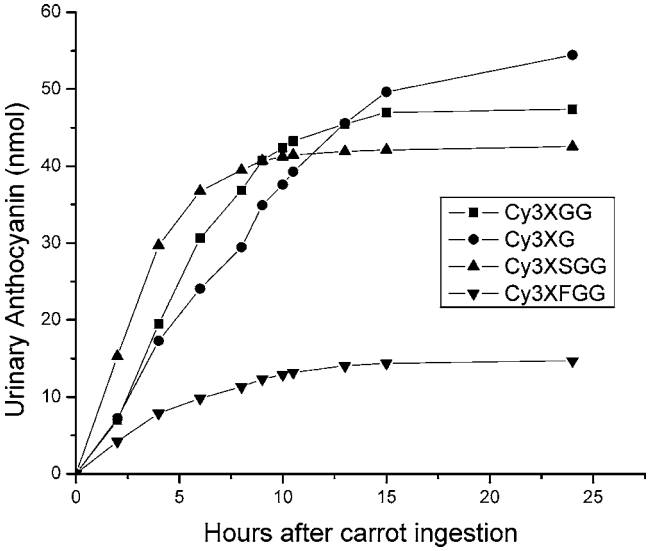

Figure 2. Cumulative urinary anthocyanin appearance of a representative subject after consumption of 250 g of raw purple carrot.

Table 3. Plasma Total Anthocyanin Response[a]

treatment	peak concn (nmol/L)	% recovered at peak	plasma AUC (nmol/L h)
250 g raw	5.8 ± 1.7	0.0041 ± 0.0016[a]	26.9 ± 7.2
250 g cook	5.3 ± 1.9	0.0048 ± 0.0017[a]	26.6 ± 9.3
500 g cook	5.0 ± 1.4	0.0023 ± 0.0006[b]	27.4 ± 7.2

[a] Values are expressed as means ± SD. Values with different letters are significantly different between treatments ($p < 0.05$).

Table 4. Plasma Acylated and Nonacylated Anthocyanin Response[a]

	peak concn (nmol/L)		percent recovered	
treatment	acylated	nonacylated	acylated	nonacylated
250 g raw	2.52 ± 0.93 m	3.44 ± 0.77 n	0.0021 ± 0.0009 am	0.018 ± 0.006 an
250 g cook	2.07 ± 0.71 m	3.47 ± 1.20 n	0.0022 ± 0.0007 am	0.023 ± 0.007 bn
500 g cook	2.05 ± 0.59 m	3.24 ± 0.92 n	0.0011 ± 0.0003 bm	0.011 ± 0.002 cn

[a] Values are expressed as means ± SD. Values with different superscripts are significantly different ($p < 0.05$). Superscripts a, b, and c show treatment differences (comparison down columns). Superscripts m and n show differences between acylated and nonacylated anthocyanins for a given treatment (comparison across rows).

lower for the 500 g dose as compared to the two 250 g doses, due to the larger dose resulting in similar plasma responses.

Neither cooking nor dose size influenced the mass of anthocyanin appearing in the urine during the 24 h collection period (**Table 5**). This held true for acylated, nonacylated, and total anthocyanins. In contrast, cooking and dose size did affect percent recovery in urine. Cooking increased percent recovery of nonacylated and total anthocyanins but not acylated anthocyanins. Increasing dose size reduced percent recovery of acylated, nonacylated, and total anthocyanins.

For urinary and plasma responses, acylated and nonacylated anthocyanins differed from each other. Masses of nonacylated anthocyanins measured in plasma and urine were significantly higher than masses of acylated anthocyanins (for all treatments), even though the doses contained far more acylated anthocyanins than nonacylated. When expressed as percent of dose, plasma recovery of nonacylated anthocyanins at peak was 8−10-fold greater than recovery of acylated anthocyanins, and recovery of nonacylated anthocyanins in urine was 11−14-fold higher than that of acylated anthocyanins.

Table 5. Urinary Recovery of Acylated and Nonacylated Anthocyanins[a]

	mass (nmol)			percent recovered		
treatment	acylated	nonacylated	total	acylated	nonacylated	total
250 g raw	51.1 ± 21.9 m	88.5 ± 17.4 n	139.6 ± 33.6	0.013 ± 0.005 am	0.14 ± 0.03 an	0.030 ± 0.008 a
250 g cook	44.0 ± 13.2 m	91.2 ± 27.0 n	135.2 ± 39.5	0.014 ± 0.004 am	0.19 ± 0.05 bn	0.038 ± 0.011b
500 g cook	48.3 ± 12.9 m	97.3 ± 19.6 n	145.6 ± 30.0	0.008 ± 0.002 bm	0.10 ± 0.02 cn	0.020 ± 0.004c

[a] Values are expressed as means ± SD. Values with different superscripts are significantly different ($p < 0.05$). Superscripts a, b, and c show treatment differences (comparison down columns). Superscripts m and n show differences between acylated and nonacylated anthocyanins for a given treatment (comparison across rows).

DISCUSSION

In humans, bioavailability of anthocyanins from several sources has been studied and the details of absorption are beginning to be understood; however, most of these studies fed extracts/concentrates rather than whole foods and, as was seen in the case of carotenoids, the food matrix as well as dose and food preparation method may impact bioavailability (*31*−*35*). In addition, most previous studies have focused on nonacylated anthocyanins and included only a single dose. Our study has added new information by examining bioavailability from whole carrots and by examining the effect of acylation, cooking, and dose on anthocyanin absorption.

Both plasma and urine of study subjects showed that the carrot anthocyanins were absorbed intact as cyanindin glycosides, as confirmed by mass spectrometric analysis. Previous studies have also shown absorption of intact glycosolated anthocyanins as confirmed by mass spectrometry (*21*, *24*, *36*−*39*). In contrast, Sesink et al. (*40*) have presented evidence against the intact absorption of quercetin glycosides, so the mechanisms for absorption of quercetin derivatives and anthocyanin derivatives likely differ.

The recovery of anthocyanins in our study was similar to that found in previous studies. Wu and co-workers (*24*) reported urinary recovery of anthocyanins to be about 0.077% from elderberry extract and 0.004% from blueberries. Bub and colleagues (*18*) reported that 0.023% of Mv-3-gal was recovered in urine after a single ingestion of red wine, dealcoholized red wine, and grape juice. Matsumoto et al. (*41*) recovered 0.11% of delphinidin-3-rutinoside and cyanidin-3-rutinoside in urine after consumption of a black currant concentrate. Urinary recoveries of total anthocyanins in our study ranged from 0.020 to 0.038% of oral dose. Because previous studies have been conducted predominantly with nonacylated anthocyanins, it is of interest to note that the urinary recovery of nonacylated anthocyanins from carrots is among the highest reported, reaching 0.19% of dose for the 250 g cooked treatment.

A few recent studies have shown that acylated anthocyanins can cross the gastrointestinal tract intact (*26*−*28*). Suda et al. (*26*) observed mono- and diacylated anthocyanins in rat plasma after consumption of a concentrated extract made from purple-fleshed sweet potato. Harada et al. (*28*) detected acylated anthocyanins in plasma and urine of both rats and humans after ingestion of an extract from purple sweet potato tuber. Our study suggests that acylation may be a major determinant of bioavailability. In purple carrot, approximately 86% of the anthocyanins are acylated with a feruloyl group, a sinapoyl group, or a coumuroyl group. Urinary recoveries of the acylated anthocyanins were only about 10% those of nonacylated anthocyanins. This is in accord with the findings of Wu et al. (*27*), who studied anthocyanin absorption from freeze-dried marionberry powder to find that the single acylated anthcyanin was recovered at lower levels than the three nonacylated anthocyanins. Mazza et al. (*19*) were unable to quantify acylated anthocyanins in serum of subjects after consumption of lowbush blueberries, while many nonacylated anthocyanins in serum were measurable.

A possible explanation for the lesser recovery of acylated anthocyanins in urine is that the acylations may have been cleaved to produce Cy3XGG. If this were the case, one would expect the recovery of Cy3XGG to be greater than that of Cy3XG (unless Cy3XGG is cleaved to Cy3XG). Instead, the recoveries of Cy3XGG (0.149%) and Cy3XG (0.139%) in urine were not significantly different. In addition, if the acylations had been cleaved, one would also expect the ratio of Cy3XGG to Cy3XG in urine to be greater than that in the carrots. Instead, the average ratio of Cy3XGG to Cy3XG in the urine was very similar to that seen in carrots (1.01 in urine vs 0.95 in carrots). So, if cleavage of acylations was occurring, these data suggest that such cleavage did not occur to a major extent. Additionally, the previously published studies with purple sweet potato (*26*, *28*) did not show evidence of acylated anthocyanins being converted to their nonacylated counterparts. While extraction recovery has been found to be lower for acylated as compared to nonacylated anthocyanins (*19*), the reduction was slight, so differences in extraction recoveries cannot account for the differences in urinary recoveries.

An additional factor that may have influenced the comparative recovery of acylated and nonacylated anthocyanins is the differing amounts in the treatments, with acylated anthocyanins being 6-fold higher in the carrots than nonacylated anthocyanins. Because the acylated anthocyanins were presented to the GI tract in larger masses, the possibility of saturation of absorption for the more concentrated form but not the less concentrated form must be considered. However, the two dose levels (250 vs 500 g carrots) resulted in very similar masses of nonacylated anthocyanins in urine, suggesting that even the nonacylated anthocyanins may have experienced saturation of absorption. Thus, it appears that the differing masses of acylated and nonacylated anthocyanins in the treatments cannot explain the differing recoveries found in plasma and urine.

There was a trend for cooking to reduce the anthocyanin content of the purple carrots, but the trend was not statistically significant in this data set. Other studies have shown that anthocyanins in juice or extracts can be thermally unstable (*42*, *43*), but studies have not previously considered thermal stability of anthocyanins in whole foods. The overall distribution of acylated and nonacylated anthocyanins was not affected by cooking. Cooking improved the urinary recovery of nonacylated anthocyanins by about 36% but had no affect on acylated anthocyanins. Cooking and processing have also been found to increase carotenoid bioavailability (*44*, *45*). Thermal processing may disrupt cell walls, making compounds more accessible for absorption.

Dose size was an important factor with respect to anthocyanin recovery in biological samples. Urinary recoveries of total anthocyanins, as well as both acylated and nonacylated antho-

cyanins, were lower for the larger carrot dose as compared to the smaller doses. The percent of total anthocyanin dose recovered in plasma at the peak concentration was also lower for the large carrot dose as compared to the smaller doses. This suggests that absorption may be saturated at levels of 350 μmol (or lower, which we cannot discern because 350 μmol was the lowest dose fed). Saturation of an absorption mechanism has been observed previously for carotenoids (*35*). The apparent saturation supports carrier involvement in anthocyanin absorption. Additionally, the large size and the polarity of these compounds make them less likely to partition into a lipid bilayer for passive transport.

Previous studies have provided evidence for carrier transport of anthocyanins. The work of Mülleder and colleagues (*37*) supports the hypothesis of a sugar-related carrier. They studied the influence of sucrose on urinary excretion of elderberry anthocyanins in 16 volunteers and found that the addition of sucrose delayed excretion, suggesting that anthocyanins compete with sugar moieties for transport to the portal blood. There is also evidence that bilitranslocase, a carrier expressed in gastric epithelium (*46*), may be involved. Passamonti and co-workers (*47*) studied the ability of several mono- and diglucosyl anthocyanins as well as an acylated anthocyanin to compete for bilitranslocase transport activity in vitro and found that 17 of the 20 anthocyanins tested behaved as competitive inhibitors of bilitranslocase. Among the 20 anthocyanins tested for the ability to interact with bilitranslocase, two were acylated. One (the acetic ester of malvidin 3-glucoside) acted as a noncompetitive inhibitor, suggesting that this compound interacted with the enzyme at a site other than the active site and at a proximity close enough to interfere with the active site. In contrast, the coumaroyl derivative of malvidin 3-glucoside seemed to prevent any interaction of the test ligand with bilitranslocase. For the two acylated compounds studied, the results of Passamonti et al. (*47*) suggest that the bilitranslocase would not play a role in their transport across the gut epithelium.

In conclusion, this study shows that several cyanidin derivatives from purple carrot are absorbed and excreted intact. We have also demonstrated that saturation of absorption may occur at doses of 350 μmol (and possibly lower). Cooking slightly improved the recovery of nonacylated anthocyanins but had no affect on acylated anthocyanins. Acylation of the anthocyanin derivatives appears to be a key characteristic in determining bioavailability.

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