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A pilot sweet cherry feeding study in overweight men: Tolerance, safety, and anthocyanin exposure

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ABSTRACT

Sweet cherries are rich in bioactive anthocyanins (ACN) and thus are considered a functional food. Yet, tolerance to and bioavailability of a significant daily dose of cherries has not been evaluated in humans. This pilot study aimed to assess compliance, tolerance, safety, and change in circulating/excreted ACNs due to daily consumption of sweet cherries. Change in bowel habits, quality of life, urinary/circulating concentration ACNs, and inflammatory biomarkers were measured in overweight, older men (n = 37) before and after daily consumption of three cups of sweet cherries for 4 weeks. Cherry fruit ACN content was also measured. Tolerance to intervention was high in this study. An increase in several plasma/urine ACNs was observed, but did not correlate with the amount of ACN measured in the fruits. No significant changes in inflammatory biomarkers were observed. This intervention was feasible and increased anthocyanin exposure in overweight men.

1. Introduction

Sweet cherries (*Prunus avium L.*) are commonly consumed in the U.S. and contain several bioactive compounds, including polyphenols (McCune, Kubota, Stendell-Hollis, & Thomson, 2011). As such, sweet cherries are considered a functional food with the potential to improve human health by modifying inflammation and oxidative stress (Kelley et al., 2013; Kelley, Rasooly, Jacob, Kader, & Mackey, 2006).

Anthocyanins (ACNs) are the major class of polyphenols in cherries (Kelley et al., 2013; Tapiero, Tew, Ba, & Mathe, 2002). Cyanidin is the major ACN found in cherries (Ferretti, Bacchetti, Belleggia, & Neri, 2010). The primary cyanidin found in sweet

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cherries is cyanidin-3-rutinoside (C3RUT), followed by cyanidin-3-glucoside (C3GLU), with low levels of other ACNs also present (Kim, Heo, Kim, Yang, & Lee, 2005; Saric et al., 2009). Additionally, protocatechuic acid (PCA) is a common metabolite of cyanidin in humans (Vitaglione et al., 2007).

Sweet cherries and their ACNs are being investigated for use in cancer, cardiovascular disease, and other inflammation-related diseases. In animal models, cherry bioactives reduce biomarkers of inflammation, including those mediated by cyclooxygenase (COX)-2 (He et al., 2006; Hou, Yanagita, Uto, Masuzaki, & Fujii, 2005; Piccolella et al., 2008; Tall et al., 2004). A few small, human studies suggest that cherry consumption may modify inflammatory biomarkers, such as C-reactive protein (CRP), in healthy adults (Connolly, McHugh, Padilla-Zakour, Carlson, & Sayers, 2006; Kelley et al., 2006, 2013). On the other hand, rats administered a single oral dose of 100 mg of ACN have been reported to experience a near-immediate rise in homocysteine levels (Nakagawa, Maruyama, & Miyazawa, 2002), indicating that over-exposure to ACNs is potentially problematic. Taken together, studies are limited and data are insufficient to evaluate the safety and efficacy of regular, higher-dose intake of sweet cherries and other ACN rich functional foods in humans.

Our primary aim was to measure compliance, tolerance, and change in plasma/urine ACN levels in response to daily intake of three cups of fresh, sweet cherries for 4 weeks. Our secondary aim was hypothesis generating and was to determine if there was any evidence of a change in biomarkers of inflammation in response to ACN dose. When designing human feeding studies with whole foods it is imperative to evaluate a reasonable dose for regular intake as well as a dose that is likely to have biological benefit. This is especially true as ACNs may have a dose-dependent effect on metabolism (Carkeet, Clevidence, & Novotny, 2008). Since humans consume whole foods and there is some argument that the synergy of bioactives in whole foods may outweigh benefits of any single bioactive compound, we sought to test whole cherry feeding. It is well-recognized that the concentrations of ACN will vary in relation to storage conditions and ripening of the cherry, as well as cultivar selected (Goncalves et al., 2004) and so we also measured ACN content in the cherries provided to our study participants in order to control for variable ACN levels. To our knowledge, this study provides the first preliminary data on a commonly consumed function food's tolerance, safety and ability to increase ACN exposure in humans that will inform future studies on this fruit.

2. Subjects and methods

2.1. Participant eligibility and study design

This feeding trial was conducted in relatively healthy older, overweight/obese men because this population exhibits mild basal inflammation (Kantor, Lampe, Kratz, & White, 2013) as compared to the normal-weight population. Respondents (n = 105) to print, television, and electronic advertising were screened via telephone for eligibility (Fig. 1). Inclusion criteria were men age >50 years, body mass index (BMI) 25–45 kg/m^2, abstinence from tobacco products, willingness to discontinue dietary supplements with the exception of a multivitamin for 1 week prior to enrollment and during the trial, willingness to discontinue anti-inflammatory medication with the exception of low-dose aspirin, absence of co-morbidities, and cancer-free >12 months. The most common exclusion criterion was a history of major chronic illness (n = 12). Written informed consent was obtained from all study participants prior to enrollment (n = 39). Of these, 37 successfully completed the washout period. The University of Arizona Institutional Review Board approved this study protocol. The study was conducted between May and August of 2011.

Upon consent, participants began a 1-week washout where they were instructed to avoid foods rich in ACNs and limit consumption of fruits and vegetables to ≤5 servings/day (Fig. 1). Next, participants began the 4-week intervention of consuming a one-cup (142 g) serving of sweet cherries three times per day, while still limiting intake of other fruits and vegetables to ≤5 servings/day. Subjects were advised to maintain regular body weight, diet, and physical activity levels throughout the study.

2.2. Compliance, tolerance, and safety

Participants returned the uneaten cherries, pits, and stems to the clinic weekly, where waste weight was subtracted from the weight of cherries provided to each participant weekly to estimate compliance to the intervention. To measure tolerance, participants completed a quality-of-life questionnaire (MOS 36-item short-form survey; SF-36) (Ware & Kosinski, 2001) at baseline and end-of-study, and bowel habits were recorded throughout the intervention using a previously validated daily bowel habits questionnaire (BHQ) (Bassotti et al., 2004). To measure safety, serum levels of homocysteine were measured at baseline and end-of-study. Samples were processed at Lab Corp (Tucson, AZ, USA), which defined an abnormal value as >15 μmol/L.

2.3. Anthropometrics

Anthropometry was performed at baseline and end-of-study by a registered dietitian. Waist and hip circumference were measured using previously published methods (Lohman, Roche, & Martorell, 1988). Body fat was estimated using a handheld Omron Body Fat Analyzer HBF-306 (Omron Healthcare, Inc., Vernon Hills, IL, USA). Resting blood pressure was measured in duplicate by an automatic blood pressure monitor by ReliOn HEM-780REL (Omron, Inc., Bannockburn, IL). The average of two systolic and diastolic blood pressure (mmHg) and heart rate (beats/min) measurements were recorded.

2.4. Dietary intake analysis

Dietary intake was assessed using the previously validated Arizona food frequency questionnaire (AFFQ) (Thomson et al., 2003) at baseline (estimated dietary intake in the past 3 months) and end-of-study (estimated dietary intake during the 4-week intervention). Metabolize (the proprietary dietary data reduction system developed at the University of Arizona, Tucson, AZ, USA) was used to determine average daily food/nutrient intake.