



This is a peer-reviewed, post-print (final draft post-refereeing) version of the following published document, This is an Accepted Manuscript of an article published by Taylor & Francis in European Journal of Sport Science on 2 January 2019, available online: <http://www.tandfonline.com/10.1080/17461391.2018.1502360> and is licensed under All Rights Reserved license:

Brown, Meghan A ORCID logoORCID: <https://orcid.org/0000-0003-3260-977X>, Stevenson, Emma J. and Howatson, Glyn (2019) Montmorency tart cherry (*Prunus cerasus* L.) supplementation accelerates recovery from exercise-induced muscle damage in females. European Journal of Sport Science, 19 (1). pp. 95-102. doi:10.1080/17461391.2018.1502360

Official URL: <https://doi.org/10.1080/17461391.2018.1502360>

DOI: <http://dx.doi.org/10.1080/17461391.2018.1502360>

EPrint URI: <https://eprints.glos.ac.uk/id/eprint/5814>

Disclaimer

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

Title

Montmorency tart cherry (*Prunus cerasus* L.) supplementation accelerates recovery from exercise-induced muscle damage in females.

Running head

Tart cherry juice and recovery in females.

Authors and affiliations

Meghan A. Brown. School of Sport and Exercise, University of Gloucestershire, Gloucester, GL2 9HW, United Kingdom. Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom. E-mail: mbrown15@glos.ac.uk. Telephone: 0 (+44) 1242 715205.

Emma J. Stevenson. Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle, NE2 4HH, United Kingdom. E-mail: Emma.Stevenson@newcastle.ac.uk. Telephone: 0 (+44) 191 208 7865.

Glyn Howatson. Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom. Water Research Group, School of Environmental Sciences and Development, Northwest University, Potchefstroom, South Africa. E-mail: glyn.howatson@northumbria.ac.uk. Telephone: 0 (+44) 191 227 3575.

Corresponding author

Meghan A. Brown

Affiliation where research was conducted

Northumbria University

Abstract

Tart Montmorency cherry concentrate (MC) has been reported to attenuate the symptoms of exercise-induced muscle damage (EIMD) and to accelerate exercise recovery, which has been attributed to its high anti-inflammatory and antioxidant properties. Although these data are promising, there are no data regarding exclusively female populations. Therefore, the aim of this investigation was to examine the efficacy of MC on recovery following EIMD in females. In a randomised, double-blind, placebo-controlled study, twenty physically active females (mean \pm SD age 19 ± 1 y; stature 167 ± 6 cm; body mass 61.4 ± 5.7 kg) consumed MC or a placebo (PL) for eight days (30 mL twice per day). Following four days of supplementation, participants completed a repeated-sprint protocol and measures of muscle soreness (DOMS), pain pressure threshold (PPT), limb girth, flexibility, muscle function, and systemic indices of muscle damage and inflammation were collected pre, immediately post (0 h) and 24, 48 and 72 h post-exercise. Time effects were observed for all dependent variables ($p < 0.05$) except limb girth and high sensitivity C-reactive protein. Recovery of countermovement jump height was improved in the MC group compared to PL ($p = 0.016$). There was also a trend for lower DOMS ($p = 0.070$) and for higher PPT in the MC group at the rectus femoris ($p = 0.071$). The data demonstrate that MC supplementation may be a practical nutritional intervention to help attenuate the symptoms of muscle damage and improve recovery on subsequent days in females.

Key words

Repeated sprint, muscle soreness, algometry, muscle function, sour cherry

Introduction

Initial muscle damage is thought to be caused by a combination of mechanical disruption to the myofibrils and oxidative stress; the latter owing to an increase in the production of reactive oxygen and nitrogen species (RONS) and nitric oxide (NO) derivatives which may exceed antioxidant capacity (Powers & Jackson, 2008). Moreover, the secondary inflammatory response to muscle injury involves the degradation of damaged muscle by immune cells which release pro-inflammatory cytokines and further RONS and NO derivatives; exacerbating muscle damage (Clarkson & Hubal, 2002). The role of RONS and NO derivatives in the oxidative stress, inflammatory, and muscle damage responses which manifest during and following exercise has raised substantial interest in antioxidant supplementation. Of particular interest is tart Montmorency cherry concentrate (MC), which has been proposed to be an effective recovery aid due to its high anti-inflammatory and antioxidant properties (Bell et al., 2014; Bell et al., 2015; Keane et al., 2015a; Kirakosyan et al., 2015; Seeram et al., 2001; Wang et al., 1999). Montmorency tart cherries and their derivatives contain numerous polyphenols that include flavonoids; for example, the flavonol quercetin and anthocyanins (Kim et al., 2005; Kirakosyan et al., 2009). Certainly, the polyphenolic compounds that MC contain result in higher oxygen radical absorbance capacity (ORAC) values compared to several other antioxidant beverages such as Concord grape, acai, and blueberry juice (Bell et al., 2013; Howatson et al., 2010; Seeram et al., 2008).

To date, research in exercise and recovery paradigms has demonstrated that MC can improve recovery from damaging bouts of exercise in isolated muscle groups by attenuating decrements in muscle strength and/or soreness and pain (Bowtell et al., 2011; Connolly et al., 2006; Levers et al., 2015). Additionally, following damaging running activity, research has identified MC to be beneficial in reducing pain (Kuehl et al., 2010) and improving indices of inflammation, oxidative stress, antioxidant status and muscle function (Howatson et al., 2010). More recently,

MC has also been shown to facilitate recovery following cycling (Bell et al., 2014; Bell et al., 2015) and an adapted Loughborough Intermittent Shuttle Test (LIST) protocol (Bell et al., 2016).

Collectively, these lines of investigation have application to athletic populations that would benefit from reduced symptoms of muscle damage following strenuous activity. However, the effects of MC beyond isolated muscle, running and cycling activity are limited, and conceptually other sports and activities could benefit from this intervention. In addition, whilst females have been included in mixed-sex populations (Howatson et al., 2010; Kuehl et al., 2010), there are no data regarding exclusively female populations, largely due to the potential for oestrogen to influence outcome variables (Kendall & Eston, 2002). A growing body of evidence suggests that oestrogen has antioxidant properties (Tiidus et al., 2005; Wolf et al., 2012) and may help to maintain muscle membrane integrity consequent to muscle damage. As a result, the initial physiological stress and ensuing recovery associated with exercise-induced muscle damage (EIMD) in females is likely to differ compared to male populations. Indeed, recent evidence suggests that EIMD and recovery may differ between menstrual cycle phases given the fluctuating oestrogen concentrations (Markofski & Braun, 2014). Moreover, given their structural similarities to oestrogen, polyphenolic secondary plant metabolites (including the flavonoids that MC contains) appear to exert oestrogenic effects (Miksicek, 1995), and thus modulate and affect the bioavailability of endogenous oestrogens (Ward & Kuhnle, 2010). As such, since oestrogen is thought to play a key role in the observed sex differences in EIMD, currently the lack of studies investigating the supplementation of MC in a female only population is surprising and warrants research.

Therefore, the aim of this investigation was to examine the efficacy of MC on recovery from EIMD in females. It was hypothesised that indices of EIMD would be attenuated by the consumption of MC.

Methods

Participants

Twenty physically active females (mean \pm SD age 19 ± 1 y; stature 167 ± 6 cm; body mass 61.4 ± 5.7 kg; BMI 22.1 ± 1.9 kg·m⁻²) were recruited from a university dance team and gave written informed consent. The sample size was determined by completing a power analysis (power=0.8, α =0.05) based on isometric strength data from Bowtell et al. (2011). This determined a sample size of five in each group would provide statistical power above 80%, with an alpha level of 0.05. Exclusion criteria were; epilepsy, bronchitis, severe asthma, cardiac complaints, bacterial or viral infection in the 2 weeks preceding, injury or recovering from an injury sustained in the preceding 4 weeks, pregnancy, food allergy relating to the study supplements (as discussed with the investigator), or anything that may prevent them from successfully completing the study that was described. Participants had been training in dance regularly for 13 ± 4 years and were currently exercising for 8.3 ± 5 h per week; with no significant differences between groups (independent samples *t*-test $p=0.567$ and $p=0.598$, respectively). A single self-reported menstrual cycle questionnaire (as used previously by Brown et al., 2016a) identified the current contraceptive use of participants; nine were using an oral combination pill (all monophasic; $n=6$ in Montmorency cherry concentrate (MC) group and $n=3$ in placebo (PL) group), six were using a progesterone only pill/implant/injection ($n=3$ in both MC and PL groups), and five were menstruating normally ($n=1$ in MC group and $n=4$ in PL group). This also estimated menstrual cycle phase and allowed testing days to be assigned; all data collection took place during the early to mid-luteal phase, or where applicable in the 14 days before a withdrawal bleed. For 24 h prior to, and for each of the testing days, participants were asked to avoid strenuous exercise, alcohol, caffeine, nutritional supplements, and any anti-inflammatory drugs or alternative treatments. Participants completed a weighed food diary (analysed using dietary analysis software (Nutritics Ltd, Swords, Ireland)) and

activity log throughout all trial periods. Aside from the restrictions outlined previously, participants were not restricted in their consumption of polyphenolic rich foods and were instructed to consume their habitual diets. However, portions of foods thought to contain antioxidants were totalled for each day and averaged across the experimental period (Howatson et al., 2012; Howatson et al., 2010). The study was conducted according to the guidelines of the Declaration of Helsinki and all experimental procedures were approved by the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria.

Experimental protocol

Participants were allocated to either tart Montmorency cherry concentrate (MC; $n=10$) or placebo (PL; $n=10$) supplementation in a double-blind manner using stratified randomisation to ensure that groups were matched and counterbalanced for muscle function. A pre-supplementation blood sample (baseline) was taken in order to detect any changes in total creatine kinase (CK) and high sensitivity C-reactive protein (hsCRP) with preload supplementation. Participants fasted for ≥ 10 h prior to each visit, except for water (consumed *ad libitum*) and the morning supplement. On arrival at the laboratory, baseline measures of dependant variables were recorded. Participants completed the exercise protocol, and after a 2 min rest, measurement of dependent variables was repeated. Before leaving the laboratory, participants were reminded to consume a supplement prior to their evening meal. Supplementation and measurement of dependent variables were then repeated at the same time of day (± 1 h) for the following 3 days (24, 48 and 72 h post EIMD).

Supplementation

Participants consumed their habitual diets during all trial periods. Participants were provided with eight days of supplementation along with instructions on ingestion frequency and timing. This period was for four days prior to muscle-damaging exercise, the day of exercise, and for

three days of recovery. The daily dose was two servings of the MC or PL; one dose taken prior to breakfast (or 1-2 h prior to laboratory visits), and one dose prior to evening meal (except for the final day where only one supplement was consumed before the final visit). This is based on previous work showing a positive effect on recovery following strenuous exercise (Bell et al., 2016; Bell et al., 2014; Bell et al., 2015).

The MC beverage was prepared with 30 mL of concentrate (CherryActive, Sunbury, UK) diluted in 100 mL of water. According to the manufacturer's information, a 30 mL dose of concentrate is equivalent to approximately 90 whole cherries and has been previously reported to contain a total anthocyanin content of $73.5 \text{ mg} \cdot \text{L}^{-1}$ of cyanidin-3-glucoside, a total phenolic content of $178.8 \text{ gallic acid equivalent} \cdot \text{L}^{-1}$ and an antioxidant capacity (TEAC) of $0.58 \text{ trolox equivalents} \cdot \text{L}^{-1}$ (Keane et al., 2016). The PL was prepared with 25 mL of a synthetically derived fruit flavoured concentrate with negligible phytochemical content (Kia-Ora, Uxbridge, UK) in 100 mL of water and was fortified with flavourless maltodextrin (Myprotein, Manchester, UK) and flavourless whey protein powder (Arla Foods, Amba, Denmark). This was in order to match test beverages as closely as possible for volume (130 ml), consistency, colour, and macronutrient (24.5 g carbohydrate and 1.1 g protein) and energy content (102 kcal and 103 kcal for the MC and PL beverages, respectively).

Exercise protocol

Following a standardised warm up, participants completed a repeated-sprint protocol which comprised 15 x 30 m maximal sprints with a rapid 10 m deceleration phase, each separated by 60 s rest (Brown et al., 2016a; Brown et al., 2016b; Howatson & Milak, 2009; Keane et al., 2015b). Rate of perceived exertion (RPE; Borg, (1982)) and heart rate (HR; Model RS-400, Polar, Kempele, Finland) were collected after each sprint effort. Sprint times were recorded

using timing gates (Brower telemetric timers, Brower timing systems, Draper, USA) to determine total sprint time, mean sprint time, and rate of fatigue (Fitzsimons et al., 1993).

Dependant variables

Muscle soreness

Subjective ratings of muscle soreness (DOMS) were measured using a 200 mm visual analogue scale. Participants were required to indicate the level of perceived active lower limb soreness felt during a 90° squat. Pain pressure threshold (PPT) was measured with a digital algometer (Model FDX, Wagner Instruments, Greenwich, USA) at three muscle locations on the right leg; the rectus femoris (RF), the vastus lateralis (VL), and medial head of the gastrocnemius (GM) (Clifford et al., 2016). To determine PPT, participants were asked to verbally indicate when the pressure applied to the muscle (at an approximate rate of 5 N·s⁻¹) became too uncomfortable to tolerate. Intra-trial and inter-trial percentage coefficient of variation (%CV) were <8% for all locations.

Limb girth

An anthropometric tape measure (Bodycare Products, Warwickshire, United Kingdom) was used to determine calf (measured at its largest girth at baseline) and mid-thigh (located as midway between the inguinal fold and the superior border of the patella) girths of the right leg. Calf and mid-thigh girth intra-examiner %CVs were <1%.

Flexibility

Hamstring stiffness and flexibility was measured using the sit and reach test. The knees were fully extended with the feet together against the sit and reach box. Participants were instructed to stretch as far as possible (but not to the point of pain) and to hold their ‘best stretch’ for

approximately 2 s (American College of Sports Medicine, 2013) (recorded to the nearest 0.5 cm). Intra-trial and inter-trial %CV were <5%.

Muscle function

Participants completed three countermovement jumps (CMJ) and three drop jumps (for measurement of reactive strength index (RSI)) using a light timing system (Optojump, Microgate, Bolzano, Italy), keeping their hands on their hips throughout. For CMJ, participants were asked to squat down and jump vertically and maximally. For RSI (jump height (cm) ÷ ground contact time (s)), participants were instructed to drop from a height of 30 cm and upon landing, to perform a two-footed jump maximally with minimum contact time. Each effort was separated by 60 s rest and the peak CMJ and RSI were used for analysis. Intra-trial and inter-trial %CV were both <4% and <12% for CMJ and RSI respectively.

Maximum voluntary isometric contraction (MVC) of the right knee extensors was measured using a strain gauge (MIE Digital Myometer, MIE Medical Research Ltd, Leeds, UK). While in a seated position, the knee joint angle was standardised at 90° of flexion before each contraction. The peak force (Newtons, N) of three MVCs (of 3 s duration interspersed with 30 s rest) was used for analysis. Intra-trial and inter-trial %CV were <4%.

Participants completed a single maximal effort 30 m sprint, and sprint time was recorded (Brower telemetric timers, Brower timing systems, Draper, USA). Both intra-trial and inter-trial %CV were <2%.

Blood sampling and analysis

Blood samples (10 mL) were collected via venepuncture from the antecubital fossa area into serum gel vacutainers. Samples were rested at room temperature for 20 min, and centrifuged for 15 min (4°C) at 3000 RCF (Allegra X-22 Centrifuge, Beckman Coulter, Bucks, UK). Aliquots of serum were stored at -80°C for later analysis of CK and hsCRP; determined spectrophotometrically using an automated system (Roche Modular, Roche Diagnostics, Burgess Hill, UK). Due to technical issues, 5 blood samples (<5%) were not collected. When lower detection limits were not reached for hsCRP, the lowest detectable concentration was used (0.15 mg·L⁻¹). Inter-assay and intra-assay %CV for both total CK and hsCRP were <9%.

Statistical analysis

For the purpose of data analysis, all dependant variables except for DOMS, CK and hsCRP are expressed as a percentage relative to pre muscle damage values to account for inter-individual variability. Statistical software (IBM Statistical Package for Social Sciences (SPSS) V22 IBM, Armonk, USA) was used for inferential analysis and statistical significance accepted at the $p \leq 0.05$ level *a priori*. Mixed factor repeated-measures analysis of variance were performed for each dependent variable. Mauchly's test assessed the sphericity of the data and, where appropriate, violations were corrected using the Greenhouse–Geisser correction. Significant main effects were analysed using the Least Significant Difference test for adjustment for multiple comparisons. Paired samples *t*-tests were conducted to assess differences between total CK and hsCRP levels pre-supplementation (baseline) and pre-exercise, in order to detect any changes in systemic indices with preload supplementation. Independent samples *t*-tests were conducted on peak HR, peak RPE, fatigue, and total and mean sprint time to examine differences in exercise intensity during the repeated-sprint protocol between groups. Where appropriate, Cohen's *d* effect sizes (ES) were calculated with the magnitude of effects considered small (0.2), moderate (0.5) and large (>0.8).

Results

There were no differences in the total energy intake and macronutrient intake presented as a percentage of total energy intake (all $p>0.05$, $ES>0.47$), and the number of portions of foods containing antioxidants consumed by participants in both treatment groups (6 ± 2 in both MC and PL groups; $p=0.731$) during the supplementation period. Following all data collection periods, only $n=4$ participants correctly identified which supplement they had consumed. There were no differences between MC and PL groups for total (80.74 ± 4.02 vs 81.69 ± 3.67 s) and mean (5.38 ± 0.27 vs 5.45 ± 0.24 s) sprint time, fatigue (5.23 ± 2.02 vs $4.54 \pm 2.16\%$), peak HR (176 ± 15 vs 178 ± 8 bpm), and peak RPE (17 ± 2 vs 18 ± 1) during the repeated-sprint protocol (all $p>0.05$); demonstrating that the exercise stimulus was comparable between groups. All raw data not illustrated in figures are presented in Table 1.

Table 1. Values for dependent variables in response to muscle-damaging exercise, mean \pm SD.

Variable	Group	Time post muscle-damaging exercise (h)				
		Pre	0	24	48	72
RF PPT, N	MC	48.1 ± 8.8	46.9 ± 11.6	42.5 ± 10.8	48.7 ± 12.9	56.0 ± 17.5
	PL	35.2 ± 12.4	30.8 ± 10.6	25.9 ± 6.9	28.7 ± 10.0	35.1 ± 8.8
VL PPT, N	MC	42.6 ± 9.8	47.3 ± 18.0	34.9 ± 13.6	41.6 ± 12.6	53.3 ± 23.3
	PL	30.2 ± 10.1	29.7 ± 11.8	25.5 ± 7.2	28.5 ± 10.2	32.5 ± 10.5
GM PPT, N	MC	41.4 ± 11.2	42.4 ± 15.5	31.8 ± 8.8	39.4 ± 9.4	50.2 ± 12.2
	PL					

	PL	27.3 ± 12.1	25.0 ± 12.1	21.9 ± 8.7	24.9 ± 10.2	30.1 ± 9.9
Thigh	MC	50.1 ± 3.1	50.4 ± 3.0	50.3 ± 3.0	50.3 ± 3.0	50.3 ± 2.8
girth, cm	PL	50.6 ± 2.2	50.9 ± 2.6	51.0 ± 2.4	51.0 ± 2.5	50.9 ± 2.4
Calf girth,	MC	36.1 ± 2.3	36.2 ± 2.4	36.1 ± 2.4	36.1 ± 2.3	36.2 ± 2.2
cm	PL	36.2 ± 2.1	36.1 ± 1.9	36.2 ± 2.0	36.2 ± 2.2	36.2 ± 2.0
Flexibility	MC	29.1 ± 5.4	28.3 ± 6.4	25.0 ± 7.2	26.4 ± 9.3	29.0 ± 7.8
, cm	PL	20.3 ± 9.0	17.7 ± 9.0	14.5 ± 6.1	17.4 ± 8.3	17.6 ± 9.0
RSI, cm·s ⁻¹	MC	102.8 ± 22.5	88.6 ± 21.5	97.2 ± 28.5	99.0 ± 22.2	107.0 ± 27.1
	PL	81.5 ± 17.6	74.7 ± 13.7	72.0 ± 13.8	73.0 ± 14.4	80.2 ± 20.1
MVC, N	MC	394.3 ± 59.3	347.1 ± 82.5	362.7 ± 87.1	381.3 ± 87.2	376.5 ± 73.6
	PL	392.2 ± 89.4	354.1 ± 72.7	355.4 ± 73.8	361.8 ± 70.2	375.9 ± 63.9
30 m	MC					
sprint		5.32 ± 0.35	5.42 ± 0.36	5.39 ± 0.31	5.45 ± 0.29	5.37 ± 0.40
time, s	PL	5.28 ± 0.26	5.46 ± 0.30	5.37 ± 0.27	5.58 ± 0.41	5.43 ± 0.29

hsCRP, mg·L ⁻¹	MC	1.63 ± 1.99	1.79 ± 1.87	2.15 ± 0.24	2.13 ± 1.97	1.56 ± 1.35
	PL	1.81 ± 1.93	1.80 ± 1.87	1.73 ± 1.73	1.29 ± 1.14	1.71 ± 1.31

MC, Montmorency cherry group ($n=10$); PL, placebo group ($n=10$); Pre, pre-exercise; RF, rectus femoris; VL, vastus lateralis; GM, medial head of the gastrocnemius; PPT, pain pressure threshold; CMJ, countermovement jump; RSI, reactive strength index; MVC, maximal voluntary isometric contraction; hsCRP, high sensitivity C-reactive protein.

Muscle soreness increased post-exercise ($p<0.001$), peaking at 24 h and remained elevated throughout recovery in both groups (Figure 1); however, there was a trend and moderate effect for lower DOMS in the MC group ($p=0.070$, $ES=0.58$). At all three locations, PPT was reduced post-exercise (all $p<0.001$), reached lowest levels at 24 h and then increased throughout recovery. There were no group differences in PPT at the VL or GM, but a trend and moderate effect for higher PPT in the MC group at the RF was observed ($p=0.071$, $ES=0.59$).

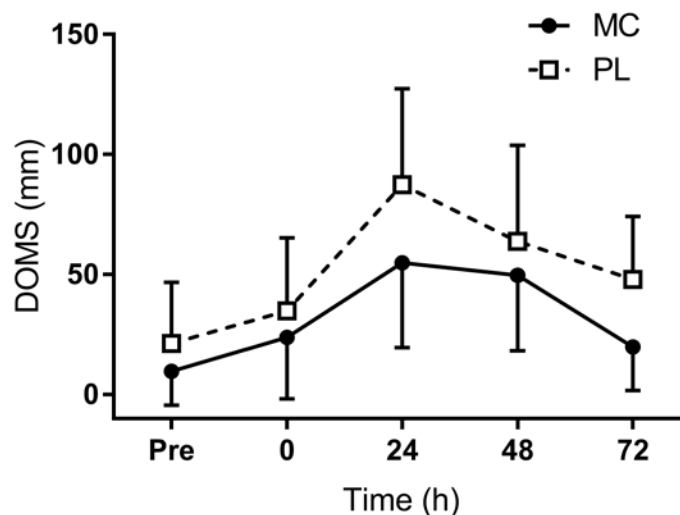


Figure 1 Muscle soreness (DOMS) post exercise-induced muscle damage in the Montmorency cherry (MC) ($n=10$) and placebo (PL) ($n=10$) groups. Values presented as mean \pm SD.

Thigh and calf girths were unaffected post-exercise and there were no differences between treatment groups (all $p>0.05$). Flexibility was reduced for 48 h post-exercise but returned to baseline levels at 72 h in both groups ($p=0.022$) with no group differences.

All measures of muscle function (CMJ, RSI, MVC and 30 m sprint time) were reduced post-exercise and progressively recovered throughout recovery (all $p<0.05$). While recovery of these measures appeared to accelerate with MC, a group effect was only evident with CMJ ($p=0.016$, $ES=0.66$) (Figure 2).

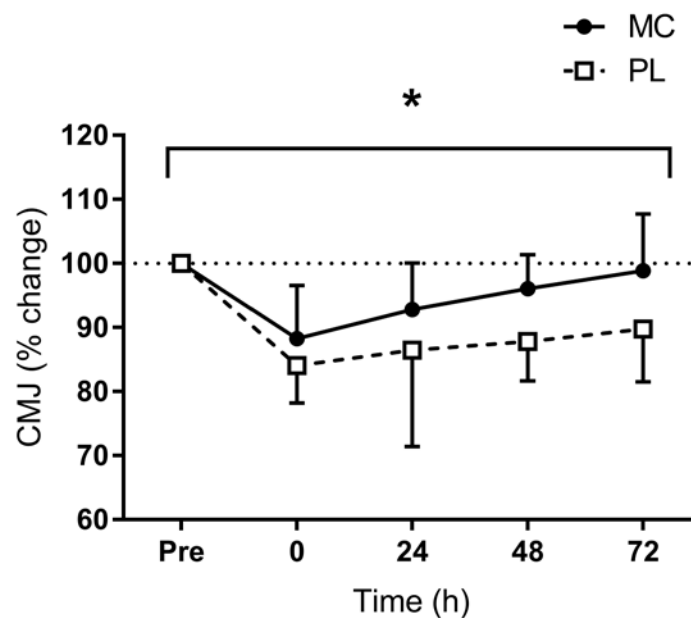


Figure 2 Percentage relative to pre-exercise (Pre) countermovement jump height (CMJ) post exercise-induced muscle damage in the Montmorency cherry (MC) (n=10) and placebo (PL) (n=10) groups. Values presented as mean \pm SD.

*denotes significantly higher CMJ in MC.

Total CK and hsCRP concentrations were not different between baseline and pre-exercise time-points for both MC and PL groups (all $p>0.05$). Both groups experienced an increase in circulating CK, which peaked 24 h post-exercise and remained elevated for 72 h post-exercise ($p<0.001$) with no differences between groups (Figure 3). Circulating hsCRP was unaffected by exercise and was not different between groups.

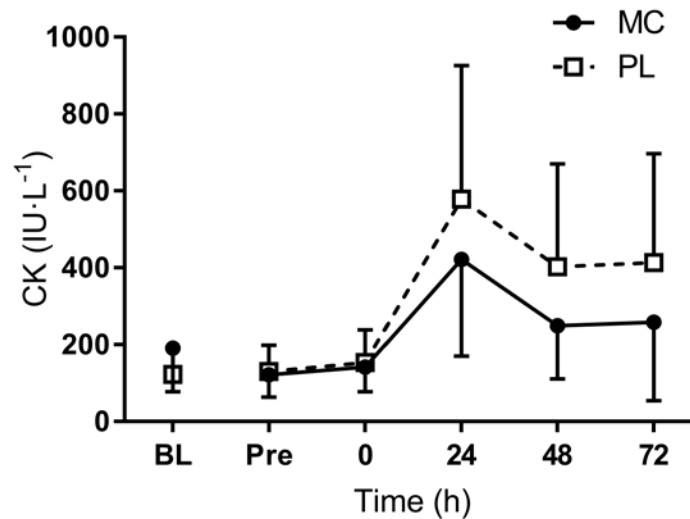


Figure 3 Total creatine kinase (CK) at baseline pre-supplementation (BL), before (Pre) and post exercise-induced muscle damage in the Montmorency cherry (MC) (n=10) and placebo (PL) groups (n=10). Values presented as mean \pm SD.

Discussion

This study sought to examine the efficacy of 8-day MC supplementation on recovery from EIMD in females. The data demonstrate that MC supplementation accelerated the recovery of CMJ and was associated with trends of reduced muscle soreness.

The clear acceleration in recovery of CMJ following MC supplementation supports a recent study demonstrating an improvement in CMJ (Bell et al., 2016), and a number of studies demonstrating an accelerated recovery in other measures of muscle function with MC consumption (Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010). The improvement of CMJ with MC may be explained by a protection against oxidative injury to the type II fibres recruited for such activity. Eccentric exercise is thought to preferentially damage type II muscle fibres (Macaluso et al., 2012) and this has implications on the muscles' force-generating capacity (Byrne et al., 2004). Interestingly, evidence suggests that mitochondrial ROS production and/or release is potentiated in type II fibres (Anderson & Neufer, 2006) and the activity of endogenous antioxidant enzymes including superoxide

dismutase (SOD) (Criswell et al., 1993; Powers et al., 1994) and glutathione peroxidase (GPX) (Lawler et al., 1994; Powers et al., 1994) are lower compared with type I fibres in rodent models. During periods of increased oxidant production (for instance intense exercise), both enzymatic and non-enzymatic antioxidants collectively provide some protection to muscle fibres from oxidative injury (Powers & Jackson, 2008). Therefore, conceptually the supplementation of non-enzymatic antioxidants contributed to the free radical scavenging capacity of type II fibres and facilitated recovery of CMJ. However, the functional measures in the present investigation all require type II fibre recruitment, so intuitively, we would have expected the measures to be equally affected by MC. Indeed, the lack of an accelerated recovery of MVC with MC is in contrast to a number of previous studies (Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010). Certainly, the role of MC in accelerating the recovery of muscle function in this population following EIMD in the current study remains unclear.

Supplementation with MC resulted in a moderate effect for reductions in DOMS and increases in PPT at the RF; which could have played a role in the observed improvements in CMJ. This is in line with a number of investigations reporting reduced soreness and pain with MC supplementation (Bell et al., 2016; Connolly et al., 2006; Kuehl et al., 2010; Levers et al., 2015). However, these reductions previously reported have not always been accompanied with improvements in muscle function, and vice versa (Bell et al., 2015; Bowtell et al., 2011; Levers et al., 2015), and others have found no reduction in DOMS with MC consumption (Bell et al., 2015; Bowtell et al., 2011; Howatson et al., 2010). The inconsistencies in the literature could be explained by the disparities in exercise protocol employed. Indeed, muscle soreness has been associated with increases in inflammation following exercise (Kraemer et al., 2004). In the current investigation, hsCRP was not different between groups, and limb girth (an indirect measure of inflammation, swelling and oedema (Smith, 1991)) was unaffected by the exercise.

Compared to marathon running (Howatson et al., 2010) and high intensity cycling exercise (Bell et al., 2015), where CRP has been shown to increase 24 and 48 h post-exercise, the repeated-sprint protocol does not appear to induce a large inflammatory response. It is conceivable that the exercise stimulus was insufficient to detect larger magnitudes of change in soreness and PPT with MC. Similarly, the CK values elicited by the exercise protocol in the current study are lower than other damaging protocols, suggesting a moderate muscle damage response, and likely influencing the ability to demonstrate differences. In addition, females have been shown to demonstrate lower basal circulating concentrations of CK, and a lower CK response following exercise compared to males (Wolf et al., 2012), owing in part to the antioxidant properties of oestrogen (Tang, Abplanalp, Ayres, & Subbiah, 1996).

The data which demonstrate no group differences in hsCRP and CK do not wholly support the literature which traditionally suggests attenuated symptoms of EIMD with MC is attributable to reduced muscle damage and inflammation; at least following repeated-sprint exercise in females. However, the current study was limited by a lack of measurement of oxidative stress and antioxidant capacity. Indeed, some have identified differences in oxidative stress and antioxidant status following strenuous exercise with supplementation of MC compared to PL (Bell et al., 2014; Bowtell et al., 2011; Howatson et al., 2010). It is possible that this is magnified in females given that oestrogen is also thought to have antioxidant properties (Tiidus et al., 2005; Wolf et al., 2012) as oestrogens, similar to vitamin E, display a hydroxyl group on their phenolic ring (Tiidus et al., 2001). It is thought that oestrogen donates the hydrogen atom to lipid peroxyl radicals, limiting lipid peroxidation in the cell membrane (Kendall & Eston, 2002). Perhaps enhanced antioxidant status and redox balance may have contributed to the improvements in CMJ and trends in reduced soreness observed with MC in this study. Future research should include measurement of a variety of systemic indices to provide greater insight into specific mechanisms influencing improved muscle function and pain with MC. Moreover,

more accurate determination of menstrual cycle phase (urine ovulation prediction tests for example) and measurement of concentrations of oestrogen (particularly of oestradiol) in female participants is warranted to assist with the interpretation of results.

This study demonstrated that 8-day MC supplementation alongside a habitual diet improved recovery of CMJ and tended to lower muscle soreness compared to PL. This research adds to the existing body of knowledge whilst providing new information for the potential application of MC to wider groups. In particular, it would appear that female populations might benefit from this nutritional intervention to help attenuate the symptoms of EIMD and improve recovery on subsequent days.

References

- Anderson, E. J., & Neufer, P. D. (2006). Type II skeletal myofibers possess unique properties that potentiate mitochondrial H₂O₂ generation. *American Journal of Physiology - Cell Physiology*, 290(3), C844-851.
- American College of Sports Medicine. (2013). *ACSM's guidelines for exercise testing and prescription*. 9th edn. Philadelphia, PA: Lippincott Williams & Wilkins.
- Bell, P. G., McHugh, M. P., Stevenson, E., & Howatson, G. (2013). The role of cherries in exercise and health. *Scandinavian Journal of Medicine & Science in Sports*, 24(3), 477-490.
- Bell, P. G., Stevenson, E., Davison, G. W., & Howatson, G. (2016). The Effects of Montmorency Tart Cherry Concentrate Supplementation on Recovery Following Prolonged, Intermittent Exercise. *Nutrients*, 8(7).
- Bell, P. G., Walshe, I. H., Davison, G. W., Stevenson, E., & Howatson, G. (2014). Montmorency Cherries Reduce the Oxidative Stress and Inflammatory Responses to Repeated Days High-Intensity Stochastic Cycling. *Nutrients*, 6(2), 829-843.
- Bell, P. G., Walshe, I. H., Davison, G. W., Stevenson, E. J., & Howatson, G. (2015). Recovery facilitation with Montmorency cherries following high-intensity, metabolically challenging exercise. *Applied Physiology, Nutrition & Metabolism*, 40(4), 414-423.
- Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise*, 14(5), 377-381.
- Bowtell, J. L., Sumners, D. P., Dyer, A., Fox, P., & Mileva, K. N. (2011). Montmorency Cherry Juice Reduces Muscle Damage Caused by Intensive Strength Exercise. *Medicine & Science in Sports & Exercise*, 43(8), 1544-1551.
- Brown, M. A., Howatson, G., Keane K., & Stevenson, E. J. (2016a). Exercise induced muscle damage following dance and sprint exercise in females. *Journal of Sports Medicine & Physical Fitness*, 56(11), 1376-1383.

- Brown, M. A., Howatson, G., Keane, K., & Stevenson, E. J. (2016b). Adaptation to damaging dance and repeated sprint activity in females. *Journal of Strength & Conditioning Research*, 30(9), 2574-2581.
- Byrne, C., Twist, C., & Eston, R. (2004). Neuromuscular function after exercise-induced muscle damage - Theoretical and applied implications. *Sports Medicine*, 34(1), 49-69.
- Clarkson, P. M., & Hubal, M. J. (2002). Exercise-induced muscle damage in humans. *American Journal of Physical Medicine & Rehabilitation*, 81(11), S52-S69.
- Clifford, T., Bell, O., West, D. J., Howatson, G., & Stevenson, E. J. (2016). The effects of beetroot juice supplementation on indices of muscle damage following eccentric exercise. *European Journal of Applied Physiology*, 116(2), 353-362.
- Connolly, D. A. J., McHugh, M. P., & Padilla-Zakour, O. I. (2006). Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *British Journal of Sports Medicine*, 40(8), 679-683.
- Criswell, D., Powers, S., Dodd, S., Lawler, J., Edwards, W., Renshler, K., & Grinton, S. (1993). High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. *Medicine & Science in Sports & Exercise*, 25(10), 1135-1140.
- Fitzsimons, M., Dawson, B., Ward, D., & Wilkinson, A. (1993). Cycling and running tests of repeated sprint ability. *Australian Journal of Science & Medicine in Sport*, 25(4), 82-87.
- Howatson, G., Bell, P. G., Tallent, J., Middleton, B., McHugh, M. P., & Ellis, J. (2012). Effect of tart cherry juice (*Prunus cerasus*) on melatonin levels and enhanced sleep quality. *European Journal of Nutrition*, 51(8), 909-916.
- Howatson, G., McHugh, M. P., Hill, J. A., Brouner, J., Jewell, A. P., van Someren, K. A., . . . Howatson, S. A. (2010). Influence of tart cherry juice on indices of recovery following marathon running. *Scandinavian Journal of Medicine & Science in Sports*, 20(6), 843-852.

- Howatson, G., & Milak, A. (2009). Exercise-induced muscle damage following a bout of sport specific repeated sprints. *Journal of Strength & Conditioning Research*, 23(8), 2419-2424.
- Keane, K. M., Bell, P. G., Lodge, J. K., Constantinou, C. L., Jenkinson, S. E., Bass, R., & Howatson, G. (2015a). Phytochemical uptake following human consumption of Montmorency tart cherry (*L. Prunus cerasus*) and influence of phenolic acids on vascular smooth muscle cells in vitro. *European Journal of Nutrition*.
- Keane, K., Salicki, R., Goodall, S., Thomas, K., & Howatson, G. (2015b). The muscle damage response in female collegiate athletes following repeated sprint activity. *Journal of Strength & Conditioning Research*.
- Keane, K. M., George, T. W., Constantinou, C. L., Brown, M. A., Clifford, T., & Howatson, G. (2016). Effects of Montmorency tart cherry (*Prunus Cerasus L.*) consumption on vascular function in men with early hypertension. *American Journal of Clinical Nutrition*.
- Kendall, B., & Eston, R. (2002). Exercise-induced muscle damage and the potential protective role of estrogen. *Sports Medicine*, 32(2), 103-123.
- Kim, D. O., Heo, H. J., Kim, Y. J., Yang, H. S., & Lee, C. Y. (2005). Sweet and sour cherry phenolics and their protective effects on neuronal cells. *J Agric Food Chem*, 53(26), 9921-9927.
- Kirakosyan, A., Seymour, E. M., Llanes, D. E. U., Kaufman, P. B., & Bolling, S. F. (2009). Chemical profile and antioxidant capacities of tart cherry products. *Food Chem*, 115(1), 20-25.
- Kirakosyan, A., Seymour, E. M., Wolforth, J., McNish, R., Kaufman, P. B., & Bolling, S. F. (2015). Tissue bioavailability of anthocyanins from whole tart cherry in healthy rats. *Food Chemistry*, 171, 26-31.

- Kraemer, W. J., French, D. N., & Spiering, B. A. (2004). Compression in the treatment of acute muscle injuries in sport : review article. *International SportMed Journal*, 5(3), 200-208.
- Kuehl, K. S., Perrier, E. T., Elliot, D. L., & Chesnutt, J. C. (2010). Efficacy of tart cherry juice in reducing muscle pain during running: a randomized controlled trial. *Journal of the International Society of Sports Nutrition*, 7, 17-17.
- Lawler, J. M., Powers, S. K., Van Dijk, H., Visser, T., Kordus, M. J., & Ji, L. L. (1994). Metabolic and antioxidant enzyme activities in the diaphragm: effects of acute exercise. *Respiration Physiology*, 96(2-3), 139-149.
- Levers, K., Dalton, R., Galvan, E., Goodenough, C., O'Connor, A., Simbo, S., . . . Kreider, R. (2015). Powdered tart cherry supplementation surrounding a single bout of intense resistance exercise demonstrates potential attenuation of recovery strength decrement with no definitive oxidative or inflammatory effect. *Journal of the International Society of Sports Nutrition*, 12(Suppl 1), P25-P25.
- Macaluso, F., Isaacs, A. W., & Myburgh, K. H. (2012). Preferential type II muscle fiber damage from plyometric exercise. *Journal of Athletic Training*, 47(4), 414-420.
- Markofski, M. M., & Braun, W. A. (2014). Influence of menstrual cycle on indices of contraction-induced muscle damage. *Journal of Strength & Conditioning Research*, 28(9), 2649-2656.
- Miksicek, R. J. (1995). Estrogenic flavonoids: structural requirements for biological activity. *Proceedings of the Society for Experimental Biology & Medicine*, 208(1), 44-50.
- Powers, S. K., Criswell, D., Lawler, J., Ji, L. L., Martin, D., Herb, R. A., & Dudley, G. (1994). Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. *Americal Journal of Physiology*, 266(2 Pt 2), R375-380.

- Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiological Reviews*, 88(4), 1243-1276.
- Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Feng, L., Dreher, M., & Heber, D. (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of Agricultural & Food Chemistry*, 56(4), 1415-1422.
- Seeram, N. P., Momin, R. A., Nair, M. G., & Bourquin, L. D. (2001). Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine*, 8(5), 362-369.
- Smith, L. L. (1991). Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Medicine & Science in Sports & Exercise*, 23(5), 542-551.
- Tang, M., Abplanalp, W., Ayres, S., & Subbiah, M. T. (1996). Superior and distinct antioxidant effects of selected estrogen metabolites on lipid peroxidation. *Metabolism*, 45(4), 411-414.
- Tiidus, P. M., Deller, M., & Liu, X. L. (2005). Oestrogen influence on myogenic satellite cells following downhill running in male rats: a preliminary study. *Acta Physiol Scand*, 184(1), 67-72.
- Tiidus, P. M., Holden, D., Bombardier, E., Zajchowski, S., Enns, D., & Belcastro, A. (2001). Estrogen effect on post-exercise skeletal muscle neutrophil infiltration and calpain activity. *Can J Physiol Pharmacol*, 79(5), 400-406.
- Wang, H., Nair, M. G., Strasburg, G. M., Chang, Y. C., Booren, A. M., Gray, J. I., & DeWitt, D. L. (1999). Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *Journal of Natural Products*, 62(2), 294-296.

- Ward, H. A., & Kuhnle, G. G. (2010). Phytoestrogen consumption and association with breast, prostate and colorectal cancer in EPIC Norfolk. *Archives of Biochemistry & Biophysics*, 501(1), 170-175.
- Wolf, M. R., Fragala, M. S., Volek, J. S., Denegar, C. R., Anderson, J. M., Comstock, B. A., . . . Kraemer, W. J. (2012). Sex differences in creatine kinase after acute heavy resistance exercise on circulating granulocyte estradiol receptors. *Eur J Appl Physiol*, 112(9), 3335-3340.