JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2008, 56, 713–719 713

Bioavailability of Pelargonidin-3-O-glucoside and Its Metabolites in Humans Following the Ingestion of Strawberries with and without Cream

WILLIAM MULLEN,[†] CHRISTINE A. EDWARDS,[§] MAURO SERAFINI,[#] AND ALAN CROZIER*,[†]

Plant Products and Human Nutrition Group, Graham Kerr Building, Division of Environmental and Evolutionary Biology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, United Kingdom; Human Nutrition Section, University of Glasgow Division of Developmental Medicine, Yorkhill Hospital, Glasgow G3 8SJ, United Kingdom; and Antioxidant Research Laboratory at the Unit of Human Nutrition, Istituto Nazionale di Ricerca Alimenti e Nutrizione, Via Ardeatina 546, 00178 Rome, Italy

Plasma and urine were collected over a 24 h period after the consumption by humans of 200 g of strawberries, containing 222 μmol of pelargonidin-3-*O*-glucoside, with and without cream. The main metabolite, a pelargonidin-*O*-glucuronide, reached a peak plasma concentration (C_{max}) of 274 ± 24 nmol/L after 1.1 ± 0.4 h (t_{max}) when only strawberries were ingested. When the strawberries were eaten with cream, the C_{max} was not statistically different but the t_{max} at 2.4 ± 0.5 h was delayed significantly ($p < 0.001$). The pelargonidin-*O*-glucuronide, along with smaller quantities of other metabolites, was also excreted in urine in quantities corresponding to ca. 1% of anthocyanin intake. The quantities excreted over the 0–24 h collection period were not influenced significantly by cream. However, the 0–2 h excretion of anthocyanin metabolites was significantly lower when the strawberries were eaten with cream, whereas the reverse occurred during with the 5–8 h excretion period. In keeping with these observations, measurement of plasma paracetamol and breath hydrogen revealed that cream delayed gastric emptying and extended mouth to cecum transit time.

KEYWORDS: Strawberries; cream; bioavailability; pelargonidin-3-glucoside; pelargonidin glucuronides; plasma pharmacokinetics; urinary excretion

INTRODUCTION

Anthocyanins are a major, water-soluble flavonoid subgroup that occurs widely in the plant kingdom; they are responsible for the vibrant red, purple, and blue colors of many fruits and flowers. They are present in a wide range of fruits, vegetables, and beverages and as such are normal dietary components (*1*). Anthocyanins have antioxidant (*2–4*), anticarcinogenic (*5–7*), vasoprotective (*8*), and anti-inflammatory properties (*9*). They are also reported to enhance vision (*10*) and improve memory (*11*) and to have antiobesity effects (*12*).

Kuhnau (*13*) estimated a daily intake in the United States of 180–255 mg. This now appears to have been based on inaccurate methodology, and a more recent study indicates that anthocyanin consumption in the United States is 12.5 mg/day (*14*). It is, however, relatively easy for consumers to markedly increase their consumption of anthocyanins as levels in red wine of 120 mg/L are not unusual (*15*) and a 100 g serving of berries can often deliver >100 mg (*14, 16*).

Anthocyanins, for people who drink red wine and eat berries on a routine basis, are major dietary components, and information on their fate following ingestion is an important topic in attempts to elucidate their potential protective effects in vivo. Although there are exceptions, unlike other flavonoids that are absorbed and excreted, most anthocyanins do not appear to undergo extensive metabolism of the parent glycosides to glucurono, sulfo, or methyl derivatives (*17–21*). In feeding studies with animals and humans, typically ca. 0.1% of the quantities ingested, and sometimes much less, has been detected in plasma and urine. The available data imply that the determinants of absorption and excretion of anthocyanins are influenced by not only the nature of the sugar moiety but also the structure of the anthocyanidin aglycone (*19, 22*).

The complex array of information on anthocyanin bioavailability obtained with humans and animal test systems has recently been reviewed (*23*). One of the reasons for the complicated picture that emerges is that many feeds have involved berry and fruit supplements containing several structurally diverse anthocyanins. For instance, black raspberries contain

* Corresponding author (telephone +44-141-330-4613; fax +44-141-330-5394; e-mail a.crozier@bio.gla.ac.uk).

[†] University of Glasgow.

[§] University of Glasgow Division of Developmental Medicine.

[#] Istituto Nazionale di Ricerca Alimenti e Nutrizione.

10.1021/jf072000p CCC: $40.75 © 2008 American Chemical Society
Published on Web 01/23/2008

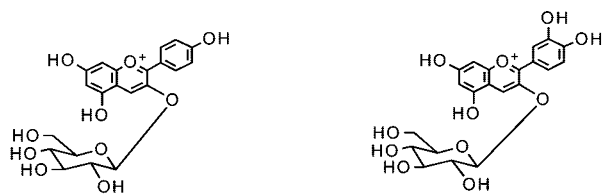

Figure 1. Structures of pelagonidin-3-glucoside (**I**) and cyanidin-3-glucoside (**II**). The former is the predominant anthocyanin in strawberries and the latter that in blackberries.

3-*O*-glucoside, 3-*O*-sophoroside, 3-*O*-rutinoside, 3-*O*-2^G-glucosylrutinoside, and 3-*O*-2^G-xylosylrutinoside derivatives of cyanidin, whereas the 3-*O*-glucosides, 3-*O*-galactosides, and 3-*O*-arabinosides of malvidin, petunidin, cyanidin, and delphinidin, a total of 12 anthocyanins, are found in blueberries (*19, 24*). Much simpler anthocyanin profiles are found in strawberries and blackberries, both of which contain one predominant anthocyanin, pelargonidin-3-*O*-glucoside (**I** in **Figure 1**) in the former and cyanidin-3-*O*-glucoside (**II**) in the latter (*14*). As a consequence, data on the appearance of anthocyanins in plasma and urine after ingestion of these berries by humans are more straightforward to interpret. Strawberry pelargonidin-3-*O*-glucoside is excreted in urine principally as metabolites, pelargonidin glucuronides and a pelargonidin sulfate, together with trace quantities of the parent glucoside and its aglycone, pelargonidin. The major component was one of the pelargonidin glucuronides, which accounted for >80% of the urinary anthocyanins. Overall, the anthocyanins excreted over a 24 h post-ingestion period corresponded to 1.8% of intake, a figure much higher than that obtained in other studies (*25*). Blackberry cyanidin-3-*O*-glucoside, in contrast to it 3′-deoxy derivative, pelargonidin-3-*O*-glucoside, appears to be ca. 10-fold less bioavailable with an overall excretion level corresponding to 0.16% of intake. Cyanidin-3-*O*-glucoside accounted for ca. 13% of the excreted anthocyanins with the remainder comprising a mixture of glucurono, sulfo, and methylated metabolites (*26*). Increased levels of pelargonidin-based anthocyanins compared to cyanidin-based derivatives in both plasma and urine have also been observed in studies in which marionberries were fed to weanling pigs (*27*).

This paper is concerned with the bioavailability of pelargonidin-3-*O*-glucoside (**I**) from strawberries in humans and how the process is affected when, as traditionally occurs during the Wimbledon lawn tennis championship in the United Kingdom, the strawberries are consumed with cream. As well as urine, plasma was collected at several time points over a 24 h period post-ingestion and the data subjected to detailed pharmacokinetic analysis. This is a major expansion on the only previous feeding study with strawberries, which restricted anthocyanin analysis to urine (*25*). The study is also of interest because of earlier work on the effects of milk on the absorption of chocolate polyphenolics (*28*), which has been the subject of much conjecture (*29–31*).

MATERIALS AND METHODS

Strawberries and Chemicals. Strawberries (*Fragaria* × *ananassa*) were purchased online from ASDA, frozen on site, and defrosted prior to use. HPLC grade methanol and acetonitrile were obtained from Rathburn Chemicals (Walkerburn, Borders, U.K.). Formic acid was purchased from Riedel-DeHaen (Seelze, Germany) and acetic acid from BDH (Poole, U.K.), whereas L-(+)-ascorbic acid, cyanidin-3-*O*-glucoside, and pelargonidin-3-*O*-glucoside were purchased from Extrasynthese (Genay, France).

Study Design. Six male and two female volunteers, who were healthy nonsmokers and not on any medication, gave their written consent and participated in the study. They were aged between 23 and 48 years and had a mean body mass index of 23.7 ± 1.2 (range 20.9−27.6). They followed a low-flavonoid diet, which excluded fruits and vegetables and beverages such as tea, coffee, fruit juices, and wine, for 2 days before the study. After an overnight fast, volunteers first consumed 1 g of paracetamol, 5 g of lactulose, and 200 g of strawberries and 4 weeks later under identical conditions ate the same supplement together with 100 mL of double cream (ASDA, Bearsden, Strathclyde, U.K.).

Twelve milliliters of venous blood was collected in heparinized tubes from all volunteers 0, 0.5, 1, 2, 3, 4, 6, 8, and 24 h post-ingestion, and plasma was obtained by centrifugation at 4000*g* for 10 min at 4 °C. Two 1 mL aliquots of plasma were acidified to pH 3 with 15 μL of 50% formic acid, and 50 μL of 10 mmol/L ascorbic acid was also added to prevent oxidation. The samples were used for analysis of anthocyanins by HPLC with photodiode array (PDA) and tandem mass spectrometric (MS^2) detection. All of the samples were frozen in liquid nitrogen and stored at −80 °C prior to analysis. For paracetamol analysis, venous blood samples obtained 0, 15, 30, 45, 60, 75, 90, 105, and 120 min post-ingestion were collected in EDTA tubes and the plasma obtained was stored as two 1 mL aliquots at −80 °C prior to analysis.

Urine was collected prior to supplementation and over four time periods, 0–2, 2–5, 5–8, and 8–24 h, after the ingestion of the strawberry meal. The total volume for each period was recorded. Immediately after collection, urine samples were acidified to pH 3 with formic acid, and within 1 h, aliquots were placed in an autosampler and analyses by HPLC-PDA-MS commenced. Additional acidified aliquots were taken and stored at −80 °C.

End expiratory breath hydrogen levels were monitored every 15 min for 8 h after ingestion of the strawberry supplement. Measurements were made by blowing into an EC60 Breath Hydrogen Monitor (Bedfont Scientific Ltd., Rochester, Kent, U.K.). The time of first sustained rise in breath hydrogen was taken as the mouth to cecum transit time. Volunteers ate ham or turkey with white bread rolls 3 h after consuming the strawberry supplement and thereafter remained on a low-flavonoid diet until the final 24 h samples were collected.

The study protocol was approved by the Glasgow Royal Infirmary Local Research Ethics Committee.

Extraction of Strawberries. Aliquots of strawberries (90–100 g) were taken for quantitative analysis of their anthocyanin content. Each aliquot was homogenized for 1 min using an Ultra-Turrax T 25 (IKA[R]-Werke, Staufen, Germany). Three 5 g aliquots were then extracted for 15 min with methanol containing 5% formic acid. The mixture was then centrifuged at 3000*g* at 4 °C for 15 min. The supernatant was collected and the pellet further extracted and centrifuged twice. The three supernatants were combined, and the anthocyanin content of 5 μL aliquots was analyzed by HPLC-PDA-MS^2.

Extraction of Plasma. Triplicate plasma samples were extracted using a solid-phase extraction procedure. Five hundred microliters of plasma, to which had been added a 50 ng cyanidin-3-*O*-glucoside internal standard, was applied to a 1 g Strata C18E (Phenomonex, Macclesfield, U.K.) cartridge that had been conditioned with 10 mL (4 column volumes) of 5% formic acid in methanol and then 10 mL of 5% aqueous formic acid. The cartridge was then washed with 5% aqueous formic acid before elution of anthocyanins with 10 mL of methanol containing 5% formic acid. The methanolic fraction was reduced to ca. 200 μL in a centrifugal vacuum concentrator Speedvac Concentrator SPD111V (Thermo Electron Corp., San Jose, CA) at 38 °C, before being lyophilized in a SuperModulyo freeze-dryer (Thermo Electron Corp.). The dried samples were then resuspended in 30 μL of 5% formic acid in methanol plus 220 μL of 5% aqueous formic acid. Samples were then filtered through a 0.2 μm VectaSpin Micro centrifugal filter (Whatman, Maidstone, U.K.) and centrifuged at 25000 *g* at 4 °C for 15 min prior to analysis. Aliquots of 200 μL were analyzed by HPLC-PDA-MS^2 on the day of extraction. Recoveries of the cyanidin-3-glucoside internal standard were typically 85%.

Processing of Urine. One hour after collection, 200 μL samples of acidified urine were analyzed by HPLC-PDA-MS^2.

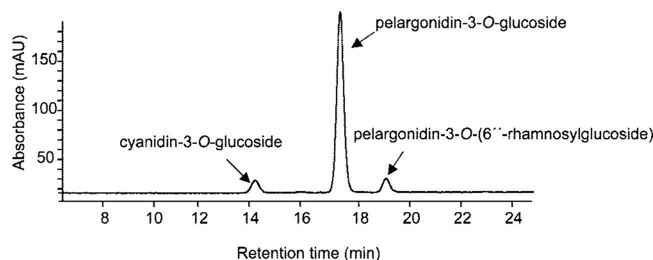

Figure 2. Gradient reverse phase HPLC of anthocyanins in a strawberry extract with detection at 520 nm.

HPLC with PDA and MS^2 Detection. Samples were analyzed on a Surveyor HPLC system comprising a HPLC pump, a diode array absorbance detector, scanning from 250 to 700 nm, and an autosampler cooled to 4 °C (Thermo Electron Corp.). Separation was carried out using a 250 × 4.6 mm i.d., 4 μm Synergi RP-Max column (Phenomenex) eluted with a gradient over 30 min of 8–18% acetonitrile in 2% formic acid at a flow rate of 1 mL/min and maintained at 40 °C. After passing through the flow cell of the diode array detector, the column eluate was split, and 0.3 mL min was directed to an LCQ Advantage ion trap mass spectrometer fitted with an electrospray interface (Thermo Electron Corp.). Analyses utilized the positive ion mode as this provided the best limits of detection for anthocyanins. Each sample was analyzed in the mass spectrometer in two separate ways: first, using full scan, data-dependent MS^2 scanning from *m*/*z* 250 to 750, then using a two-segment selected reaction monitoring (SRM) method looking for ions *m*/*z* 351, 433, and 447 from 0 to 24 min and at *m*/*z* 271 and 351 from 24 to 30 min. Capillary temperature was 150 °C, sheath gas and auxiliary gas were 40 and 20 units, respectively, and the source voltage was 3 kV.

All quantitative estimates were based on 520 nm absorbance traces with data expressed as pelargonidin-3-*O*-glucoside equivalents and peak identifications confirmed by full-scan MS^2 and/or SRM.

Analysis of Plasma Paracetamol. Plasma paracetamol levels were measured using an acetaminophen assay kit (Cambridge Life Sciences, Cambridge, U.K.) with amounts of standard adapted to predicted paracetamol concentrations from initial measurements in this study (*32*). The time to peak was taken as a marker of the rate of gastric emptying.

Pharmacokinetic Analysis of Anthocyanins in Plasma. Maximum plasma concentration of the metabolites from 0 to 8 h postdose was defined as C_{max}. The time to maximum plasma concentration (t_{max}) was defined as the time in hours at which C_{max} was reached. The elimination half-life for the metabolites was computed by using the following formula: $t_{1/2} = 0.693/K_e$ where K_e is the slope of the linear regression of the plasma metabolite concentrations. Area-under-the-curve calculations were determined using a Kinetica software package (Thermo Electron Corp.).

Statistical Analysis. Data on anthocyanin levels are represented as mean values ± standard error (SE) ($n = 8$). When appropriate, data were subjected to statistical analysis using analysis of variance (ANOVA) and paired *t* test with Minitab software, version 13 (Minitab Inc., Addison-Wesley Publishing, Reading, MA).

RESULTS

Strawberries. Gradient reverse phase HPLC with PDA detection and full-scan data-dependent MS^2 was used to identify and quantify the anthocyanin content of the strawberry meals. The main peak in the absorbance chromatogram at A_{520nm} (**Figure 2**) is the pelargonidin-3-*O*-glucoside based on cochromatography with a standard and mass spectral showing that a *m*/*z* 433 molecular ion ([M − H]$^+$) fragmented with a loss of 162 amu (glucose) to produce a *m*/*z* 271 (pelargonidin) daughter ion. Two smaller peaks were also present. The earlier eluting peak (retention time of 14.2 mn) cochromatographed with and had the same mass spectrum (*m*/*z* 449 → 287) as cyanidin-3-*O*-glucoside. The later eluting component (retention time of 19.2 min) yielded a mass spectrum

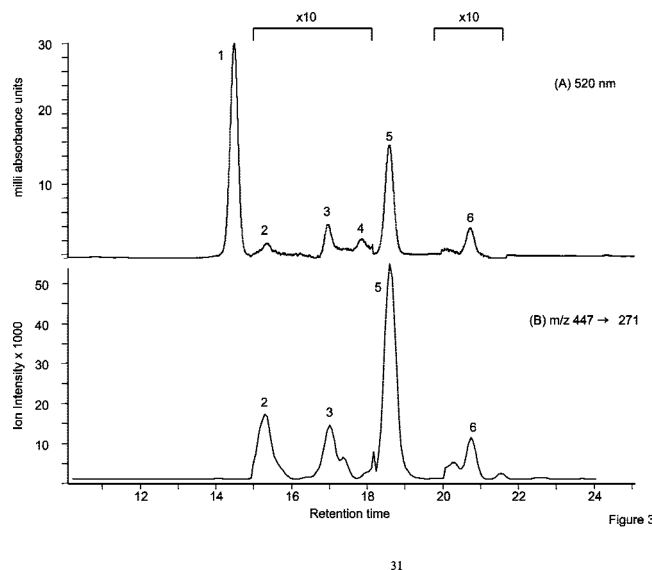

Figure 3. Gradient reversed phase HPLC of a plasma extract collected 1 h after the ingestion of 200 g of strawberries by a human volunteer: detection at (**A**) 520 nm and (**B**) by MS^2 with SRM (*m*/*z* 447 → 271). Peak 1 is the cyanidin-3-*O*-glucoside internal standard. Peaks 2, 3, 5, and 6 are pelargonidin-*O*-glucuronides, whereas peak 4 is pelargonidin-3-*O*-glucoside (see **Table 1**). Sections of the chromatogram where scaling was increased 10-fold to detect minor peaks are indicated.

(*m*/*z* 579 → 422 and 271) indicative of the presence of pelargonidin-3-*O*-6″-rhamnosylglucoside. These minor components were previously detected in strawberries by Lukton et al. (*33*) and Lopes da Silva et al. (*34*). The pelargonidin-3-*O*-glucoside content in the meal was 222 ± 19 μmol along with trace amounts of pelargonidin-3-*O*-6″-rhamnosylglucoside (13 ± 1 μmol) and cyanidin-3-*O*-glucoside (6.2 ± 0.2 μmol).

Qualitative Analysis of Plasma and Urine. Plasma extracts and urine samples collected after the ingestion of 200 g of strawberries with and without cream were all analyzed by HPLC-PDA-MS^2. **Figure 3** illustrates typical HPLC profiles obtained with plasma which, in addition to the cyanidin-3-*O*-glucoside (peak 1) internal standard, contained four pelargonidin-*O*-glucuronides (peaks 2, 3, 5, and 6) and pelargonidin-3-*O*-glucoside (peak 4) (**Table 1**). All of these compounds were detected in urine except cyanidin-3-*O*-glucoside, which was not used as an internal standard. Urine also contained a pelargonidin-*O*-sulfate (peak 7) and the aglycone pelargonidin (peak 8) (**Table 1**). Identifications were based on mass spectral data and cochromatography with authentic standards in the case of pelargonidin and pelargonidin-3-*O*-glucoside. Full-scan MS^2 and SRM were used to partially identify the pelargonidin sulfate and glucuronide metabolites. The pelargonidin-*O*-sulfate had a [M − H]$^+$ at *m*/*z* 351, which with an 80 amu cleavage of the sulfate moiety gave rise to a *m*/*z* 271 pelargonidin daughter ion. The four glucuronide metabolites were characterized by a [M − H]$^+$ at *m*/*z* 447, which fragmented with a 176 amu loss to yield a MS^2 fragment at *m*/*z* 271 (**Table 1**).

Although four pelargonidin-*O*-glucuronides were detected, it was not possible to determine the point of attachment of the glucuronide units because reference compounds were not available. The fact that pelargonidin has hydroxyl groups at the 4′-, 3-, 5-, and 7-positions implies that the four pelargonidin isomers are glucuronidated at these positions. Earlier work with flavonol metabolites established that quercetin-3-*O*-glucuronide elutes from the reversed phase HPLC column used in the present study very close to quercetin-3-*O*-glucoside and much

Table 1. HPLC-MS^2 Identification of Anthocyanins in Plasma and Urine Collected after the Ingestion of Strawberries by Human Volunteers[a]

peak	t_R (min)	$[M + H]^+$ (m/z)	MS^2 (m/z)	identity	location
1	14.2	449	287	cyanidin-3-*O*-glucoside (internal standard)	
2	16.1	447	271	pelargonidin-*O*-monoglucuronide	plasma, urine
3	16.5	447	271	pelargonidin-*O*-monoglucuronide	plasma, urine
4	17.5	433	271	pelargonidin-3-*O*-glucoside	plasma, urine
5	18.3	447	271	pelargonidin-*O*-monoglucuronide	plasma, urine
6	20.3	447	271	pelargonidin-*O*-monoglucuronide	plasma, urine
7	25.4	351	271	pelargonidin-*O*-monosulfate	urine
8	27.0	271		pelargonidin	urine

[a] For peaks 1−6 see **Figure 3**. t_R, retention time; $[M + H]^+$, positively charged molecular ion; MS^2 daughter ion produced by fragmentation of $[M + H]^+$.

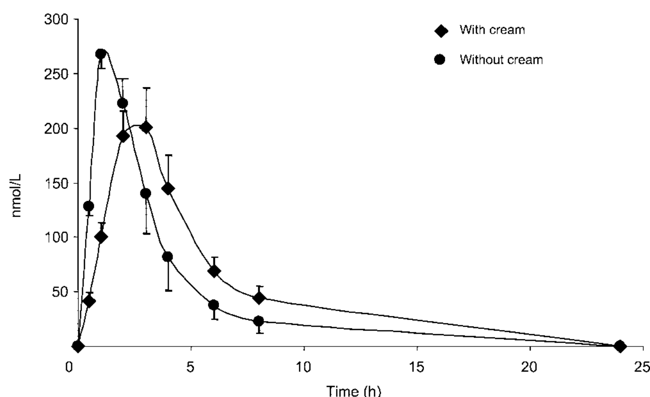

Figure 4. Concentration of a pelargonidin-*O*-glucuronide (peak 5; see **Table 1**) in the plasma of eight human subjects 0–24 h after the ingestion of 200 g of strawberries with (◆) and without 100 mL of double cream (●). Data expressed as nanomoles per liter are presented as mean values ± standard errors depicted by vertical bars ($n = 8$).

earlier than the 3′- and 4′-*O*-glucuronides (*35*). This implies that in the present study the main plasma and urinary metabolite, peak 5, which elutes immediately after pelargonidin-3-*O*-glucoside (peak 4), may be pelargonidin-3-*O*-glucuronide (**Table 1**; **Figure 3**).

Quantitative Analysis of Anthocyanins in Plasma. The anthocyanin that appeared in plasma in by far the largest amounts was a metabolite, pelargonidin-*O*-glucuronide (peak 5) (**Figure 3**; **Table 1**). The other three plasma pelargonidin glucuronides and pelargonidin-3-*O*-glucoside were present in only trace amounts and could not be quantified accurately in most samples. The pharmacokinetic data presented in **Figure 4** are, therefore, restricted to the peak 5 glucuronide metabolite. As illustrated in **Figure 4**, the 0 h plasma samples contained no detectable anthocyanins, and 30 min after ingestion of the strawberries without cream, the pelargonidin-*O*-glucuronide was present in plasma at a mean concentration of 128 ± 16 nmol/L, and after 1 h a concentration 268 ± 24 nmol/L was achieved. Thereafter, the concentration of the glucuronide declined. After 8 h, only 8% of the 1 h level remained, and 24 h after supplementation, it was not detectable. The pelargonidin glucuronide concentration increased more slowly when the strawberries were consumed with cream, reaching a maximum level of 227 ± 35 nmol/L 2–3 h after intake, after which there was again a decline with 43 ± 11 nmol/L remaining at 8 h, 19% of the peak value, and at 24 h the metabolite fell below the limit of detection (**Figure 4**).

Pharmacokinetic analysis of the pelargonidin glucuronide plasma data summarized in **Figure 4** is presented in **Table 2**. The C_{max} without cream, 274 ± 24 nmol/L, was higher than that with cream, 227 ± 35 nmol/L, but because of subject-to-subject variations the two figures are not statistically different. However, the t_{max} data indicate that cream significantly ($p < 0.001$) delayed the time of the C_{max} of the strawberry anthocyanin metabolites from 1.1 ± 0.4 to 2.4 ± 0.5 h. The elimination half-life with strawberries alone was 2.1 ± 0.7 h but was 2.8 ± 1.1 h when the strawberries were consumed with cream. Like the values for area under the curve, these figures are not significantly different.

Quantitative Analysis of Anthocyanins in Urine. Data on the excretion of anthocyanins in urine after the ingestion of 200 g of strawberries with and without cream are presented in **Table 3**. Like the plasma, the main anthocyanin in all urine samples was peak 5, the pelargonidin glucuronide metabolite. The native strawberry anthocyanin, pelargonidin-3-*O*-glucoside, was also present but in much smaller quantities, as were pelargonidin, a pelargonidin-*O*-sulfate and the other three pelargonidin glucuronides (**Table 3**). In keeping with the later plasma t_{max}, there was a significantly lower 0–2 h excretion of total anthocyanins from volunteers who had consumed the strawberries with cream ($p < 0.01$). In contrast, at the 2–5, 5–8, and 8–24 h collection points more anthocyanins were excreted by the subjects who ate the strawberries with cream, but these differences were significant only for the 5–8 h period ($p < 0.02$). The overall excretion of anthocyanins in urine over the 24 h period, with respect to the 222 μmol of pelargonidin-3-*O*-glucoside ingested, was 0.75 ± 0.25% of intake without cream and 1.00 ± 0.33% when the strawberries were consumed with cream. These values are not significantly different. The pelargonidin-*O*-glucuronide, peak 5, comprised 90% of the anthocyanins excreted over the 24 h post-ingestion period, irrespective of whether the strawberries were consumed on their own or with cream.

Breakdown of Anthocyanins in Plasma and Urine. Felgines et al. (*25*) reported that pelargonidin metabolites appearing in urine after the ingestion of strawberries disappeared or were substantially decreased if the samples were frozen prior to analysis. We also observed this phenomenon and, as a consequence, like Felgines et al., we analyzed urine samples 1 h after collection and acidification of urine. We suspect this breakdown is due to the presence of compounds in urine derived from the strawberries as such degradation did not occur with urinary anthocyanins collected after the ingestions of raspberries (data not shown). It was also observed that the pelargonidin glucuronide content of plasma samples collected after strawberry ingestion and stored at −80 °C for 1 month prior to analysis was the same as that of samples analyzed immediately upon collection (data not shown).

Effect of Cream on Gastric Emptying and Mouth to Cecum Transit Time. When the strawberries were eaten with cream, the time to peak paracetamol increased by an average of 42.5 ± 9.0 min ($p < 0.005$), indicating a delay in gastric emptying. The mouth to cecum transit time, as determined by breath hydrogen measurements, was also significantly delayed by the cream, 3.04 ± 0.26 versus 1.21 ± 0.29 h ($p < 0.001$).

Table 2. Pharmacokinetic Parameters of a Pelargonidin-*O*-glucuronide (Peak 5) in the Plasma of Eight Human Subjects after the Consumption of 200 g of Strawberries, Containing 222 μmol of Pelargonidin-3-glucoside, with and without 100 mL of Double Cream[a]

supplement	C_{max} (nmol/L)	t_{max} (h)	$t_{1/2}$ (h)	AUC (nmol/L/h)	C_{max}/dose (nmol/L/μmol)	AUC/dose (nmol/L/μmol)
strawberries without cream	274 ± 24	1.1 ± 0.4	2.1 ± 0.7	856 ± 42	1.13 ± 0.10	3.86 ± 0.19
strawberries with cream	227 ± 35	2.4 ± 0.5	2.8 ± 1.1	895 ± 112	0.94 ± 0.14	4.03 ± 0.48

[a] Data expressed as mean values ± standard error ($n = 8$); C_{max}, maximum post-ingestion plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, elimination half-life; AUC, area under the curve (0–8 h). The pelargonidin glucuronide is peak 5 in **Figure 3** and **Table 1**.

Table 3. Quantities of Anthocyanins in the Urine of Eight Human Subjects 0–24 h after the Consumption of 200 g of Strawberries with and without 100 mL of Double Cream[a]

	0–2 h		2–5 h		5–8 h		8–24 h		total	
anthocyanin (peak no.)	w/o	with	w/o	with	w/o	with	w/o	with	w/o	with
pelargonidin-*O*-glucuronide (2)	6 ± 5	nd	3 ± 4	nd	nd	nd	nd	nd	9 ± 5	nd
pelargonidin-*O*-glucuronide (3)	20 ± 11	5 ± 5	26 ± 28	6 ± 4	1 ± 2	6 ± 5	nd	nd	47 ± 30	17 ± 7
pelargonidin-3-*O*-glucoside (4)	16 ± 6	23 ± 17	10 ± 9	20 ± 10	3 ± 2	12 ± 6	3 ± 6	nd	31 ± 16	55 ± 22
pelargonidin-*O*-glucuronide (5)	636 ± 162	363 ± 226	515 ± 195	884 ± 375	241 ± 145	610 ± 283	107 ± 88	142 ± 78	1498 ± 472	1999 ± 592
pelargonidin-*O*-glucuronide (6)	28 ± 16	40 ± 38	23 ± 30	23 ± 27	5 ± 2	16 ± 9	1 ± 2	4 ± 7	57 ± 35	82 ± 49
pelargonidin-*O*-sulfate (7)	nd	2 ± 4	nd	1 ± 3	1 ± 3	nd	2 ± 4	nd	3 ± 7	3 ± 4
pelargonidin (8)	12 ± 8	10 ± 19	9 ± 15	40 ± 55	nd	6 ± 9	5 ± 14	12 ± 16	26 ± 27	68 ± 90
total	718 ± 156 (42.9)	442 ± 265 (19.9)	585 ± 257 (35.0)	968 ± 383 (43.7)	251 ± 146 (15.0)	649 ± 282 (29.3)	117 ± 104 (7.0)	158 ± 85 (7.1)	1672 ± 551	2217 ± 735

[a] Data expressed as nmol ± standard error ($n = 8$). Figures in parentheses represent anthocyanins excreted as a percentage of the total excreted over the 0–24 h collection period. w/o, strawberries without cream; with, strawberries with cream; nd, not detected. For anthocyanins and peak numbers refer to **Table 1**.

DISCUSSION

There are few reports on the effect of the food matrix on the absorption/excretion of anthocyanins by humans. There are conflicting conclusions, based on studies with red wine, dealcoholized red wine, and red grape juice, as to whether alcohol does or does not impair the absorption of anthocyanins (*36, 37*). If there is an effect, at best it is marginal (*23*). In keeping with this view, absorption and urinary excretion of black currant anthocyanin are not affected by the ingestion of a carbohydrate-rich rice cake (*38*). It has been claimed that ingestion of sugar with an elderberry concentrate leads to a reduced excretion of anthocyanins (*39*). In reality, the differences in cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside in urine appear to be minimal and in any case were not evaluated statistically. It is similarly difficult to gauge the effect of a high-fat diet on the absorption of elderberry anthocyanins as the volunteers on a low-fat diet did not participate in the study (*40*).

The data obtained in the current study demonstrate that following ingestion of strawberries by human subjects pelargonidin-3-*O*-glucoside is converted primarily to a pelargonidin-*O*-glucuronide (metabolite 5). It is this metabolite rather than the parent glucoside that is the predominant anthocyanin in both plasma and urine. When fed without cream, the metabolite appeared rapidly in the bloodstream and reached a C_{max} of 274 ± 24 nmol/L after 1.1 ± 0.4 h. When the strawberries were consumed with 100 mL of double cream, the t_{max} at 2.4 ± 0.5 h was delayed by more than 1 h and the C_{max} was lower, 227 ± 35 nmol/L, but not significantly so (**Figure 4**; **Table 2**). The excretion of the anthocyanins in urine is in keeping with these observations. In the absence of cream anthocyanins are excreted more rapidly 0–2 h after consumption of the strawberries ($p < 0.01$). Thereafter, over the 2–5, 5–8, and 8–24 h collection periods, greater quantities of anthocyanins appeared in the urine of volunteers who consumed the strawberries with cream, although the only period when this difference was significant ($p < 0.02$) was 5–8 h (**Table 3**). As expected, the paracetamol and breath hydrogen data confirm a delay in both gastric emptying and mouth to cecum transit time by the cream. This is caused by duodenal and ileal brakes activated by receptors to the fat in the cream (*41*).

The influence of the cream, thus, appears to be to slow transit through the gastrointestinal tract and result in delayed absorption of the peak 5 pelargonidin-*O*-glucuronide from the small intestine, but overall the amounts that are absorbed are not greatly different in the presence or absence of cream. Hence, a delayed gastric emptying would slow the appearance of anthocyanins in the small intestine and, therefore, their emergence in the blood. However, the delay in mouth to cecum transit would give more time for their absorption in the small intestine. This is similar to the delay seen in sugar absorption when eaten with viscous soluble fiber, where urinary xylose demonstrated no change in the total amount absorbed (*42*).

It has previously been reported that after the ingestion of strawberries by human volunteers, total urinary excretion of anthocyanins, principally as a pelargonidin-*O*-glucuronide metabolite, corresponded to 1.80% of pelargonidin-3-*O*-glucoside intake (*25*). This is a high figure compared to the typical urinary recovery of dietary anthocyanins, which is usually ca. <0.1% of intake. In the present study with strawberries, urinary excretion of anthocyanins was 0.75 ± 0.25% of pelargonidin-3-*O*-glucoside intake without cream and 1.00 ± 0.33% with cream, lower than the recovery found by Felgines et al. (*25*), but nonetheless still comparatively very high. These recoveries after the ingestion of strawberries suggest that pelargonidin-3-*O*-glucoside is more readily absorbed than other anthocyanins that have been investigated to date, and feeding studies with blackberries indicate it has greater access to the blood stream than its 3′-hydroxy derivative, cyanidin-3-*O*-glucoside, which appears in plasma principally in an unmetabolized form rather than as a glucuronide (*26*).

This is the first study in which anthocyanins in plasma as well as urine have been analyzed after strawberry ingestion by humans. This provided further evidence of the comparatively high bioavailability of the strawberry pelargonidin-3-*O*-glucoside. Both C_{max} values were in excess of 200 nmol/L (**Table 2**). This is considerably higher than C_{max} values obtained in

any other human feeding study with anthocyanins that are conveniently reviewed in Table 1 of Prior and Wu (*23*). The figures for C_{max}/dose and AUC/dose (**Table 2**) are also substantially higher values than those obtained in other studies (*23*).

The plasma data demonstrate that prior to absorption into the bloodstream pelargonidin-3-*O*-glucoside is converted to three minor and one major pelargonidin glucuronide. The main metabolite may be pelargonidin-3-*O*-glucuronide. As has been shown with flavonols (*35, 43*), these conversions may begin with the glucoside being hydrolyzed by lactase phlorizen hydrolase located in the apical membrane of the small intestine epithelial cells and/or cytosolic β-glucosidase in the small intestine (*44*). Through the action of glucuronyltransferases in the enterocyte, the released aglycone is glucuronidated and the products, the putative pelargonidin-3-*O*-glucuronide and trace amounts of its three isomers and unhydrolyzed pelargonidin-3-*O*-glucoside, enter the portal vein (*25*). Another possibility is that the pelargonidin-3-*O*-glucoside is converted directly to pelargonidin-3-*O*-glucuronide by the action of UDP glucose dehydrogenase (*22*), although this is unlikely as it would account for the appearance of one, not four, pelargonidin glucuronides. The low urinary excretion of anthocyanins after the ingestion of blackberries (*26*) indicates that cyanidin-3-*O*-glucoside (**II**) is not hydrolyzed and glucuronidated with the same efficiency as pelargonidin-3-*O*-glucoside (**I**). It remains to be determined whether this is a consequence of greater instability of the cyanidin-3-*O*-glucoside in the small intestine or the inability of hydrolyzing enzymes to cleave the aglycone.

Once in the circulatory system the pelargonidin glucuronides and pelargonidin-3-*O*-glucoside appear to undergo little modification before being excreted in urine along with trace quantities (ca. 1–3%) of pelargonidin and a pelargonidin-*O*-sulfate (**Table 3**), neither of which was detected in plasma (**Table 1**). In this regard the fate of pelargonidin-3-*O*-glucosides in strawberries following ingestion by humans is very different from that of quercetin-4′-*O*-glucoside and quercetin-3,4′-*O*-diglucoside in onions. The quercetin glucosides are rapidly hydrolyzed and undergo not only 3-glucuronidation but also 3′-methylation and extensive 3′-sulfation and appear in the bloodstream as quercetin-3′-*O*-sulfate, quercetin-3-*O*-glucuronide, isorhamnetin-3-*O*-glucuronide, and a quercetin diglucuronide (*35*). Substantial sulfation of quercetin appears to take place in the wall of the small intestine (*45*), but clearly this does not occur with pelargonidin. Once in the bloodstream, the quercetin derivatives undergo extensive phase II metabolism, which results in the profile of urinary metabolites being very different from the plasma metabolite profile, the most notable distinction being the absence of quercetin-3′-*O*-sulfate in urine (*35*). This is in marked contrast with the plasma pelargonidin glucuronides, which are excreted having undergone almost no phase II metabolism.

ACKNOWLEDGMENT

We thank the volunteers who participated in the study.

LITERATURE CITED

(1) Crozier, A.; Yokota, T.; Jaganath, I. B.; Marks, S. C.; Saltmarsh, M.; Clifford, M. N. Secondary metabolites in fruits, vegetables, beverages and other plant based dietary components. In *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet*; Crozier, A., Clifford, M. N., Ashihara, H., Eds.; Blackwell Publishing: Oxford, U.K., 2006; pp 208–302.
(2) Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 304–309.
(3) Ichikawa, H.; Ichiyanagi, T.; Xu, B.; Yoshi, Y.; Naakajima, M.; Konishi, T. Antioxidant activity of anthocyanin extract from purple black rice. *J. Med. Food* **2001**, *4*, 211–218.
(4) Kahkonen, M. P.; Heinonen, M. Antioxidant activity of anthocyanins and their aglycones. *J. Agric. Food Chem.* **2003**, *51*, 628–633.
(5) Hou, D. X. Potential mechanisms of cancer chemoprevention by anthocyanins. *Curr. Mol. Med.* **2003**, *3*, 149–159.
(6) Lala, G.; Malik, M.; Zhao, C.; He, J.; Kwon, Y.; Giusti, M. M.; Magnuson, B. A. Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. *Nutr. Cancer* **2006**, *54*, 84–93.
(7) Wu, Q. K.; Koponen, J. M.; Mykkänen, H. M.; Törrönen, A. R. Berry phenolics extracts modulate the expression of p21^{WAF1} and Bax but not Bcl-2 in HT-29 colon cancer cells. *J. Agric. Food Chem.* **2007**, *55*, 1156–1163.
(8) Xu, J.-W.; Ikeda, K.; Yamori, Y. Cyanidin-3-glucoside regulates phosphorylation of endothelial nitric oxide synthase. *FEBS Lett.* **2005**, *574*, 176–180.
(9) Seeram, N. P.; Bourquin, L. D.; Nair, M. G. Degradation products of cyanidin glycosides from tart cherries and their bioactivities. *J. Agric. Food Chem.* **2001**, *49*, 4924–4929.
(10) Mercier, A.; Perdriel, G.; Rozier, J.; Cheraleraud, J. Note concerning the action of anthocyanin glycosides on the human electroretinogram. *Bull. Soc. Opthalmol. Fr.* **1965**, *65*, 1049–1053.
(11) Joseph, J. A.; Denisova, N. A.; Arendash, G.; Gordon, M.; Diamond, D.; Shukitt-Hale, B.; Morgan, D. Blueberry supplementation enhances signalling and prevents behavioural deficits in an Alzheimer disease model. *Nutr. Neurosci.* **2003**, *6*, 153–162.
(12) Tsuda, T.; Horio, F.; Uchida, K.; Aoki, T.; Osawa, T. Dietary cyanidin-3-*O*-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J. Nutr.* **2003**, *133*, 2125–2130.
(13) Kuhnau, J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet* **1976**, *24*, 117–191.
(14) Wu, X.; Beecher, G.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Concentration of anthocyanins in common foods in the United States and estimation of normal consumption. *J. Agric. Food Chem.* **2006**, *54*, 4069–4075.
(15) Burns, J.; Gardner, P. T.; McPhail, D. B.; O'Neil, J.; Crawford, S.; Morecroft, I.; Lister, C.; Matthews, D.; MacLean, M. R.; Lean, M. E. J.; Duthie, G. G.; Crozier, A. Relationship among antioxidant activity, vasodilation capacity and phenolic content of red wines. *J. Agric. Food Chem.* **2000**, *48*, 220–230.
(16) Koponen, J. M.; Happonen, A. M.; Mattila, P. H.; Törrönen, A. R. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *J. Agric. Food Chem.* **2007**, *55*, 1612–1619.
(17) Miyazawa, T.; Nakagawa, K.; Kudo, M.; Muraishi, K.; Someya, K. Direct intestinal absorption of red fruit anthocyanins, cyandin-3-glucoside and cyandin-3,5-diglucoside, into rats and humans. *J. Agric. Food Chem.* **1999**, *47*, 1083–1091.
(18) Milbury, P. E.; Cao, G.; Prior, R. L.; Blumberg, J. Bioavailability of elderberry anthocyanins. *Mech. Ageing Dev.* **2002**, *123*, 997–1006.
(19) McGhie, T. K.; Ainge, G. D.; Barnett, L. E.; Cooney, J. M.; Jensen, D. J. Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolized by both humans and rats. *J. Agric. Food Chem.* **2003**, *51*, 4539–4548.
(20) Cooney, J. M.; Jensen, J. D.; McGhie, T. K. LC-MS identification of anthocyanins in boysenberry extract and anthocyanin metabolites in human urine following dosing. *J. Sci. Food Agric.* **2004**, *84*, 237–245.
(21) Ichiyanagi, T.; Shida, Y.; Rahman, M. M.; Hatano, Y.; Konishi, T. Extended glucuronidation is another major path of cyanidin 3-*O*-β-D-glucopyranoside metabolism in rats. *J. Agric. Food Chem.* **2005**, *53*, 7312–7319.
(22) Wu, X.; Pittman, H. E.; McKay, S.; Prior, R. L. Aglycones and sugar moieties alter anthocyanin absorption and metabolism after

berry consumption in weanling pigs. *J. Nutr.* **2005**, *135*, 2417–2424.
(23) Prior, R.; Wu, X. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Res.* **2006**, *40*, 1014–1028.
(24) Cho, M. J.; Howard, L. R.; Prior, R. L.; Clark, J. R. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Sci. Food Agric.* **2004**, *84*, 1771–1782.
(25) Felgines, C.; Talavéra, S.; Gonthier, M.-P.; Texier, O.; Scalbert, A.; Lamaison, J.-L.; Rémésy, C. Strawberry anthocyanins are recovered in urine as glucurono and sulphoconjugates in humans. *J. Nutr.* **2003**, *133*, 1269–1301.
(26) Felgines, C.; Talavéra, S.; Texier, O.; Gil-Izquierdo, A.; Lamaison, J.-L.; Rémésy, C. Blackberry anthocyanins are mainly recovered from urine as methylated and glucuronidated conjugates in humans. *J. Agric. Food Chem.* **2005**, *53*, 7721–7727.
(27) Wu, X.; Pittman, H. E.; Prior, R. L. Pelargonidin is absorbed and metabolised differently than cyanidin after marionberry consumption in pigs. *J. Nutr.* **2004**, *134*, 2603–2610.
(28) Serafini, M.; Bugianesi, R.; Maiani, G.; Valtuena, S.; De Santis, S.; Crozier, A. Plasma antioxidants from chocolate. Dark chocolate may offer its consumers health benefits the milk variety cannot match. *Nature* **2003**, *424*, 1013.
(29) Halliwell, B. Health benefits of eating dark chocolate. *Nature* **2003**, *426*, 787.
(30) Schroeter, H.; Holt, R. R.; Orozco, T. J.; Schmidtz, H. H.; Keen, C. L. Milk and absorption of dietary flavanols. *Nature* **2003**, *426*, 787–788.
(31) Serafini, M.; Crozier, A. Milk and absorption of dietary flavanols − reply. *Nature* **2003**, *426*, 788.
(32) Clements, J. A.; Heading, R. C.; Nimmo, W. S.; Prescott, L. F. Kinetics of acetaminophen absorption and gastric emptying in man. *Clin. Pharmacol. Ther.* **1978**, *24*, 420–431.
(33) Lukton, A.; Chichester, C. O.; MacKinney, G. Characterisation of a second pigment in strawberries. *Nature* **1955**, *176*, 790.
(34) Lopes da Silva, F.; de Pascual-Teresa, S.; Rivas-Gonzalo, J.; Santos-Buelga, C. Identification of anthocyanin pigments in strawberry (cv. Camarosa) by LC using DAD and ESI-MS detection. *Eur. Food Res. Technol.* **2002**, *214*, 248–253.
(35) Mullen, W.; Edwards, C. A.; Crozier, A. Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. *Br. J. Nutr.* **2006**, *96*, 107–116.
(36) Bub, A.; Watzl, B.; Heeb, D.; Bechkemmer, G.; Briviba, K. Malvidin-3-glucoside bioavailability in humans after the ingestion of red wine, dealcoholized red wine and red grape juice. *Eur. J. Nutr.* **2001**, *40*, 113–120.
(37) Frank, T.; Netzel, M.; Strass, G.; Bitsch, R.; Bitsch, I. Bioavailability of anthocyanin-3-glucosides following consumption of red wine and red grape juice. *Can. J. Physiol. Pharmacol.* **2003**, *81*, 423–435.
(38) Nielsen, I. L. F.; Dragsted, L. O.; Rav-Haren, G.; Freese, R.; Rasmussen, S. E. Absorption and excretion of black currant anthocyanins in humans and Watanabe heritable hyperlipidemic rabbits. *J. Agric. Food Chem.* **2003**, *51*, 2813–2820.
(39) Mülleder, U.; Murkovic, M.; Pflannhauser, W. Urinary excretion of cyanidin glycosides. *J. Biochem. Biophys. Methods* **2002**, *53*, 61–66.
(40) Murkovic, M.; Toplak, H.; Adam, U.; Pflannhauser, W. Analysis of anthocyanins in plasma for determination of their bioavailability. *J. Food Compos. Anal.* **2000**, *13*, 291–296.
(41) Welch, I. M.; Bruce, C.; Hill, S. E.; Read, N. W. Duodenal and ilea lipid suppresses postprandial blood glucose and insulin responses in man: possible implications for the dietary management of diabetes mellitus. *Clin. Sci.* **1987**, *77*, 209–216.
(42) Jenkins, D. J. A.; Wolever, T. M. S.; Leeds, A. R.; Gasull, M. A.; Haisman, P.; Dilawari, J.; Goff, D. V.; Metz, G. L.; Alberti, K. G. M. M. Dietary fibers, fiber analogues and glucose tolerance: importance of viscosity. *Br. Med. J.* **1978**, *1*, 1392–1394.
(43) Gee, J. M.; DuPont, M. S.; Day, A. J.; Plumb, G. W.; Williamson, G.; Johnson, I. T. Intestinal tansport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. *J. Nutr.* **2000**, *130*, 2765–2771.
(44) Németh, K.; Plumb, G. W.; Berrin, J.-G.; Juge, N.; Jacob, R.; Naim, H. Y.; Williamson, G.; Swallow, D. M.; Kroon, P. A. Deglycosylation by small intestinal epithelial cell β-glucosidase is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *Eur. J. Nutr.* **2003**, *42*, 29–42.
(45) Jaganath, I. B.; Mullen, W.; Edwards, C. A.; Crozier, A. The relative contribution of the small and large intestine to the absorption and metabolism of rutin in man. *Free Radical Res.* **2006**, *40*, 1035–1046.

Received for review July 3, 2007. Revised manuscript received August 7, 2007. Accepted November 27, 2007. This project was funded by a BBSRC grant to A.C. and C.A.E.

JF072000P