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Montmorency tart cherry (*Prunus cerasus L.*) concentrate lowers uric acid, independent of plasma cyanidin-3-O-glucosiderutinoside

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ABSTRACT

Two doses (30 and 60 mL) of Montmorency tart cherry concentrate (MC) were used to investigate their impact on physiological indices of uric acid activity, inflammation and the bioavailability of the major anthocyanin (CYA-3-O-GluRut). Following MC supplementation plasma CYA-3-O-GluRut increased ($P < 0.05$), with a greater uptake in the 60 mL dose found at 1 h. Serum urate was decreased ($P < 0.001$) with a peak change (% of baseline) of 178 $\mu mol \cdot L^{-1}$ (36%) at 8 h; urinary urate excretion ($P < 0.05$) was increased, peaking at 2 h (178 $\mu Mol \cdot mMol\ creatinine^{-1}$ [250%]). Serum hsCRP ($P < 0.001$) was decreased with peak decrements of 3.19 $mg \cdot L^{-1}$ (29%).

These data show that MC impacts upon the activity of uric acid and lowers hsCRP, previously proposed to be useful in managing pathological conditions such as gouty arthritis; the findings suggest that changes in the observed variables are independent of the dose provided.

1. Introduction

In recent years, nutritional research has focussed more on the use of foods that might provide benefits for improving human health (Bitsch, Netzel, Frank, Strass, & Bitsch, 2004; Blando, Gerardi, & Nicoletti, 2004; Charron, Clevidence, Britz, & Novotny, 2007; Hollands, Brett, Dainty, Teucher, & Kroon, 2008; Kay, Mazza, Holub, & Wang, 2004; Wallace, 2011). In particular, there is a growing interest in fruits and vegetables high in phytochemical compounds that have been implicated as an alternative or additive to pharmaceutical medications (Wallace, 2011). Particular attention has been placed upon foods containing high concentrations of anthocyanins, which are glycosides of the flavonoid sub-group anthocyanidins (Hollands et al., 2008). Flavonoids are known to have a high antioxidant activity and the ability to scavenge reactive oxygen and nitrogen species (RONS); more specifically, anthocyanins possess strong anti-inflammatory and antioxidant properties (Blando et al., 2004; Hollands et al., 2008; Wang et al., 1999). Anthocyanins have also been shown to inhibit cancer cell growth (Kamei et al., 1995; Kang, Seeram, Nair, & Bourquin, 2003), reduce airway

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inflammation (Park, Shin, Seo, & Kim, 2007; Rossi et al., 2003) and to exhibit antiplatelet (Rechner & Kroner, 2005) and vasomodulatory activity (Bell & Gochenaur, 2006).

Many foods containing anthocyanins, such as red grape juice, red wine (Bitsch et al., 2004), purple carrots (Charron et al., 2009), red cabbage (Charron et al., 2007), berries (Del Rio, Borges, & Crozier, 2010), strawberries (Felgines et al., 2003), blueberries (Norberto et al., 2013) sour cherries (Blando et al., 2004), Bing sweet cherries (Jacob et al., 2003; Kelley et al., 2013; Kelley, Rasooly, Jacob, Kader, & Mackey, 2006) and tart cherry juice concentrate (Schlesinger, Rabinowitz, & Schlesinger, 2012), have received attention regarding the biokinetic properties and possible impact on human health. Recently, a growing emphasis has been placed on cherries from a range of cultivars due to the high concentration of anthocyanins in comparison to other functional foods; in particular Montmorency tart cherries are high in anthocyanin content and compare favourably with other varieties of cherries and fruits (Bell, McHugh, Stevenson, & Howatson, 2013; McCune, Kubota, Stendell-Hollis, & Thomson, 2011). Direct comparisons are difficult to ascertain given that studies have focussed on different supplementation mediums such as whole fruit, fresh pressed juice blends or concentrates; however per serving, Montmorency cherries show good efficacy across a number of experimental paradigms. Specifically, Montmorency cherries have been shown to reduce exercise-induced pain (Connolly, McHugh, & Padilla-Zakour, 2006; Kuehl, Perrier, Elliot, & Chesnutt, 2010), inflammation (Howatson et al., 2010), oxidative stress (Howatson et al., 2010; Traustadottir et al., 2009) and aid in the recovery of human muscle function (Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Connolly et al., 2006; Howatson et al., 2010). Given the evidence for Montmorency tart cherries to reduce inflammation and oxidative stress, it seems plausible they could be a suitable intervention for inflammatory conditions (Bell et al., 2013). Reports from clinical populations have indicated a reduced incidence of gout attacks following regular consumption of tart cherry juice concentrate across a 4 month period (Schlesinger et al., 2012) and in epidemiological work, patients' self-reported cherry consumption was associated with a 35% reduction in gout attacks (Zhang et al., 2012). Additionally, gout flare-ups are associated with elevated systemic uric acid levels (Moriwaki et al., 2011); however, decreased plasma urate, increased urinary urate and a trend towards lowered C-reactive protein have been acutely demonstrated following consumption of 280 g of Bing cherries in humans (Jacob et al., 2003). The mechanism responsible for the uric acid and inflammatory-lowering effects of cherries is not clear, however anthocyanins might play a major role (Jacob et al., 2003; Zhang et al., 2012).

Given (1) the reported high concentrations of phytochemicals in Montmorency tart cherries (Seeram, Momin, Nair, & Bourquin, 2001; Wang et al., 1999); (2) previous evidence showing a reduction in plasma urate following consumption of Bing sweet cherries (Jacob et al., 2003); and (3) the reported lower incidence of gout flare-ups with tart cherry consumption (Schlesinger et al., 2012; Zhang et al., 2012), the aim of this study was to examine the influence of Montmorency tart cherries on uric acid activity and inflammation. In this investigation, the pharmacokinetics of the principal anthocyanin cyanidin-3-O-glucosiderutinoside (CYA-3-O-GluRut), and its influence on urate, antioxidant status, high sensitivity C-reactive protein and urinary urate were investigated following two doses of Montmorency tart cherry concentrate. It was hypothesised that the consumption of Montmorency tart cherry concentrate would reduce serum uric acid and the magnitude of clearance and CYA-3-O-GluRut bioavailability would be associated with the dose.

2. Experimental methods

2.1. *Participants*

Twelve healthy participants (age: 26 ± 3 years, 11 male and 1 female) volunteered for the study. Prior to gaining written informed consent, all volunteers confirmed they were non-smokers, had no known food allergies and no history of gastrointestinal, renal or cardiovascular disease or use of food supplementations. Ethical approval was gained from the Faculty's Research Ethics Committee and the trial was registered with clinicaltrials.gov (NCT01825070).

2.2. *Study design*

The study utilised a single blind, two-phase, randomised, cross-over design in order to identify the bioavailability of anthocyanins following the ingestion of two different doses of Montmorency tart cherry juice concentrate (MC). A washout period of at least 10 days between phases was administered, and counterbalancing participants eliminated order effects. The dosages used were 30 or 60 mL of the MC supplement (CherryActive, Hanworth, UK) mixed with 100 mL of water. According to the manufacturer, a 30 mL dose was equivalent to approximately 90 whole Montmorency tart cherries; independent analysis provided compositional data as follows: fat – 0.028 mg·mL^{-1}, protein 31.47 mg·mL^{-1}, carbohydrate – 669.4 mg·mL^{-1}, cholesterol – <0.01 mg·mL^{-1}, sodium – 0.691 mg·mL^{-1}, calcium – mg·mL^{-1} and iron – 0.026 mg·mL^{-1}. The total anthocyanin content of the MC was previously reported as 9.117 mg·mL^{-1} (Howatson et al., 2012) – specifically, contributions from six parent anthocyanins were stated as: cyanidin – 3.346 mg·mL^{-1}, malvidin – 4.696 mg·mL^{-1}, pelargonidin – 0.428 mg·mL^{-1}, peonidin – 0.221 mg·mL^{-1}, delphinidin – 0.169 mg·mL^{-1}, petunidin – 0.257 mg·mL^{-1}.

Participants were requested to follow a low-polyphenolic diet by avoiding fruits, vegetables, tea, coffee, alcohol, chocolate, cereals, wholemeal bread, grains and spices for 48 h prior to, and throughout each arm of the trial. Food diaries were completed for 48 h before and throughout the testing phase in order to assess the diet for compliance (Howatson et al., 2012). Participants were required to attend the start of each phase of the study at 9 am following a 10 h overnight fast in order to account for diurnal variation. Each phase was comprised of 2 days supplementation with MC; one supplement was taken immediately following the morning blood and urine sample and a second consumed prior to each evening meal. Multiple-supplementation was administered in order to identify any cumulative effects on the dependent variables. The length of the supplementation phase was chosen due to the short period of time in which anthocyanins are metabolised (Bitsch et al., 2004; Kurilich, Clevidence, Britz, Simon, & Novotny, 2005).

Venous blood samples (~27 mL) were collected from the antecubital fossa into sodium heparin (2 × 6 mL), ethylenediaminetetraacetic acid (EDTA) (10 mL) and serum separator (5 mL) tubes, immediately before supplementation and, 1, 2, 3, 5, 8, 24, 26, and 48 h following ingestion of the supplement. Samples were immediately centrifuged (2400 × *g*) at 4 °C for 15 min and the supernatant was aspirated in to aliquots. Aliquots assigned for anthocyanin analysis were treated with 30 μL of 50% aqueous formic acid and 100 μL of ascorbic acid (10 mMol) prior to 1 mL of plasma being added from the heparinised tubes and then stored at –80 °C. Following each blood sample, urine was collected using a sterilised measuring cylinder; a 10 mL aliquot of the urine sample was immediately stored at –80 °C for analysis of urinary urate.

2.3. *Processing and analysis of blood*

2.3.1. *High sensitivity C-reactive protein and urate (uric acid)*

High sensitivity C-reactive protein (hsCRP), urea and uric acid were determined using a clinical chemistry analyser (Advia 2400, Siemens Healthcare Diagnostics, Frimley, UK). Briefly, serum hsCRP was determined by latex enhanced immunoturbidimetry. CRP containing serum was added to a uniform polystyrene latex particle reagent, coated with anti-CRP antibody. Agglutination occurs between the antibody-coated particle and CRP containing serum, creating an increase in turbidity, which was measured at 571 nm. The total assay coefficient of variation (CV) was 1.6–6.6% in the range 0.17–10.65 mg·L^{-1}. The limit of detection (LoD) was 0.05 mg·L^{-1} and limit of quantification (LoQ) was 0.16 mg·L^{-1}. The assay range was 0.16–10 mg.L^{-1}. Where appropriate, samples with CRP higher than 10 mg·L^{-1} were diluted with saline and re-run.

Determination of serum and urine uric acid (expressed relative to creatinine) was based on a previous method (Fossati, Prencipe, & Berti, 1980) using uricase and a Trinder-like endpoint. Uric acid was converted to allantoin and H_2O_2 by uricase. A coloured complex was formed from H_2O_2, 4-aminophenazone and TOOS (*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline) under the catalytic influence of peroxidase. The absorbance of the coloured complex was measured as an endpoint reaction at 545/694 nm. The method was traceable to the CDC reference method utilising reference materials from NIST. The total assay CV was 1.1–3.2% in the range 0.25–0.54 mMol·L^{-1} for serum and 3.1–3.3% in the range 0.72–1.30 mMol·L^{-1} for urine. The LoD was 0.03 mMol·L^{-1} and had a linear range up to 1.19 mMol·L^{-1} for serum and a range of 0.06–10.71 mMol.L^{-1} for urine.

2.3.2. *Lipid soluble antioxidants*

Lipid soluble antioxidants (LSA) were quantified in plasma due to the ability of antioxidants to synergistically recycle each other and to control for the possible interaction between the ingested hydrophilic antioxidants in MC and endogenous lipophilic antioxidants (Niki, Noguchi, Tsuchihashi, & Gotoh, 1995). LSA were determined by high performance liquid chromatography (HPLC), according to the combined methods of Catignani and Bieri (1983) and Thurnham, Smith, and Flora (1988) simultaneously. Ultraviolet detection, following sample extraction into heptane, was used to quantify concentrations of retinol, lycopene, α-tocopherol, γ-tocopherol, α-carotene and β-carotene. Briefly, 200 μL of plasma and 200 μL of α-tocopherol acetate were mixed as an internal standard, whilst ethanol precipitated the sample protein; heptane (Analar or 99% – 700 mL – Labscan, Gliwice, Poland) containing 0.5 g·L^{-1} butylated hydroxytoluene was added to the mix prior to being vortexed vigorously for 1 min. The tube was then centrifuged (2400 × *g*) for 10 min before transferring 500 μL of a heptane supernatant layer to an identical glass tube. The complete contents of the standard tubes were evaporated using a rotary evaporator (Howe, Gyrovap, UK) under vacuum (Edwards – stage 2 pump) for 2 h. Following reconstitution in 100 μL ethanol, samples were vortexed and subsequently assayed using HPLC ultra-violet detection. Analysis by HPLC utilised a Hypersil ODS Alltech 250 mm × 4.6 mm × 5 μM column during the mobile phase. The mobile phase consisted of 45% methanol:45% acetonitrile:10% dichloromethane (200 mL, not filtered, degassed for exactly 5 min using Romil HPLC grade solvents, Analab, Dromore, N.I.) at a flow rate of 1.8 mL·min^{-1}. Samples were analysed at wavelengths of 325, 292 and 450 nm to detect the retinol, tocopherol and carotenoid peaks, respectively. The concentration of retinol, lycopene, α-tocopherol, γ-tocopherol, α-carotene and β-carotene in each sample was subsequently calculated. Intra- and inter-assay CV were <10%.

2.3.3. *Plasma anthocyanins*

The HPLC-photodiode array was used to identify plasma concentrations of anthocyanins (pre supplementation through to 8 h post supplementation). Briefly, 1 mL of plasma was mixed with 4 mL of 10 mM oxalic acid and 100 μL of 6 M HCl, vortexed and centrifuged (2400 × *g*) for 5 min. The supernatant was adsorbed onto a primed solid phase extraction cartridge (Waters Oasis® HLB 6 cc, 200 mg), washed with 2 × 5 mL of 10 mM oxalic acid followed by 2 mL of ethyl acetate. The anthocyanins were eluted with 3 mL of 0.1% trifluoroacetic acid in methanol, dried under N_2 at 30 °C, reconstituted in 200 μL of 50:50 (*v*/*v*) 0.1% formic acid:2% HCl in methanol and filtered through a 0.2 μm polytetrafluoroethylene (PTFE) filter prior to HPLC analysis. For quality assurance purposes, a blank control plasma sample was run with each analytical batch along with control plasma spiked at *ca*. 13 μg·L^{-1} (low) and *ca*. 132 μg·L^{-1} (high). Analysis by HPLC utilised a Phenomenex® Luna® C_{18} 250 mm × 2.0 mm × 5 μm column, maintained at 25 °C with a mobile phase comprising solvent A:1% formic acid in water and solvent B:1% formic acid in acetonitrile, run under gradient elution conditions at a flow rate of 0.2 mL·min^{-1}. The injection volume was 20 μL. Photodiode array (PDA) detection conditions were set at a monitoring wavelength of 520 nm × 20 nm bandwidth and the spectral wavelength range was 250–640 nm. The HPLC-PDA system was calibrated using cyanidin-3-O-glucoside chloride analytical standard (96%, Extrasynthese, Genay, France) and the anthocyanin content of plasma subsequently expressed in terms of cyanidin-3-O-glucoside (CYA-3-O-Glu).

2.4. *Montmorency tart cherry juice*

HPLC-PDA was used to identify concentrations of anthocyanins within MC. A 1% solution of MC was prepared (1:1 (*v*/*v*), 0.1% formic acid: 2% HCl in methanol mixed solvent, filtered (0.2 μm PTFE) and analysed by HPLC-PDA using a 150 × 4.6 mm × 5 μm

column (Agilent Zorbax Eclipse XDB-C_{18}, Aglient Technologies, Stockport, UK)) under the same conditions as plasma samples, except for the mobile phase flow rate, which was adjusted to 1 mL·min^{-1} and the injection volume which was 50 µL. To describe the major anthocyanins present, a sample was analysed by HPLC with time-of-flight (TOF) mass spectrometric (MS) detection employing Agilent MassHunter software (Ver. B.06.00) and under similar LC conditions as the MC by HPLC-PDA.

2.4.1. *Hydrolysis of MC to parent anthocyanidins*

In order to identify parent anthocyanidins, a 1% solution (20 mL) of MC was hydrolysed in 2 M HCl for 2 h at 90 °C in a sealed glass vial. The solution was filtered (0.2 µm PTFE), diluted with methanol (1:1 (*v*/*v*)) and analysed by the same HPLC-PDA as the MC. The anthocyanidins peaks were identified by comparison with standard anthocyanidins and relative retention time.

2.5. *Statistical analyses*

All data analyses were conducted using SPSS Statistics v.20 (IBM, Surrey, UK) and were reported as mean ± standard deviation. All samples were assessed in duplicate, with the mean value of each set of replicates used for analysis. Dependent variables were analysed by using a condition (30 *vs.* 60 mL) by time-point (baseline, 1, 2, 3, 5, 8, 24, 26 and 48 h) mixed model analysis of variance (ANOVA). Anthocyanin concentration was not measured at 24, 26 and 48 h because we were interested in the acute kinetics following consumption and therefore had three fewer levels. Based on the variability (Howatson et al., 2010) in our primary measure, uric acid, it was estimated that with a sample of 12 subjects there was 80% power to detect a 115 µL decrease (17%) at an alpha level of 0.05. Mauchley's test of sphericity was carried out to assess homogeneity of the data and where violations were present; corrections were made using Greenhouse–Geiser adjustment. Where necessary, interaction effects were examined using an LSD *post-hoc* analysis. Prior to all analyses, a significance level of $P < 0.05$ was set.

3. Results

Serum urate (Fig. 1A) showed significant time effects ($F_{(1,8)}$ =10.626, $P < 0.001$) with lower values at all time points after 2 h; the greatest mean change (% of baseline) of 178 µmol·L^{-1} (36%) was at 8 h post-supplement. Urinary urate increased over time ($F_{(1,8)}$ =16.262, $P < 0.001$) post-supplementation with the greatest change (178 µMol·mMol creatinine^{-1}; ~250%) at 2 h (Fig. 1B). Following the third dose at 24 h, there was also an increase in urinary urate of 102 µMol·mMol creatinine^{-1} (~165%) at 26 h. No group or interaction effects were found for serum or urinary urate.

Serum hsCRP decreased ($F_{(1,8)}$ =31.192, $P < 0.001$) from baseline across the initial 8 h period (Fig. 1C) following Montmorency cherry juice consumption; the largest decrements of 3.19 mg·L^{-1} (29%) at 5 h. There were no group or interaction effects observed. Serum hsCRP returned close to basal levels at 24 h, with

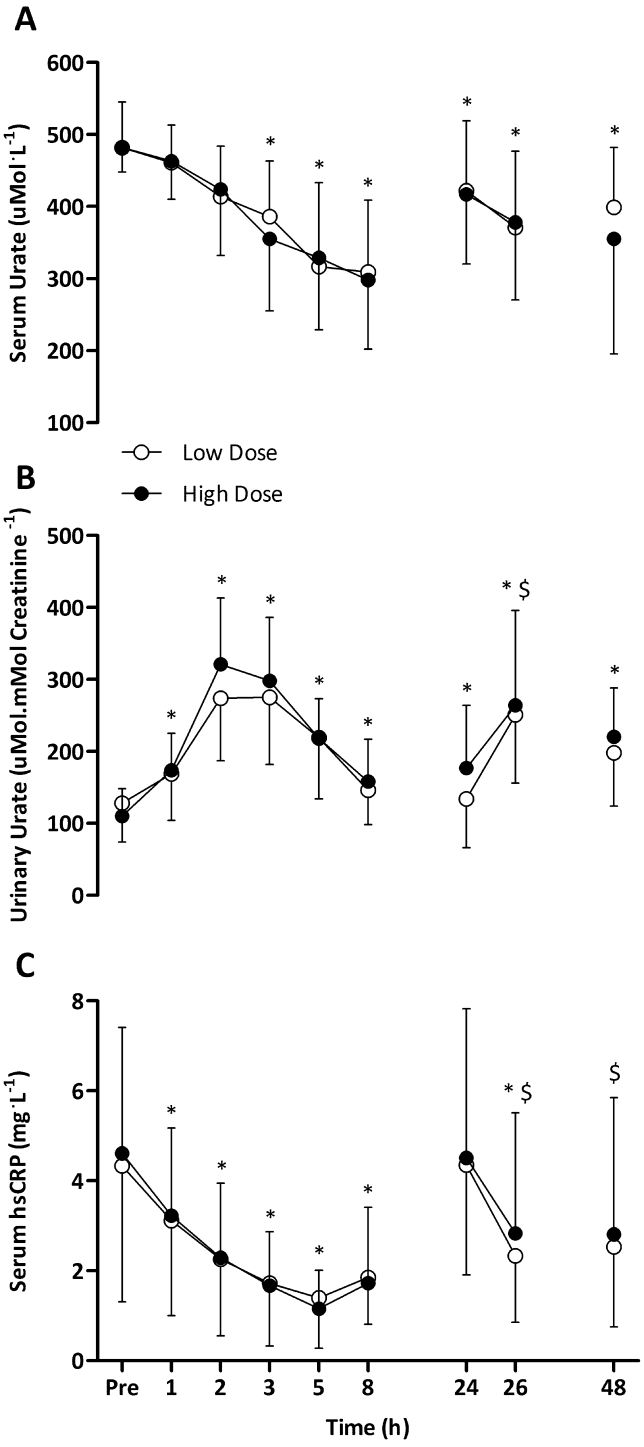

Fig. 1 – Indices of uric acid metabolism (serum urate – panel A; urinary urate – panel B) and inflammation (hsCRP – panel C) before and following the ingestion of Montmorency tart cherry concentrate. *Significantly decreased mean concentration ($P < 0.05$). Urinary urate (panel B) response to cherry juice consumption. * denotes significantly different from baseline and 24 h ($P < 0.05$). All values are expressed as mean ± SD.

a further drop of 1.89 mg·L^{-1} (42%) at 26 h following the third dose.

The main anthocyanin identified in the Montmorency tart cherry juice concentrate was CYA-3-O-glucosiderutinoside, which was confirmed by previous literature (Blando et al., 2004). Further peaks were identified as PEO-3-O-rutinoside and cyanidin-3-O-rutinside, respectively, but were below the limits of quantification. Using a surrogate standard (CYA-3-O-glucoside) and assuming equal response factors, the concentration of total anthocyanins with the juice was estimated at 60 mg·L^{-1}. Following hydrolysis, qualitative analysis identified three major parent anthocyanidins: cyanidin, peonidin and malvidin within the Montmorency tart cherry juice concentrate. In agreement with previous work analysing tart cherry phytochemical content (Ou, Bosak, Brickner, Iezzoni, & Seymour, 2012), cyanidin accounted for the overwhelming majority of anthocyanins with significantly smaller amounts of peonidin and malvidin; however, both peonidin and malvidin were beyond the limits of quantification. Other aforementioned (Howatson et al., 2012) anthocyanins that have been reported to be present in the Montmorency cherry concentrate (pelargonidin, delphinidin and petunidin) were not detected.

HPLC–TOF analysis by accurate mass and molecular formula generation showed the major anthocyanins to be CYA-3-O-glucosiderutinoside, CYA-3-O-rutinoside and PEO-3-O-rutinoside, which concurs with previously reported data (Blando et al., 2004). Total anthocyanins content of the plasma was expressed as CYA-3-O-Glu equivalents. There was no group difference (30 mL *vs*. 60 mL) in plasma CYA-3-O-Glu, although there was a significant time ($F_{(1,5)}$ =6.786, $P < 0.001$) and interaction ($F_{(1,5)}$ =3.164, $P < 0.05$) effect. Higher plasma CYA-3-O-Glu concentrations were found at 3 h ($P = 0.033$) and 5 h ($P = 0.044$) in comparison to baseline. At 1 h, the 60 mL group showed a higher ($P < 0.001$) increase of plasma CYA-3-O-Glu than 30 mL condition (Table 1).

Lipid soluble antioxidants provided a measure of plasma antioxidative capacity. No differences were found between conditions or time-points for α-tocopherol, γ-tocopherol, retinol and lycopene ($P > 0.05$). α-carotene and β-carotene were negligible for both doses at all time-points. Finally, although dietary analysis was not used to recall macro and micronutrient intake, participants reported a 100% adherence rate with regards to dietary restrictions. Two cases of acute, but mild gastrointestinal discomfort were reported in the 60 mL condition.

4. Discussion

It was hypothesised that Montmorency tart cherry concentrate would reduce serum uric acid and inflammation in a dose-dependent manner. The primary anthocyanin (CYA-3-O-GluRut) detected in the concentrate was greater at 1 h in blood plasma following the higher dose of the concentrate. In support of our hypothesis, serum urate and hsCRP were reduced and urinary urate was increased following both doses of MC; however, the magnitude of the change was independent of the dose given.

HPLC analysis was conducted to give an accurate representation of MC content from the batch used in the current

Table 1 – Absolute values of plasma biomarkers prior to and following Montmorency tart cherry juice consumption; values are reported as mean ± SD.

	Pre		1 h		2 h		3 h		5 h		8 h		24 h		26 h		48 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CYA-3-O-Glu (nMol·L^{-1})*																		
30 mL	0.02	0.06	0.1	0.12↑	0.07	0.11	0.13	0.17	0.11	0.13	0.05	0.09	NA		NA		NA	
60 mL	0.00	0.00	2.84	5.35↑	1.37	2.31	0.89	1.36	0.43	0.73	0.23	0.39	NA		NA		NA	
γ-tocopherol (μMol·L^{-1})																		
30 mL	1.44	0.5	1.4	0.82	1.47	0.81	1.46	0.74	1.62	0.56	1.86	0.58	1.91	0.98	1.86	0.75	1.82	0.94
60 mL	1.36	0.84	1.66	1.82	1.89	1.88	1.26	0.51	1.63	0.52	2.02	1.14	1.81	0.64	1.67	1.14	1.55	0.52
α-tocopherol (μMol·L^{-1})																		
30 mL	12.53	4.03	11.8	3.71	12.29	1.77	11.19	2.68	15.44	6.95	14.49	7.07	14.47	5.49	14.74	6.02	11.12	2.01
60 mL	12.89	3.37	12.63	3.00	10.97	4.03	10.52	5.19	12.5	4.62	12.73	3.68	13.88	4.14	13.41	4.38	10.16	1.69
Retinol (μMol·L^{-1})																		
30 mL	0.09	0.18	0.03	0.02	0.05	0.02	0.04	0.03	0.05	0.03	0.05	0.03	0.04	0.02	0.04	0.02	0.04	0.02
60 mL	0.09	0.17	0.04	0.02	0.04	0.03	0.06	0.05	0.05	0.04	0.05	0.03	0.04	0.02	0.04	0.02	0.05	0.03
Lycopene (μMol·L^{-1})																		
30 mL	0.04	0.08	0.02	0.02	0.03	0.04	0.01	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.02
60 mL	0.04	0.09	0.02	0.01	0.05	0.10	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02

CYA-3-O-Glu, cyanidin-3-O-glucoside equivalents.
* Significant main effects for time ($P < 0.05$).
↑ Significantly greater than the 30 mL condition ($P < 0.05$).

study. In agreement with previous work, the principal anthocyanin found was cyanidin (Blando et al., 2004), with the major downstream metabolite identified as cyanidin-3-O-glucosiderutinoside. Interestingly, we were unable to quantify any other previously reported (Howatson et al., 2012) parent anthocyanins (malvidin, pelargonidin, peonidin, delphinidin and petunidin) in the MC; although it should be noted that peonidin-3-O-rutinoside and cyanidin-3-O-rutinside were identified, but were not quantifiable. The contrast in reported MC anthocyanin content could be ascribed to inter-batch differences, for example, variations in the harvest year and time could impact significantly upon anthocyanin content of cherries (Poll, Petersen, & Nielsen, 2003), with variations as large as 500–2300 mg·L^{-1} of cyanidin-3-glucoside equivalents in harvested cherry juice (McCune et al., 2011). Additionally, subtle differences in the analysis methods might contribute to the variation in the anthocyanins reported between studies. Notwithstanding methodological or seasonal variations, the analysis of anthocyanins in the batch used in the current study provided an indication of the overwhelming anthocyanin (cyanidin-3-O-glucosiderutinoside) present in the MC and serves to inform the subsequent discussion.

The reductions in serum urate and increases in urinary urate concur with previous findings (Jacob et al., 2003). Although making direct comparisons between the whole fruit (Jacob et al., 2003) and the current investigation is difficult, the greater magnitude of change suggests that a single serving of Montmorency cherry concentrate might be more beneficial than a single serving of whole Bing sweet cherries in managing hyperuricemia. Montmorency cherry concentrate showed a greater reduction in serum urate than Bing cherries (30 *vs.* 15%, respectively at 5 h post consumption); additionally, the rate of serum urate reduction was faster with Montmorency cherries (3 *vs.* 5 h). Moreover, the appearance of increased urinary urate was earlier (1 *vs.* 1.5 h) and was a greater (44% *vs.* 37% increase, respectively) than Bing cherries (Jacob et al., 2003).

Decreased serum urate and increased urinary urate were maintained at 24 h and modulated further at 26 h, suggesting a potential cumulative effect of multiple doses on uric acid clearance and excretion. Despite the maintenance of decreased serum urate between baseline and 24 h, levels between 8 and 24 h rose towards baseline values. This was the longest period between supplementation, which suggested the active compounds in MC assert an effect on serum urate for a limited period and return to basal levels. Importantly, the magnitude of change was not different between doses, which suggested that despite plasma availability of CYA-3-O-GluRut being higher with a larger dose, the uric acid activity was not affected.

This work is the first to report the plasma uptake of anthocyanin compounds following Montmorency cherry concentrate consumption, which has previously been suggested (Jacob et al., 2003) to be responsible for changes in uric acid metabolism. Whilst our work does not refute this suggestion, it seems that there is a finite amount of anthocyanin compounds required to elicit a change. Conceivably, it is possible that there is an interaction with other phenolics or other undetected anthocyanin conjugates and derivatives that bring about these observations. Indeed, previous research has demonstrated multiple degradation products including protocatechuic acid (PCA), phloroglucinaldehyde (PGA) and hippuric acid following consumption of 500 mg of 13C labelled cyanidin-3-glucoside (de Ferrars et al., 2014), which might play a role in the observed changes. The main anthocyanins in the current study (CYA-3-O-GluRut, CYA-3-O-Rut and PEO-3-O-Rut) were expressed as cyanidin-3-O-glucoside equivalents since no reference standards could be sourced. As a result, caution should be taken when associating findings even though CYA-3-O-GluRut was measured, but expressed as CYA-3-O-Glu, which may play a role in the changes witnessed. Additionally, it has been suggested that anthocyanins might activate a xenobiotic antioxidant responses, which may contribute further to the protective effects of cherries (Kong, Chia, Goh, Chia, & Brouillard, 2003; Traustadottir et al., 2009).

Systemic inflammation, as indicated by hsCRP, is a clinically accepted marker of whole body inflammation (Pearson et al., 2003) and is sensitive to change, independent of the appearance of inflammatory cytokines. Here, inflammation was reduced following both doses of the concentrate; this concurs with previous work that demonstrated the anti-inflammatory activity of isolated tart cherry (Wang et al., 1999) and sweet cherry (Seeram et al., 2001) anthocyanins, which were comparable to commercially available non-steroidal anti-inflammatory drugs (NSAIDs). Indeed the aglycone of the anthocyanin found in the present study (cyanidin) was found to be particularly potent in reducing the inflammatory actions of prostaglandin enzymes (Wang et al., 1999). These authors suggested that increasing the number of sugar residues in the C_3 position of cyanidin decreased the antioxidant and anti-inflammatory activity of the compound; therefore anthocyanin compounds with higher sugar residues may not be as powerful as CYA-3-O-GluRut for exerting anti-inflammatory actions. Additionally, an *in-vivo* study has demonstrated a reduction in the exercise-induced inflammatory response (hsCRP, interleukin-6) with Montmorency cherry concentrate supplementation following marathon running (Howatson et al., 2010). The impact of Montmorency cherry concentrate on inflammation may provide further efficacy for its use in the treatment of inflammatory disorders.

Gout sufferers have been reported to consume cherries to reduce the effects of gouty arthritis, that is characterised by the formation of monosodium urate crystals, which trigger an inflammatory cascade (Terkeltaub, 2010) resulting in tender and swollen joints. In contrast to Jacob et al. (2003), this investigation extended the dosing and data collection phase to 48 h in order to identify the cumulative effects of multiple doses of MC. The hsCRP returned to baseline levels at 24 h, whilst additional supplementation elicited further reductions in serum urate at 26 h. At 48 h, serum hsCRP remained reduced from 24 h, suggesting several doses of MC provide a cumulative anti-inflammation response. Additionally, this provides evidence that changes in hsCRP were not a result of diurnal variation or influenced by postprandial MC consumption. Previous literature has demonstrated that dietary carbohydrate can attenuate the post-exercise inflammatory response (IL-6) (Starkie, Arkinstall, Koukoulas, Hawley, & Febbraio, 2001); however the results in the current study showed a decline in inflammation from a rested state, rather than attenuating an increase in inflammation. As a result, it is unlikely that any discrepancies in postprandial CHO consumption impacted upon the inflammatory responses in the current study. A further point of note is the

large inter-subject variation in both hsCRP and urate, which concur with previous observations in human studies (Howatson et al., 2010; Jacob et al., 2003). In summary, these results provide rationale for the use of Montmorency cherry concentrate as an adjuvant therapy to NSAIDs in the treatment of gouty arthritis.

This study also investigated the impact of increasing the dose by twofold. No differences were observed between 30 and 60 mL doses for serum urate, urinary urate or serum hsCRP at any time point. Consequently, a 30 mL dose of Montmorency cherry concentrate was sufficient to modulate indices associated with gout outbreaks. Plasma CYA-3-O-GluRut levels were not different between doses, but the 60 mL dose showed a greater level of absorption in the first hour following supplementation; although this higher dose caused some mild gastrointestinal discomfort in two participants.

The biokinetics of anthocyanins in the current study concur with other reports examining purple carrot juice (Charron et al., 2009), raw purple carrots (Kurilich et al., 2005) and elderberry extract (Cao, Muccitelli, Sánchez-Moreno, & Prior, 2001), although the magnitude was lower. Anthocyanins appear to be rapidly absorbed and quickly eliminated (Bell et al., 2013; Fernandes, Faria, Calhau, de Freitas, & Mateus, 2014; Manach, Williamson, Morand, Scalbert, & Rémésy, 2005), which suggests Montmorency cherry concentrate anthocyanins may not be directly responsible for the urate-lowering and anti-inflammatory effects. It should be noted, however, that multiple downstream anthocyanin metabolites, such as glucuronide conjugates as well as methylated and oxidised derivatives (de Ferrars et al., 2014; Kay et al., 2004), were not examined in the current study and it is feasible that such compounds may play also a role.

Given the sustained decrease in serum urate and hsCRP over 8 h, it is likely that further mechanisms are tenable. The formation of a cyanidin-DNA complex capable of resistance to oxidative stress (Traustadottir et al., 2009), and/or activation of xenobiotic responses that up-regulate endogenous antioxidant expression (Bell et al., 2013; Shih, Yeh, & Yen, 2007; Traustadottir et al., 2009) have been suggested as alternate mechanisms by which anthocyanins might exert their protective effects. In the current investigation it was not possible to elucidate the potential mechanism by which Montmorency cherries exert their urate and inflammation-lowering effects; however, previous work suggested that an increase in glomerular filtration or a decrease in renal tubular reabsorption through cherry supplementation might be responsible (Jacob et al., 2003; Zhang et al., 2012). Additionally, reduced uric acid production has been speculated as another possible mechanism (Haidari, Mohammad Shahi, Keshavarz, & Rashidi, 2009); following tart cherry juice supplementation, decreased hepatic activity of xanthine oxidase and xanthine dehydrogenase resulted in lower uric acid in rats with hyperuricemia (Haidari et al., 2009).

The current study provides new insight into the biokinetics and effects of different doses of tart Montmorency cherry concentrate on uric acid activity and inflammatory status. Although the inclusion of a control condition might have been useful to identify natural variation in the assessed variables, there were no differences between the 30 and 60 mL conditions in any of the assessed variables at baseline. Furthermore, previous literature in human (Cameron et al., 2012) and animal (Shinosaki, Inagaki, Nakai, Yamashita, & Yonetani, 1992; Yokozawa, Kanai, & Hikokichi, 1976) studies showed no circadian differences in uric acid concentration. This study utilised a randomised, cross-over design with a washout period, which provided some confidence that the changes were real. It should also be noted that the participants were asymptomatic for hyperuricemia, so further studies are warranted in a symptomatic population. Finally, we were unable to examine the full array of phenolic compounds and downstream metabolites present in the concentrate, which may also play a role in the uric acid-lowering and anti-inflammatory effects.

In conclusion, the data from the current study provide evidence that tart Montmorency cherries reduce levels of hsCRP and serum urate and increase urinary urate excretion. Importantly, a 30 mL dose of Montmorency cherry concentrate confers the same effects as a 60 mL dose; therefore from a practical and economic standpoint, it is not necessary to increase the dose in order to elicit a greater response. Overall, these are the first data to show Montmorency cherry concentrate as an efficacious, adjuvant supplement in managing inflammatory conditions, such as those seen with gout, and these observations are independent of CYA-3-O-GluRut absorption in humans.

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