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Flavonoids and phenolic acids from cranberry juice are bioavailable and bioactive in healthy older adults

Diane L. McKay *[,1], C.-Y. Oliver Chen [1], Carly A. Zampariello, Jeffrey B. Blumberg

Antioxidants Research Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA

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ABSTRACT

Cranberries (*Vaccinium macrocarpon*) are a rich source of phenolic phytochemicals, which likely contribute to their putative health benefits. A single-dose pharmacokinetic trial was conducted in 10 healthy adults ⩾50 y to evaluate the acute (24-h) absorption and excretion of flavonoids, phenolic acids and proanthocyanidins (PACs) from a low-calorie cranberry juice cocktail (54% juice). Inter-individual variability was observed in the C_{max} and T_{max} of many of these compounds in both plasma and urine. The sum total concentration of phenolics detected in plasma reached a peak of 34.2 μg/ml between 8 and 10 h, while in urine this peak was 269.8 μg/mg creatinine, and appeared 2–4 h earlier. The presence of PAC-A2 dimers in human urine has not previously been reported. After cranberry juice consumption, plasma total antioxidant capacity assessed using ORAC and TAP assays correlated with individual metabolites. Our results show phenolic compounds in cranberry juice are bioavailable and exert antioxidant actions in healthy older adults.

1. Introduction

Observational studies have consistently shown the consumption of fruits and vegetables is inversely associated with the risk of developing chronic diseases, such as cardiovascular diseases (CVD), certain forms of cancer, and neurodegenerative diseases. The phytochemical constituents of these foods appear to contribute substantially to this benefit (Arts & Hollman, 2005). Phenolic phytochemicals have a wide range of biological actions, including the ability to act as antioxidants, ameliorate inflammation, modulate enzyme activity, and regulate gene expression.

Abbreviations: AUC, area under the curve; BMI, body mass index; BODIPY, 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-/s/-indacene-3-undecanoic acid; BP, blood pressure; CHD, coronary heart disease; CJC, cranberry juice cocktail; C_{max}, maximum concentration; CVD, cardiovascular diseases; FRAP, ferric reducing antioxidant power; HNRCA, Human Nutrition Research Center on Aging; LDL oxidation, susceptibility of LDL to Cu^{2+}-induced lipid oxidation; MeOH, methanol; ORAC, oxygen radical absorbance capacity; PAC, proanthocyanidin; TAP, total antioxidant performance; TE, Trolox equivalents; T_{max}, time to maximum concentration.

* Corresponding author. Tel.: +1 781 608 7183; fax: +1 617 556 3344.
E-mail address: diane.mckay@tufts.edu (D.L. McKay).

[1] Drs. McKay and Chen contributed equally to this article.

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Among the 20 most commonly consumed fruits in the American diet, cranberries appear to have the highest total phenol content (Vinson, Su, Zubik, & Bose, 2001). Cranberries (*Vaccinium macrocarpon*) are a rich source of phenolic compounds, particularly phenolic acids (including benzoic, hydroxycinnamic and ellagic acids) and flavonoids (including flavonols, flavan-3-ols, anthocyanins, and proanthocyanidins [PACs]). These phenolics appear responsible for the putative health benefits of cranberry consumption, such as the prevention of urinary tract infections (Howell et al., 2010) and stomach ulcers (Zhang et al., 2005), as well as improved oral health (Weiss et al., 2004). These actions may result from the ability of cranberry phenolics to interfere with the adhesion of some bacteria to select cell types and surfaces. Cranberry polyphenols may also contribute to reducing the risk of CVD (Ruel et al., 2008) and improving glucoregulation in diabetics at high risk for CVD (Wilson, Meyers, Singh, Limburg, & Vorsa, 2008).

Cranberry products and cranberry phenolics have also been shown to possess antibacterial (Caillet, Cote, Sylvain, & Lacroix, 2012), anti-mutagenic (Vattem, Jang, Levin, & Shetty, 2006), anti-carcinogenic (Vu et al., 2012), anti-angiogenic (Roy et al., 2002), and antioxidant activities (Caillet, Lorenzo, Cote, Sylvain, & Lacroix, 2012); however, most of this evidence is derived from *in vitro* studies and animal models. More information is required on the bioavailability and metabolism of cranberry bioactives in humans to better understand their impact on pertinent risk factors for chronic diseases and to inform the design of future clinical trials (McKay & Blumberg 2007).

Previously, we characterised the pharmacokinetics of anthocyanins from cranberry juice in patients with coronary heart disease (CHD), and found most appeared in plasma at nM concentrations within 1–2 h of consumption (Milbury, Vita, & Blumberg, 2010). We have expanded on this work by examining the bioavailability of a broader array of phenolics following cranberry juice consumption, as well as their bioactivity, in a small human study. Healthy older adults were selected as subjects for this study as we anticipate future trials of the putative benefits of cranberry juice on risk factors for chronic age-related diseases, including CVD, type 2 diabetes, and cancer will be conducted in this age group.

2. Materials and methods

2.1. Participants

Ten healthy, nonsmoking men and postmenopausal women age 50–70 y with a body mass index (BMI) of 18.5–29.9 kg/m^2 were recruited from the Boston area. Postmenopausal status in women was defined as the absence of menses for $\geqslant$1 y. The exclusion criteria used to screen for their eligibility included: presence of cardiovascular, endocrine, gastrointestinal, and renal diseases; use of estrogen, with or without progesterone; use of medications known to affect lipid metabolism; use of medications known or suspected to influence blood pressure (BP); gastrointestinal diseases and conditions or medications influencing gastrointestinal absorption; chronic kidney disease; endocrine disorders including diabetes and untreated thyroid disease; rheumatologic disorders; active treatment for cancer of any type (except basal cell carcinoma) $\geqslant$1 y; regular use of oral steroids; systolic blood pressure >150 mmHg and/or diastolic blood pressure >95 mmHg; regular use of any dietary supplements within previous 30 d; usual daily ethanol intake of $\geqslant$2 drinks; cigarette smoking and/or nicotine replacement use; laboratory blood or urine biochemistries outside of normal ranges; EKG abnormalities. The study design was approved by the Institutional Review Board of Tufts University Health Sciences Campus and Tufts Medical Center. All participants signed a written informed consent agreement before participating. This study was registered with the public registry ClinicalTrials.gov (ID # NCT00740077).

2.2. Study design

A single-dose pharmacokinetic trial was conducted to evaluate the acute (24-h) bioavailability of flavonoids and phenolic acids from 237 ml of a double-strength (54% juice) low-calorie, low-sugar cranberry juice cocktail (CJC). The CJC was specially formulated and produced in a single batch by Ocean Spray. Qualified subjects were asked to consume foods low in phenols and polyphenols for 48 h prior to the CJC intervention. Examples of the restricted foods include certain fruits, berries, vegetables, juices, nuts, tea, herbal tea, coffee, cocoa, chocolate and wine. The purpose of these dietary restrictions was to reduce any residual dietary phenolic compounds from the body (these compounds are typically cleared from blood and urine within 48 h of consumption) and to ensure phenolic compounds present in the blood and urine samples collected during the intervention were derived from the CJC alone. Prior to the day of the CJC intervention, subjects reported to the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University for an overnight stay no later than 7:00 PM in the evening. All subjects consumed the same low phenolics meal prepared in the HNRCA Metabolic Research Unit (MRU) kitchen, and refrained from consuming any food or beverages, except for water, for the next 12 h. Prior to administering the CJC the following morning, fasting blood and urine (first morning void) samples were collected for baseline measurements. A single dose of CJC was then administered orally under close observation by the MRU staff. No other food or beverages were provided at this time. Following administration of the CJC, blood samples were collected via indwelling catheter at 0.25, 0.5, 1–6, and 10 h. Urine was collected every 2 h for the next 10 h. Lunch and dinner meals, prepared by the kitchen, were low in phenols and polyphenols and provided 5 h and 10 h post-administration. At the end of 10 h, subjects were allowed to leave the HNRCA with 24 h urine collection materials. Subjects returned to the HNRCA the following morning after having fasted for 12 h. Fasting blood and urine (excreted since the 10 h intervention) were collected within 24 h of administering the CJC. Vital signs, including blood pressure, temperature, pulse and respiration rate, were monitored regularly following CJC consumption.

2.3. Sample preparation

Collected blood samples were assessed for phenolic acids, flavonoids (including flavonols, flavanols, anthocyanins, PAC), and selected measures of total antioxidant capacity – Oxygen Radical Absorbance Capacity (ORAC) with perchloric acid precipitation, Ferric Reducing Antioxidant Power (FRAP), Total Antioxidant Performance (TAP) – and susceptibility of LDL to Cu^{2+}-induced lipid oxidation (LDL oxidation). Blood samples for the analysis of phenolic acids, flavonoids, FRAP, TAP, and LDL oxidation were collected in EDTA-containing evacuated tubes and centrifuged within 15 min of drawing (1000×*g*, 15 min, 4 °C) with a SUR-Sep cap (Organon Teknika, Durham, NC). Blood samples for the ORAC were collected in heparin tubes, and processed similarly. Plasma samples for anthocyanin analysis were prepared by adding 30 μl of 12 mol/l HCl to 1.5 ml of plasma followed by centrifugation (1000×*g*, 15 min, 4 °C) and aliquoted immediately into 2 ml NUNC tubes (Vanguard Cryotubes, Neptune, NJ). Plasma samples for the analysis of LDL oxidation were prepared by adding 111 μl of 6% sucrose solution to 1 ml plasma, and stored at −80 °C for no longer than 8 wk before analyzing. Urine samples for anthocyanin analysis were prepared by adding 0.2 ml of 12 mol/l HCl to 50 ml aliquots. All samples were stored at −80 °C until analysis. All samples for