Anthocyanin pharmacokinetics and dose-dependent plasma antioxidant pharmacodynamics following whole tart cherry intake in healthy humans

E. Mitchell Seymour a,*, Sara M. Warber b, Ara Kirakosyan a, Kathleen R. Noon c, Brenda Gillespie d, Virginia E. Uhley e, Jenna Wunder b, Daniel E. Urcuyo f, Peter B. Kaufman a, Steven F. Bolling a

a Cardiovascular Center, University of Michigan Health System, Ann Arbor, MI, USA
b University of Michigan Integrative Medicine, Ann Arbor, MI, USA
c Mass Spectrometry Facility, Innovation Center, Medical College of Wisconsin, Milwaukee, WI, USA
d Center for Statistical Consultation and Research, University of Michigan, Ann Arbor, MI, USA
e Oakland University William Beaumont School of Medicine, Rochester, MI, USA
f Cleveland Clinic Lerner College of Medicine, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form 9 July 2014
Accepted 7 August 2014
Available online 2 September 2014

Keywords:
Tart cherry
Anthocyanins
Pharmacokinetics
Antioxidant

ABSTRACT

Anthocyanin-rich tart cherries may impart health benefits for oxidative stress and inflammation. Anthocyanin (ACN) pharmacokinetic studies often sample plasma and urine within hours of ingestion; these approaches do not reveal enterohepatic metabolites that may be critical for pharmacodynamic bioactivity. This study investigated ACN pharmacokinetics in healthy humans following intake of Montmorency tart cherries (Prunus cerasus). Using a within-subject crossover design, subjects (n = 12) ingested whole frozen tart cherries (45 or 90 cherries), and blood and urine samples were collected over 12 hours. LC-MS/MS identified two unmodified ACN in plasma and two ACN metabolites in urine. Intake of 45 cherries caused a biphasic antioxidant response, while 90 cherries caused a prolonged elevation over the 12 h period. The broad antioxidant peak beyond 8 h suggests that enterohepatic metabolites contribute to antioxidant pharmacodynamics. These findings should encourage extended pharmacokinetic studies with ACN-rich foods to reveal their breadth of bioavailability and bioactivity.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

A diet rich in plant foods is inversely correlated with cardiovascular morbidity and mortality, and non-nutritive phytochemicals in fruits, vegetables, and grains may participate in this benefit. Anthocyanins are a subclass of flavonoids that confer red, blue, purple, and black pigmentation in plants. An epidemiologic study revealed that among post-menopausal women, regular intake of certain diet-derived phytochemicals

* Corresponding author. Tel.: +1 734 763 0331; fax: +1 734 615 4377.
E-mail address: seymoure@med.umich.edu (E.M. Seymour).
http://dx.doi.org/10.1016/j.jff.2014.08.007
1756-4646/© 2014 Elsevier Ltd. All rights reserved.
was inversely related to cardiovascular disease mortality (Mink et al., 2007). Of all phytochemical classes, only anthocyanins sustained significant benefit for cardiovascular disease mortality after multivariate adjustment (age, energy intake, marital status, education, blood pressure, diabetes, BMI, waist-to-hip ratio, physical activity, smoking, and estrogen use). Currently, a knowledge gap exists for mechanisms associated with this profound benefit of anthocyanin-rich foods, but consumer interest in these fruits is high.

Oxidative stress contributes to cardiovascular disease, so improving antioxidant capacity is a common therapeutic target in functional foods research. For example, it is known that oxidation of low-density lipoprotein (LDL) contributes to its atherogenicity within blood vessel walls. Oxidative stress also affects endothelial function and vascular reactivity, both of which affect blood pressure and tissue perfusion. It is not surprising, then, that much attention is given to the possible benefits of antioxidant approaches to improve cardiovascular health.

Tart cherries (Prunus cerasus) and their processed products (like juice concentrate, juice, dried) are a rich source of dietary anthocyanins and have antioxidant activity against a range of prooxidants (Ou, Bosak, Brickner, lezzoni, & Seymour, 2012). Tart cherries and their related processed products also reduce markers of oxidative stress and inflammation in animals and humans (Bell, Walsh, Davison, Stevenson, & Howatson, 2014; Howatson et al., 2010; Lynn et al., 2014; Matchynski et al., 2013; Schumacher et al., 2013; Seymour et al., 2009; Tall et al., 2004; Traustadottir et al., 2009). Pharmacokinetics (PK) reveals the absorption and excretion of compounds, while pharmacodynamics (PD) describes biologic effects as affected by intake. Despite experimental evidence of benefits relevant to heart disease, no published studies have revealed PK of tart cherry phytochemicals and concurrent evidence of a biologic effect (PD). This is an important information gap, because high throughput, hypothesis-testing in vitro studies with tart cherry compounds should utilize the most relevant serum compounds – within a physiologically relevant concentration and time course.

Clinical PK studies with anthocyanin-rich foods are typically limited in their time course, with plasma and urine collected within 6 hours of consumption. Compounds from tart cherry not initially absorbed in the small intestine will reach the colon and can be further metabolized by colon microflora. Products of this microbial metabolism may then be absorbed by colon enterocytes and taken into systemic circulation. In addition, phytochemicals absorbed in the small intestine are metabolized by the liver and then released back into the small intestine through biliary excretion, making a second pass through the gastrointestinal tract. Enterohepatic metabolism therefore predicts that the absorption of phytochemicals and their metabolites are not limited to few hours after intake. Thus, both PK and PD studies with phytochemical-rich foods should be surveyed for longer time periods to reveal these secondary metabolites and/or any concurrent pharmacodynamic effects.

This study in healthy subjects is a randomized, within-subject crossover design using acute dosing with tart cherries, a whole food source of anthocyanins. The study design uses physiologically-relevant amounts of whole, pitted tart cherries and measures plasma and urine anthocyanin PK and plasma antioxidant PD over a 12-hour period.

2. Materials and methods

2.1. Subjects

Healthy subjects were recruited by flyers, by print ads, and by web announcements via ENGAGE, an online University of Michigan service for volunteer clinical research participants. Interested individuals were first screened by telephone (N = 66) to identify 24 potentially eligible participants to visit the University of Michigan Clinical Research Unit (MCRU) for additional screening. This study was approved by the University of Michigan Institutional Review Board, and all participants signed informed consents at the initial screening visit. Of these individuals, 12 normal weight healthy subjects were eligible based on our inclusion and exclusion criteria (Supplementary Table S1). Patient demographics are provided in Supplementary Table S2.

2.2. Design and methods

The study material was pitted, individually quick-frozen (IQF) Montmorency tart cherries (P. cerasus) supplied by the Cherry Marketing Institute (Dewitt, MI, USA). Doses were 45 whole tart cherries (~187 g, or 1.5 cups) or 90 whole tart cherries (~367 g, or 3 cups). Using a crossover design, the alternate dose was provided following a minimum 14-day washout period.

Subjects were instructed to follow a 2-day low phenol diet as directed by a research dietitian. Subjects then underwent a ≥8 hour overnight fast and had a baseline blood drawn the following morning at the MCRU, where they remained for the duration of the study. Frozen tart cherries were thawed at room temperature for 10 minutes. All cherries were consumed within 15 minutes, including a 10 mL tap water rinse of the bowl that contained juice derived from the thawing cherries. The MCRU metabolic kitchen provided standardized, low phenol meals and a snack (total of 2000 kcals) beginning 2 hours after cherry consumption and throughout the 12-hour period. Subjects consumed no other foods during this period. On average, subjects consumed lunch between 12 and 1 pm, a snack around 3–3:30 pm, and dinner around 5 pm. Given that the provided foods lacked phytochemicals, the ingested whole tart cherries were the only likely source of phytochemicals during the study period.

2.3. Tart Cherry Anthocyanin Analysis

Frozen pitted tart cherry (1 g) was macerated and extracted with 10 mL methanol/water/acetic acid (85:15:0.5 v/v/v) in 15 mL screw-cap tubes at 4 °C and placed on a gyrorotary shaker overnight in the dark. The sample was vortexed and sonicated. After filtration through a 0.22 lm filter, the extract was ready for analysis. A simple fractionation was performed using preconditioned C-18 Sep-Pak cartridges to separate anthocyanins from non-anthocyanin phenolics, as reported previously (Kim, Heo, Kim, Yang, & Lee, 2005). Liquid chromatography-tandem mass
spectrometry (LC-MS/MS) analysis for individual anthocyanin identification and quantification was done as we described previously (Kirakosyan, Seymor, Llanes, Kaufman, & Bolling, 2009). The results are in Table 1.

### 2.4 Blood and urine collection and storage

Two baseline blood collections were obtained to assess baseline coefficient of variation in all parameters; these samples were drawn 30 minutes and 1 minute before cherry ingestion. Subjects were asked to empty their bladder prior to cherry consumption. After ingestion, serial blood draws were taken at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and 12 hours. Whole blood was collected in 10 cc K2 EDTA tubes and placed on ice. Within 15 minutes, samples were centrifuged (1875 × g, 20 minutes, at 4 °C), and plasma was acidified with 1% triflouracetic acid. All urine was collected and pooled with 1% triflouracetic acid, aliquoted into chilled 2 mL cryovials, centrifuged at 4 °C, and kept on ice. Within 15 minutes, samples were centrifuged (1875 × g, 20 minutes, at 4 °C), and plasma was acidified with 1% triflouracetic acid. Aliquots were taken at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and 12 hours. Whole blood was collected in 10 cc K2 EDTA tubes and placed on ice. Within 15 minutes, samples were centrifuged (1875 × g, 20 minutes, at 4 °C), and plasma was acidified with 1% triflouracetic acid, aliquoted into chilled 2 mL cryovials, and snap-frozen in liquid nitrogen and stored at −80 °C. For 12 hours post-consumption, all urine was collected and pooled into five aliquots representing hours 0–2, 2–4, 4–6, 6–8, and 8–12. Urine samples were immediately acidified with 1% formic acid and stored at −80 °C.

### 2.5 LC-MS/MS analysis

Urine and plasma samples were extracted using a solid-phase extraction procedure. Sep-Pak C18 cartridges (Waters, Milford, MA) and were preconditioned with 5 mL (four column volumes) of 5% formic acid in methanol followed by 5 mL of 5% aqueous formic acid. Urine (4 mL) or plasma (3 mL) was then applied to the cartridge. Using this approach, typical solid-phase extraction recovery (using cyanidin-3-glucoside as an internal standard) averaged 80–85%. The eluate was evaporated to dryness in a vacuum centrifuge and reconstituted in a solution of methanol:water:formic acid (5:94:1, v/v/v). The reconstituted volumes were 0.4 mL and 0.2 mL for urine and plasma, respectively. Samples were subjected to LC-MS/MS analysis with a spiked internal standard of malvidin-3-galactoside (Extrasynthese, Genay, France) resulting in a final concentration of 0.56 µM for urine samples and 5.6 µM for plasma samples.

An Alliance 2695 HPLC (Waters) was used to generate a binary gradient with 5% formic acid in water as the aqueous solvent (A) and 5% formic acid in acetonitrile as the organic modifier (B). Chromatographic separation was achieved with a Gemini 5 µm C18 150 × 2.00 mm (Phenomenex, Torrance, CA) reverse phase column held at 35 °C using a flow rate of 0.2 mL/minute. The column was initially equilibrated to 5% B, to 15% B over 10 minutes, to 35% B over the next 15 minutes, to 55% B over 12 minutes and then returned to 5% B. The sample chamber was cooled to 10 °C and the injection volume was 20 µL.

Effluent from the HPLC column was directed into the electrospray ionization probe of a TSQ Quantum Ultra AM triple quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA). Positive ions were generated with the following parameters: metal needle spray voltage 3200 V, sheath gas 40, aux gas 5, sweep gas 5, and capillary temperature 300 °C. Data were collected in centroid mode. For urine analysis, precursor ion scans from m/z 200–1200 were conducted to detect and quantify metabolites containing the three aglycone structures present in tart cherries: cyanidin (m/z 287); peonidin (m/z 301), and pelargonidin (m/z 271). Single reaction monitoring (SRM) was used for mass analysis of the internal standard. Tube lens voltage was 116 V, collision energy was 20 eV, and collision gas pressure was held at 1.0 mTorr.

Compounds with cyanidin aglycone core structures were quantified as equivalents of cyanidin-3-glucoside, those with peonidin aglycones were quantified as equivalents of peonidin-3-glucoside, and metabolites containing the pelargonidin aglycone were quantified as equivalents of pelargonidin. For plasma samples, mass detection was achieved using SRM scan functions for the major anthocyanin species present in tart cherries: pelargonidin (m/z 271–287); cyanidin-3-glucoside (m/z 449–287); peonidin-3-glucoside (m/z 463–301); cyanidin-3-rutinoside (m/z 595–287); cyanidin-galactosylrutinoside (m/z 757–287); and the internal standard (malvidin-3-galactoside) (m/z 493–331). Tube lens voltages and collision energies were optimized for each species. Data analysis was performed with Xcalibur quantitation software (version 1.4 SR1, ThermoFinnigan).

### 2.6 Measurement of plasma antioxidant capacity

Plasma antioxidant capacity was measured at each time point using the Trolox equivalent antioxidant capacity (TEAC) assay (Cayman Chemical, Ann Arbor, MI USA) without modification. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate]) to an ABTS® radical by metmyoglobin. The capacity of the antioxidants in the sample to prevent ABTS oxidation is compared with that of Trolox, a water-soluble tocopherol analog, and is quantified as millimolar Trolox equivalents (mmol TE). To detect color development, the 96-well plate was shaken for 4 minutes at room temperature using an orbital shaker and then read at 405 nm on a BioTek-312 microplate reader (Bio-Tek Instruments, Winooski, VT).

### Table 1 – Anthocyanins in pitted, frozen, whole Montmorency tart cherry.

<table>
<thead>
<tr>
<th></th>
<th>Cyanidin-3-glucosylrutinoside</th>
<th>Cyanidin-3-rutinoside</th>
<th>Peonidin-3-rutinoside</th>
<th>Total ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen cherries, mg/g fresh weight</td>
<td>0.047 ± 0.002</td>
<td>0.02 ± 0.003</td>
<td>0.003 ± 0.001</td>
<td>0.07</td>
</tr>
<tr>
<td>Estimated ACN per 45 pitted cherries (185 g)</td>
<td>8.70</td>
<td>3.70</td>
<td>0.55</td>
<td>12.96</td>
</tr>
<tr>
<td>Estimated ACN per 90 pitted cherries (369 g)</td>
<td>17.34</td>
<td>7.38</td>
<td>1.11</td>
<td>25.83</td>
</tr>
</tbody>
</table>

* a Sum of the values of the three anthocyanins listed.

* b One pitted frozen Montmorency tart cherry is approximately 4.1 g. Mean ± SEM, n = 3 per analysis.
2.7. Statistical analysis

Plasma and urine values for each measured anthocyanin species were plotted versus time over the 12-hour period. TEAC of the unknown samples was determined following linear regression analysis of the Trolox standard curve using GraphPad PRISM 5 (GraphPad Software, La Jolla, CA, USA). Paired t-tests compared each time point TEAC to the mean baseline TEAC (determined as the mean of the −30 min and −1 min plasma sample TEAC). To determine area under the curve (AUC), points were plotted as the value of TEAC – mean baseline TEAC, and the baseline was therefore set at 0. We determined Positive TEAC (area above mean baseline TEAC), Negative TEAC (area below mean baseline TEAC), and Net TEAC (Positive TEAC – Negative TEAC). With 12 subjects, a single group t-test with a 0.05 one-sided significance level provided 82% power to detect a difference between a null hypothesis AUC of 0 (no change from baseline) and an effect size of 0.8. Statistical analysis was conducted using SAS version 9.1.3 software, and graphing was conducted with GraphPad PRISM.

3. Results

Subjects (N = 12; n = 6 males and n = 6 females) were well-distributed across gender and ethnicities (Supplementary Table S1). No subjects dropped out of the study. Anecdotally, participants found the 45 cherry doses easier to consume than the 90 cherry doses; two participants experienced loose stools at the higher dose. Consumption of either 45 or 90 whole IQF tart cherries yielded the plasma appearance tart cherry ACN cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside (Fig. 1). For 45 cherries, the tmax was 2 h. For 90 cherries, tmax differed by compound detected, occurring at 2 h for cyanidin-3-rutinoside and 4 h for cyanidin-3-glucosylrutinoside. Consumption of either 45 or 90 cherries increased urine ACN – cyanidin glycoside, peonidin glycoside or methylated cyanidin glycoside, and a pelargonidin metabolite with an undetermined conjugate moiety. Fig. 2a,b illustrates that excretion of cyanidin and peonidin species was maximal between hours 6 and 8.

Following 45 cherries, Fig. 3 shows the biphasic nature of the antioxidant activity over 12 h, with a TEAC peak at 1 h and another peak spanning the 8 h and 12 h samples. Significant differences vs. mean baseline TEAC were found at 1 h and 12 h (p < 0.05 and p < 0.001, respectively). Other time points visually appear to be significant, yet the larger variance among samples at these points likely affected the calculated p value. Following the 90 cherry dose (Fig. 4), there was an immediate
peak in TEAC, followed by a persistent elevation, and another peak at 8 h. Significant differences vs. mean baseline TEAC were found at 1 h, 8 h, and 12 h (p < 0.05; p < 0.001; and p < 0.05, respectively).

Baseline TEAC did not differ significantly between those initially assigned to the high- or low-dose groups (data not shown). Using a one-sample t-test of the null hypothesis (Ho: mean Positive AUC = 0), Fig. 5 shows that both cherry doses were significant for Positive AUC; the 90 cherry dose mean Positive AUC was 5.37 (p < 0.0001 vs. Ho), and the 45 cherry dose mean Positive AUC was 1.57 (p = 0.0003 vs. Ho). The cherry doses had different impacts on Negative TEAC. During the 45 cherry dosing, TEAC reached a nadir at hour 6; this corresponded with the elimination half-life of parent ACN in plasma and with the peak urinary excretion of ACN metabolites. Comparatively, the 90 cherry dosing largely prevented the decline in plasma TEAC at the same time period. The mean Negative TEAC was –0.53 for 90 cherries (p = 0.1220 vs. Ho) but was –2.46 for 45 cherries (p = 0.0003 vs. Ho). The cherry doses had different impacts on Net TEAC. The mean Net AUC was 4.85 for 90 cherries (p < 0.0001 vs. Ho) and –0.89 for 45 cherries (p = 0.1955 vs. Ho).

### 4. Discussion

After eating, normal metabolic and oxidative processes produced reactive oxygen species. An imbalance between oxidative generation and antioxidant defense that favors oxidants can cause postprandial oxidative stress. For example, proxidants in tissues and circulation can contribute to adverse consequences like inflammation, poor vascular reactivity, endothelial dysfunction (Wautier, Boulanger, & Wautier, 2006), and lipid oxidation (Ceriello et al., 2002). From a mechanistic perspective, postprandial oxidative stress positively correlates with post-meal hyperglycemia/insulinemia and hyperlipidemia. The accumulated effects of postprandial oxidative stress can impact the pathogenesis of many chronic diseases impacted by diet and metabolism, such as diabetes and cardiovascular disease.

Expectedly, postprandial oxidative stress reduces serum antioxidant capacity following a meal. However, antioxidants, vitamins, minerals, and phytochemicals in foods and beverages can partially attenuate this oxidative stress, elevating serum antioxidant capacity toward pre-consumption antioxidant capacity – or even exceeding it. An excellent review by Burton-Freeman (2010) discussed the impact of fruit-derived phytochemicals on postprandial metabolic events as studied in clinical trials. The majority of studies showed reduced postprandial hyperglycemia, while there were mixed effects (either reduction or no effect) on insulin, triacylglycerides, and oxidized LDL. Some of these pharmacodynamic effects were statistically significant. Almost all studies that measured increased antioxidant capacity obtained statistical significance. However, all of the clinical trials reviewed were limited to 4–6 hours after intake of the food or beverage; it is unclear what a longer study window could reveal for pharmacodynamic effects. Given that postprandial oxidative stress is ubiquitous, antioxidant-rich meals provide a functional food opportunity for protection and intervention.

Antioxidant nutrients and phytochemicals in tart cherry must then overcome the predicted negative serum antioxidant capacity caused by postprandial oxidative stress. The subjects were not fasted during the 12 hour study, postprandial oxidative stress from other snacks and meals likely affected plasma TEAC. Also, subjects only consumed low phenol meals and snacks, and this could further contribute to a Negative TEAC balance over the course of the study. The 45 cherry Net AUC (Positive AUC – Negative AUC) was –0.89; the p value was not significantly different from 0 (p = 0.196). In other words, postprandial oxidative stress was largely negated by 45 cherries when considered over the 12 h period. However, the antioxidant capacity from 90 cherries was significant throughout the study period.

To our knowledge, this is the first demonstration of increased plasma antioxidant capacity following ingestion of whole Montmorency tart cherries. Many others have shown increased antioxidant capacity in humans following ingestion of anthocyanin-rich foods (Cao, Muccitelli, Sanchez-Moreno, & Prior, 2001; Carkeet, Clevidence, & Novotny, 2008; Felgines et al., 2003, 2005; Hollands, Brett, Dainty, Teucher, & Kroon, 2008; Kay, Mazzu, Holub, & Wang, 2004; McGhie, Ainge, Barnett, Cooney, & Jensen, 2003; Mertens-Talcott et al., 2008; Wu, Cao, & Prior, 2002). However, few studies have examined this effect
beyond 6 hours, likely due to the operational challenges of controlled feeding trials. Studies generally show a peak in antioxidant capacity followed by a steady decline to baseline (or below baseline) within 4–6 hours of consumption. One study with Bing sweet cherries (acute dosing of 45 tart cherries as used here) measured serial changes in both hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) over 5 hours after consumption (Prior et al., 2007). Interestingly, serum hydrophilic ORAC was reduced versus baseline ORAC during the entire 5 hour period, while the lipophilic ORAC was elevated over baseline for that same time period. The current 12-hour design allowed measurement of a pharmacodynamic effect beyond 5 hours, which was critical for revealing the increasing antioxidant effects 8–12 hours after initial ingestion at both dose levels. This delayed increase in antioxidant capacity could reflect the production of phytochemical metabolites by gut flora and subsequent re-absorption into systemic circulation.

The extended pharmacokinetics of single anthocyanins has been explored previously, and this is worth discussing as compared to the current approach and findings. de Ferrars (de Ferrars et al., 2014) recently demonstrated the pharmacokinetics of 500 mg of radiolabeled anthocyanin cyanidin-3-glucoside (C3G). The serum T\text{max} of C3G (1.8 h) was similar to that achieved here with cyanidin-3-glucosyl rutinoside (C3GR) from 45 cherries. Interestingly, serum C3G was back to baseline at hour 6, faster than what we observed here with C3GR. The urine anthocyanins and metabolites were many, including significant levels of C3G, methyl cyanidin glucuronide, protocatechuic acid (PCA) and PCA-derived metabolites, hippuric acid, ferulic acid, and vanillic acid. Importantly, this study was carried out over 48 hours, which allowed the appearance and quantification of many secondary metabolites. From a single dosing of anthocyanin, plasma levels of ferulic and hippuric acid were still elevated above baseline 48 hours after C3G ingestion. This work contributes to our understanding of C3G metabolism for further hypothesis testing for possible health benefits.

Some important distinctions exist between the C3G study and the present study. First, the present study used a whole food model, which is affected by the matrix effect of food and by other flavonoids that impact both the metabolism and uptake of other flavonoids. Second, the present study coupled pharmacokinetic and a pharmacodynamic assessment (TEAC) that may reflect a beneficial biologic effect. Third, the present study had physiologically relevant amounts of total anthocyanins. Our total anthocyanin dosing at either cherry dose was more than an order of magnitude lower than that of de Ferrars’. Despite this difference, the more physiologically relevant doses provided here still caused dose-dependent changes in a pharmacodynamic parameter of biologic interest.

This study has certain limitations that should be considered. While the ACN were conclusively identified in plasma, the circulating concentrations were too low from this whole foods model to be reliably quantified by mass spectrometry using standard curve-based interpolation. This approach is in contrast to studies using high levels of pure anthocyanin to enable quantification of the parent ACN and metabolite compounds in plasma, urine, and feces. However, we see these experimental different approaches as complimentary for advancing our understanding of anthocyanin metabolism, physiologic effects, and health relevance of anthocyanin-rich foods.

Another limitation was the lack of data on the ACN content of pre-dosing urine samples (when subjects voided their bladder immediately before consuming the cherries) due to an experimental oversight. However, their 0–2 h urine ACN was not significantly different between groups; this suggests that the population was relatively homogenous for urine ACN at baseline. The low phenol run-in diet may have assisted in reducing heterogeneity. However, inter-subject variance in urine ACN metabolites was larger beyond hour 6, indicating that multiple factors may influence ACN absorption and excretion as expected from previous studies in other ACN-rich foods.

Finally, TEAC is based on the scavenging ability of antioxidants to the radical anion ABTS+. In this assay, ABTS is oxidized by a prooxidant to its radical cation. The ABTS radical is not found in mammals. Thermodynamically, a compound can reduce the ABTS radical if it has a redox potential lower than that of ABTS (0.68 V). Many phenolic compounds have low redox potentials and can thus react with the ABTS radical and interfere with the interpretation of results (Prior, Wu, & Schaich, 2005). We recently profiled tart cherry products, including frozen tart cherry, for antioxidant capacity as measured by the oxygen radical absorbance capacity (ORAC) and related assays targeted for other pro-oxidants – the HORAC, SORAC, and NORAC assays for detecting hydroxyl radical, superoxide, and peroxynitrite radicals, respectively (Ou et al., 2012). Further clinical studies could use these other antioxidant assays to further elucidate tart cherry pharmacodynamics against diverse radicals, including hydrophilic versus lipophilic antioxidant assays. In addition, we recognize that other phytochemicals from tart cherry and their metabolites could affect observed TEAC values, but this study is not designed to confirm causation between anthocyanin PK and antioxidant PD. In fact, the plasma levels of C3G and P3G achieved are somewhat similar between the 45 and 90 cherry dose, though the trend does favor the higher dose. As such, other anthocyanin metabolites or those of other tart cherry phytochemicals are likely contributing to the observed TEAC effects, though they are not conclusively measured here.

In conclusion, these data are the first to support the plasma presence and urine excretion of tart cherry anthocyanins from whole tart cherries, and to show dose-dependent changes in antioxidant capacity over a 12-hour period after ingestion. Results revealed a time-course of anthocyanin and metabolite absorption and excretion that could inform new in vitro studies with isolated compounds and clinical studies with anthocyanin-rich foods. These foods and their related commercial products like juices and concentrates are being increasingly marketed to the public for their potential health benefits as functional foods or nutraceuticals. As such, further hypothesis testing and mechanistic studies are warranted.

Conflict of interest

None declared.
Acknowledgments

We would like to thank: Nidhi Talwar, MA for assistance with statistical analysis and tables; Sara Al-Rawai, ND, MPH for IRB submissions, subject recruitment, and data collection; and Alyssa Northrop, MPH for assistance with literature review. In addition, we thank the staff of the Michigan Clinical Research Unit Bionutrition Core for their assistance in the dietary assessment, the feeding trials, and the sampling procedures. Support for this study was provided by an unrestricted grant from the Cherry Research Committee of Cherry Marketing Institute, Lansing, MI, USA and by the University of Michigan Clinical Research Unit of the Michigan Institute for Clinical and Health Research (M01-RR000042 and UL1TR000433 from the United States National Institutes of Health).

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2014.08.007.

REFERENCES


