

Title: Montmorency cherry juice reduces muscle damage caused by intensive strength exercise

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Cherry juice: exercise-induced muscle damage

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Abstract

Purpose: Montmorency cherries contain high levels of polyphenolic compounds including flavonoids and anthocyanins possessing anti-oxidant and anti-inflammatory effects. We investigated whether the effects of intensive unilateral leg exercise on oxidative damage and muscle function were attenuated by consumption of a Montmorency cherry juice concentrate using a crossover experimental design.

Methods: 10 well-trained male overnight fasted athletes completed two trials of 10 sets of 10 single leg knee extensions at 80% one repetition maximum (1RM). Trials were separated by 2 weeks and alternate legs were used in each trial. Participants consumed each supplement (CherryActive[®], CA or isoenergetic fruit concentrate, FC) for 7 d prior to and 48h after exercise. Knee extension maximum voluntary contractions (MVC) were performed pre, immediately, 24 and 48h after the damaging exercise. Venous blood samples were collected at each time point and serum analysed for creatine kinase activity (CK), nitrotyrosine, high sensitivity C reactive protein, total anti-oxidant capacity and protein carbonyls (PC). Two way repeated measures ANOVA were used for statistical analysis of the data.

Results: MVC force recovery was significantly faster (24h: 90.9 ± 4.2 , CA vs 84.9 ± 3.4 , FC; 48h: 92.9 ± 3.3 , CA vs 88.5 ± 2.9 , FC; % of initial MVC; mean \pm SEM; $p < 0.05$) after CA than FC consumption. Only serum CK and PC increased significantly from baseline, peaking 24h

after exercise ($p < 0.001$). The exercise-induced increase in CK activity was not different between trials. However, both the percentage (24h post: 23.8 ± 2.9 , CA; 82.7 ± 11.7 , FC; %; $p = 0.013$) and absolute (24h post: 0.31 ± 0.03 , CA; 0.60 ± 0.08 , FC; nmol/mg protein; $p = 0.079$) increase in PC was lower in CA than FC trials.

Conclusions: Montmorency cherry juice consumption improved the recovery of isometric muscle strength after intensive exercise perhaps due to attenuation of the oxidative damage induced by the damaging exercise.

Key Words: anti-oxidant; protein carbonyl; oxidative damage; muscle strength

Introduction

Paragraph 1 Novel or unaccustomed intensive exercise results in muscle damage, the symptoms of which are long lasting (2-5 d) reductions in muscle strength and muscle soreness. The decreased muscle force generating capacity has been attributed to myofibrillar disruption and structural damage to the muscle as evidenced by the increase in the blood concentration of large intracellular muscle proteins such as creatine kinase and lactate dehydrogenase. These changes have been directly associated with increased permeability of the damaged sarcolemma (18). Impaired excitation-contraction coupling related to altered intracellular calcium homeostasis has also been implicated in the reduced functional capacity of muscle after eccentric exercise. This may be due to damage to the ryanodine receptors of the sarcoplasmic reticulum resulting in elevated intracellular calcium ion concentration (for review see (38)) and altered membrane potentials. Increased intracellular calcium may also contribute to muscle damage through activation of calcium dependent proteolytic pathways and increased muscle protein degradation (36).

Paragraph 2 The exact mechanisms by which muscle damage occurs are not yet fully understood but are thought to involve both mechanical and metabolic pathways, with the relative contribution presumably varying according to the mode, intensity and duration of exercise (for reviews see (9, 15, 28, 30, 34). The initial phase of damage during exercise is suggested to occur as a consequence of both the mechanical forces to which the muscle fibres are exposed and oxidative stress due to exercise-induced increases in reactive oxygen species (ROS) and nitric oxide (NO) derivatives that exceed the antioxidant defence capacity. Acute high intensity resistance exercise (>60 % 1RM) has been shown to induce increases in lipid hydroperoxides and protein carbonyls, blood markers of oxidative damage (16). A second phase of damage occurs due to the inflammatory response to muscle injury (for review see

(34). This is characterised by neutrophil migration to muscle occurring within several hours of exercise and lasting for up to 24h, and the presence of macrophages within damaged muscle from 24 h to up to 14d after exercise. These immune cells contribute to the degradation of damaged muscle by releasing ROS and NO derivatives as well as pro-inflammatory cytokines.

Paragraph 3 As a consequence of the apparent role for ROS and NO derivatives in muscle damage, there has been considerable interest in the efficacy of antioxidant supplements such as vitamins C and E in ameliorating exercise induced muscle damage. However evidence is equivocal with some studies showing that vitamin C and/or E supplementation decreased eccentric exercise induced muscle damage (decreased CK and delayed onset muscle soreness, (5); decreased CK, (24); decreased malondialdehyde, MDA and PC, (11)) but others found no change in CK (2) or even increased CK and lactate dehydrogenase (LDH, (8). The conflicting evidence can be attributed in part to the variation in exercise mode, intensity and duration employed to induce damage as well as the dose and duration of vitamin supplementation.

Paragraph 4 More recently there has been interest in the potential of fruit derived phytochemicals with both antioxidant and anti-inflammatory properties to improve recovery from exercise-induced muscle damage (10, 13, 35). Connolly et al (10) found that consumption of montmorency (tart) cherry juice for 4 days prior repeated single arm elbow flexor eccentric contractions resulted in a quicker recovery of isometric force generating capacity than in a placebo trial, however no biochemical measures of muscle damage were taken. Howatson et al (13) found quicker recovery of knee extensor maximal isometric force after running a marathon, when participants consumed montmorency cherry juice rather than placebo for 6 d prior to the race. Although there was no difference in serum CK or LDH between trials, markers of inflammation (interleukin 6, IL6 and C reactive protein, CRP) and

oxidative damage (thiobarbituric acids reactive substances, TBARS) were significantly lower in the cherry juice trial. In similar fashion, Trombold et al (35) found that pomegranate derived ellagitannin consumption improved recovery of elbow flexor isometric strength after repeated eccentric contractions in resistance exercise naïve participants. However there was no difference between trials in serum markers of muscle damage (CK and myoglobin, Mb) or inflammation (IL6 and CRP). It seems therefore that fruit-derived polyphenolic compounds have the potential to improve recovery from damaging exercise, although the mechanism is as yet unclear in part because there is only limited published evidence available. It is not clear how far these existing findings can be generalised. Although cherry juice consumption has been shown to enhance functional recovery from elbow flexor eccentric exercise in recreationally active participants (10), it is unclear whether such a supplement would be similarly effective for knee extensor recovery of well-trained individuals, who are relatively resistant to damage. Howatson et al (13) quantified the effects of cherry juice consumption on markers of oxidative damage and inflammation, as well as muscle function after marathon exercise. However, these data are not available for recovery from resistance exercise where at least the initial phase of damage will be more mechanical in nature. Therefore in the present study the effect of supplementation with montmorency cherry juice concentrate on functional recovery from intensive knee extensor resistance exercise in well-trained individuals was investigated in parallel with changes in serum markers of oxidative damage and inflammation.

Methods

Participants

Paragraph 5 Ten well-trained male participants (age: 27.8 ± 1.6 y, single leg 1RM: 73 ± 4 kg, weight: 81.3 ± 4.3 kg, height: 1.76 ± 0.03 m) completed the study. Participants all competed in high intensity intermittent sports: rugby, football, or taekwondo; and regularly performed resistance training. The study was approved by the local university ethics committee, and was conducted in compliance with the World Medical Association's Declaration of Helsinki (2008). All participants were informed verbally and in writing of the experimental procedures and associated risks, prior to completing a medical health questionnaire and giving their written informed consent. Exclusion criteria included cardio-respiratory, neuromuscular problems and acute knee/ ankle injuries and pain.

Baseline Strength Testing.

Paragraph 6 Participants were fully familiarized with completing single leg, knee extension maximum voluntary isometric contractions (MVC), as well as the other experimental procedures and measures. During the week prior to the first trial the single leg knee extension one repetition maximum was determined for each leg. Subjects were seated on the knee-extension machine (TechnoGym UK Ltd), and the backrest and bar levels were adjusted for each subject and kept constant during all subsequent testing. A lapbelt restraint was used to exclude contribution from the hip musculature during the knee-extension exercise. Subjects performed a standard ramp test to identify the 1RM (12). Each weight lift was assessed by the investigator and considered successful if performed with proper technique, within the metronome-guided time interval (2.5 s) and going through the full range of knee motion of

the exercise (0.7 rad). The maximum weight lifted was identified as the 1RM. Verbal encouragement was given to each participant throughout.

Experimental Design

Paragraph 7 Participants completed two main trials separated by a two week wash-out period (Figure 1). Participants consumed 30 ml twice per day for 10 days of either montmorency cherry juice concentrate (CherryActive[®], CA) or an isoenergetic fruit concentrate placebo (FC). On each occasion participants completed the single leg intensive knee extensor training session on day 8 of the supplementation period, with different legs used for each trial in order to minimise any repeated bout effect. Trials were allocated by systematic rotation to counterbalance the study for trial order and leg dominance, with participants and investigators blind to treatment. Participants were instructed to consume their habitual diet and continue normal training activities for the first 5 days of the supplementation period but to refrain from strenuous physical activity for 48 h prior to and after the intensive exercise protocol. Participants recorded their diet for 48 h prior to the first main trial and then repeated this diet prior to the second trial.

Nutritional Supplements

Paragraph 8 Each 30 ml serving of the montmorency cherry juice concentrate contained 20g carbohydrate and provided 96 kcal. HPLC analysis (6) of the concentrate performed by Atlas Bioscience Inc found that total anthocyanin content was 9.117 mg.ml⁻¹, with malvidin (4.696 mg.ml⁻¹) and cyanidin (3.346 mg.ml⁻¹) being the most prevalent. The typical oxygen radical absorbance capacity (ORAC) of CherryActive[®] is 275 mmol/L Trolox equivalents (Brunswick Laboratories), which compares favourably with reported ORAC values (9.1-31.7

mmol/L Trolox equivalents) for other commercially available juices such as grape, pomegranate acai, blueberry and cranberry (33) and competitor montmorency cherry juice products (Cherrypharm Inc, 55mmol/L Trolox equivalents; (13).

Paragraph 9 The placebo was an isoenergetic synthetically derived fruit concentrate that was designed to have similar consistency and colour but without the phytochemical content of the cherry juice concentrate. Participants were instructed to take one serving in the morning and one in the afternoon after training.

Experimental protocol (Figure 1)

Paragraph 10 Participants arrived at the laboratory following an overnight fast, and after weighing rested in the supine position whilst a 10 ml resting blood sample was taken from an antecubital vein. Participants were then seated on the knee extension machine and pressure pain tolerance over vastus lateralis, rectus femoris and vastus medialis muscles was measured using an algometer (Wagner Instruments Inc, Connecticut, USA) as an index of muscle soreness. Measurements were made by the same investigator for each subject on each occasion.

Paragraph 11 Participants then completed a warm-up consisting of 3 sets of 5 repetitions of single leg knee extension exercise at 50 % 1RM each separated by a two-minute rest period. Participants then completed three single leg knee extension MVCs at 70 deg knee flexion angle (quadriceps muscle stretched) and separated by 2 min rest. After 5 min rest, participants completed 10 sets of 10 single leg knee extensions at 80 % of their one repetition maximum with elongated eccentric phase (lasting 3 s), each set was separated by 2 min rest. If subjects were unable to maintain the workload, the load was decreased by 10 % and this was then matched during the second trial. After completing the 10 sets, participants were asked to

repeat the 3 MVCs, with the first performed immediately after the last set, thereafter each MVC was separated by 2 min rest. Participants received verbal encouragement to perform maximally throughout the exercise protocol. Pressure pain tolerance was re-assessed after completion of the MVCs and a further blood sample was taken 10 min after completion of the last MVC.

Paragraph 12 Participants then returned to the laboratory at the same time of day 24 and 48 h later in an overnight fasted state. On each occasion a 10 ml resting blood sample was taken from an antecubital vein, whilst participants rested in a supine position; and pressure pain tolerance was re-assessed. Participants then repeated the warm-up and after 2 min rest completed three single leg knee extension MVCs separated by 2 min rest.

For the second main trial, the protocol was repeated but the exercise was performed with the contralateral leg to minimise the repeated bout effect. The experimental design was counterbalanced for trial order and for leg dominance.

Blood Analysis

Paragraph 13 At each time point fasting blood samples were collected into a 10 ml vacutainer containing no anti-coagulant and left at room temperature for one hour and then centrifuged at 4500 rpm for 15 min at 4°C. Serum samples were aliquoted into eppendorfs and stored at -80°C until analysis.

Paragraph 14 Serum samples were analysed for creatine kinase (CK), high sensitivity C reactive protein (hsCRP), total nitrotyrosine, protein carbonyls and total anti-oxidant capacity. Serum creatine kinase and CRP concentrations were determined using colorimetric

and turbidometric assays on a Siemens Advia 2400 autoanalyser and using commercially available reagents (hsCRP: PZ Cormay, Lublin, Poland, inter-assay CV 3.34%; CK: Siemens Medical Solutions Diagnostics Limited, Berks, UK, inter-assay CV 3%). Total antioxidant status (TAS) was assessed using a colorimetric assay (Randox Laboratories Ltd, Antrim, UK, inter-assay CV 3.65%). Serum nitrotyrosine (Millipore, Billerica, MA, USA, inter-assay CV 8 %) and protein carbonyls (Oxiselect™, Cell Biolabs Inc, San Diego, CA, USA, inter-assay CV 8 %) were quantified using commercially available ELISA kits. Changes over time were quantified as both percentage and absolute differences from pre-exercise values.

Biomechanical recordings

Paragraph 15 The knee-extension force was measured continuously during the experimental protocol using an inline force transducer (MCL; RDP Ltd. Wolverhampton, United Kingdom). The transducer was calibrated in the range from 0 to 100 kg using standard weights, and the force was recalculated and displayed on-line in Newtons. Data were recorded and digitised simultaneously via an analogue-to-digital converter (CED 1401power, Cambridge, UK), using Spike2 data acquisition software (CED, Cambridge, UK), with a 200 Hz sampling frequency.

Paragraph 16 Off-line data analysis was performed using custom made software developed in the script language Spike2 ver. 6.1 for CED (Cambridge, UK). The maximal isometric force was calculated as the average force over 1-s periods during the force plateau of each MVC contraction. This period did not include the first segment of the contraction (lasting about 0.5s) to exclude the period of force development. The highest of three MVCs completed before exercise was accepted as the initial MVC force. MVC force at each time point was normalised to pre-exercise value for the specific condition. Work done during the

exercise protocol was calculated by integrating the force over time trace and data were normalized to the corresponding 1 RM value to eliminate inter-individual and inter-leg variability.

Pressure Pain Threshold

Paragraph 17 Pressure pain threshold (PPT) was measured using a hand held algometer (Wagner Instruments Inc, Connecticut, USA) before, immediately, 24 and 48 h after completion of the exercise protocol. Muscle site contact was made with a cylindrical metal probe with flat head diameter of 10mm. The investigator applied a steadily increasing pressure to the muscle until the participant indicated that the point of discomfort had been reached. The applied pressure was recorded. Measurements were made over the muscle belly of rectus femoris (RF; distally to the midpoint of the line connecting the central superior aspect of the patella and anterior superior iliac spine), vastus lateralis (VL; distal half of the muscle along the line connecting the lateral superior aspect of the patella and the head of the greater trochanter) and vastus medialis (VM; 4-5 cm medial to the superior aspect of the patella). Data were normalized to pre-exercise values to reduce inter-individual variability.

Statistical Analysis

Paragraph 18 All data are reported as mean \pm SEM for pre-exercise, post-exercise, Day 1 recovery and Day 2 recovery for 10 participants unless otherwise stated. Data were analysed by two way repeated measures ANOVA (treatment (2 levels: CherryActive[®] and placebo) VS time (4 levels)) to determine whether there were any statistically significant effects of time or treatment. Mauchly's sphericity test was used to check homogeneity of covariance for all ANOVA analyses; violations of the assumption of sphericity were corrected using the Greenhouse-Geisser

adjustment. Where appropriate, the effect size statistic (η^2) was also calculated. The overall acceptable significance level of differences for all statistical tests was set at $p \leq 0.05$. The statistical analyses were performed in SPSS 14 (SPSS Inc., Chicago, IL) and Origin version 6.0 (Microcal Software Inc.) package software. Intra-class correlation analysis was used to assess the test-retest reliability of the muscle function (MVC force) measurement. A one-way random-effects single measure model [1,1] was applied on the data from repeated MVC tests to calculate the intraclass correlation coefficients (ICC).

Results

Paragraph 19 There was no difference between trials in the amount of relative work completed by subjects during the intensive exercise protocol (326 ± 31 vs 335 ± 27 , arbitrary units; $p=0.6$). Knee extension maximum voluntary contraction force decreased on average to 64% of pre-exercise levels after completing the intensive exercise protocol (main effect of time, $p<0.001$). Force recovery was significantly faster during the CherryActive[®] than placebo trials (interaction effect, $p=0.04$; η^2 0.36), with levels returning to 90.9 ± 4.0 % (vs 84.9 ± 3.2 %, placebo trial) after 24 h recovery and to 92.9 ± 2.8 % (vs 88.5 ± 3.1 %, placebo trial) after 48 h recovery respectively (Figure 2). A similar pattern was observed for non-normalised MVC force data, with both absolute change (trial by time interaction, $p=0.047$, η^2 0.32) and absolute MVC force (main trial effect, $p=0.03$, η^2 0.47, Table 1) data significantly higher in the CherryActive than Placebo trials. ICC of 0.955 was calculated from the repeated MVC tests performed by 3 participants during a preliminary and the main trial indicating high reliability of the MVC force measurement.

Paragraph 20 There was a significant reduction in the pressure pain threshold after 24 and 48 h recovery from the exercise protocol in all 3 muscles ($p<0.005$), indicating the development of muscle soreness. Similarly when a summed response across all muscles was considered there was a significant reduction in pressure pain tolerance after 24 and 48h recovery (interaction effect, $p<0.001$). However there was no significant difference between trials in pressure pain threshold of either individual muscles or the summed response (Figure 3).

Paragraph 21 There was a significant increase in serum CK activity after the exercise protocol (main effect of time, $p < 0.001$; Table 1). The percentage increase in serum CK activity from baseline was not statistically different between trials after 24h ($108.3 \pm 56.5\%$, CherryActive[®]; $127.6 \pm 54.3\%$, Placebo) and 48 h ($38.2 \pm 33.2\%$, CherryActive[®]; $73.7 \pm 31.2\%$, Placebo) recovery from the exercise protocol (Figure 4). However both total serum CK activity (trial main effect, $p = 0.055$; Table 1) and the absolute increase in serum CK activity tended to be higher during the CherryActive[®] than the Placebo trial (interaction effect, $p = 0.063$; Table 1). Protein carbonyl content also increased significantly after the exercise protocol (main effect of time, $p < 0.001$; Table 1). Both the percentage (main trial effect, $p = 0.013$, Figure 5) and the absolute (main trial effect, $p = 0.079$, Table 1) increase in protein carbonyl content from baseline was lower during the CherryActive[®] than placebo trials. However, protein carbonyl content was significantly higher throughout the experimental protocol in the CherryActive[®] than placebo trials (trial main effect, $p = 0.001$, Table 1). There was no statistically significant effect of time or condition on total serum nitrotyrosine concentration or hsCRP although there was a tendency for hsCRP to be higher during the placebo than the CherryActive[®] trials (Table 1). For 7 of the 10 subjects hsCRP concentrations were close to the detection limit of the assay 0.1 mg/L. In the remaining 3 subjects, baseline hsCRP concentration was observed to be lower in the CherryActive[®] (5.1 ± 3.0 mg/L) than Placebo (9.0 ± 2.8 mg/L) trials.

Paragraph 22 There was no significant change over time in serum total anti-oxidant capacity, nor was there any difference between trials (Table 1). The total anti-oxidant capacity values were at the top end of the normal range (1.3 - 1.77 mmol.l⁻¹) for both Placebo and CherryActive[®] trials (1.74 ± 0.03 vs 1.72 ± 0.05 mmol.l⁻¹).

Discussion

Paragraph 23 The main finding of this study was that recovery of knee extensor maximum isometric strength was enhanced after consuming montmorency cherry juice for 7 d prior to and 2 d after an intensive knee extensor resistance training session. This improvement in functional recovery was accompanied by a reduction in serum protein carbonyls indicative of reduced oxidative damage. However no other markers of muscle damage or inflammation were favourably affected by montmorency cherry juice consumption. Nor was there evidence of a significant reduction in muscle soreness since montmorency cherry juice consumption had no effect on muscle pressure pain tolerance.

Paragraph 24 These findings are in agreement with those of Connolly et al (10) and Howatson et al (13) who also found that montmorency cherry consumption enhanced maximum isometric force recovery of the elbow flexors after eccentric exercise and of the knee extensors after completing a marathon, respectively. Interestingly, Howatson et al (13) found that the magnitude of the immediate reduction in maximum isometric force production after completing the marathon was not different between trials. This was suggested to indicate that the cherry juice supplement did not prevent the initial muscle injury, which was presumably induced by a combination of mechanical disruption of the myofibrils and increased generation of ROS and NO species during exercise. Instead the cherry juice was suggested to blunt the secondary muscle damage response associated with the local inflammatory response in the damaged muscle, and this was corroborated by the finding of reduced IL6, CRP and uric acid response to the marathon race. In the present study the reduction in knee extensor maximum isometric force 4 min after completion of the exercise protocol was also very similar between trials (~ 36%), suggesting similar degrees of long lasting fatigue immediately after exercise. However, hsCRP was not elevated in response to

the single leg knee extensor exercise, perhaps the smaller muscle mass involvement was not sufficient to elevate this marker of systemic inflammation, and unfortunately no markers of local muscle inflammation were measured. Surprisingly Connolly et al (10) did not measure MVC force immediately after the eccentric elbow flexor exercise so data are not available for comparison.

Paragraph 25 Kuehl et al (23) and Connolly et al (10) reported that participants experienced less muscle soreness after the Hood to Coast relay race (mean 26km run) and eccentric elbow flexion exercise when cherry juice was consumed for a period prior to and after the exercise. However in common with Howatson et al (13), we found no reduction in muscle soreness in the cherry juice trial, which in the face of reduced inflammation, at least in the former trial, is perhaps surprising. Pressure pain tolerance on the belly of the muscle was used as the measure of muscle soreness in the present study in an attempt to ameliorate the subjective nature of the visual analogue scale measure of soreness. However, any measure of muscle soreness is by its very nature subjective and therefore subject to variability, although PPT reliability is maximised when measures are taken over the muscle belly, by the same investigator on each occasion, as in the present study (27). However rate of pressure development, which also introduces variability was not directly controlled in the present study although Kinser et al (22) found that a single investigator has a high degree of reliability in rate of pressure development. The magnitude of muscle soreness reported by our subjects was modest (at maximum ~27% reduction in PPT) compared to that induced by 40 min downhill running (~50%). The repeated bout effect is well-documented (25), thus it was perhaps unsurprising that we were only able to induce relatively modest muscle soreness in these participants who regularly performed intensive knee extensor resistance training. This small magnitude of change in PPT may have limited the ability of our experimental paradigm to detect any effect of the montmorency cherry juice.

Paragraph 26 The repeated bout effect is a well accepted phenomena, which refers to the marked reduction in muscle damage when repeated bouts of eccentric exercise are performed up to 6 months apart (26). The effect is generally considered to be peripheral in nature and isolated to the specific muscle/limb exercised. The repeated bout effect must be considered when designing studies to investigate the efficacy of strategies to counteract muscle damage. In this instance we used single limb exercise, with trials counterbalanced for supplement and leg dominance to avoid the response to exercise in trial 1 influencing results in trial 2. However, there is one published study which has demonstrated a small protective effect against eccentric elbow flexor damage and soreness in the contralateral arm (14). A second bout of single arm exercise was performed by both contralateral and ipsilateral arms 2 weeks after performing the same exercise with one arm only. The repeated bout effect was much smaller in the contralateral than ipsilateral arm, and except for muscle soreness the contralateral repeated bout effects were evident only at 96 h not 48h post-bout 2 of exercise. The observed differences in parameters during the first 48h of recovery in the present study were not therefore confounded by any repeated bout effect from Trial 1 in the contralateral leg. In line with previously published crossover phytonutrient studies, we adopted a 2 week washout period between trials (10, 35). In addition, the study was fully counterbalanced.

Paragraph 27 Despite the improvement in functional recovery with montmorency cherry juice consumption, there does not appear to be any protective effect in terms of the extent of the structural damage to the muscle since the percentage change in serum CK was not different between trials. Indeed, the absolute concentration and absolute change in CK after the intensive exercise tended to be larger during the cherry juice trial. This finding also concurs with Howatson et al (13) where despite the improved functional recovery and reduced inflammatory response to marathon running after cherry juice consumption, the increases in CK and lactate dehydrogenase were not different between trials. Unfortunately in

many studies, markers of muscle damage have been measured in the absence of markers of oxidative damage and vice versa. However in the present study and others there is a dissociation between the extent of oxidative damage induced by exercise as indicated by a variety of measures such as protein carbonyls, isoprostanes (7, 20, 21), TBARS (13), MDA (7) and the increase in serum CK and LDH. In our hands, the exercise-induced increase in PC was significantly attenuated by cherry juice consumption whilst the increase in CK was if anything exaggerated in the cherry juice condition. There is considerable variation across studies in the oxidative damage response to resistance exercise with some groups finding evidence of oxidative damage (4, 11, 16, 31, 32) and others not (1, 3). This variation may be attributable to differences in the exercise mode, duration and intensity, training status of participants as well as the use of a variety of indirect measures of oxidative damage to muscle. Veskokoukis et al (37) recently demonstrated that blood and muscle protein carbonyls after swimming to exhaustion were closely correlated in rats and therefore suggest that blood protein carbonyls, as well as catalase and reduced glutathione, provide reliable indicators of skeletal muscle redox status. In contrast TBARS, xanthine oxidase and total antioxidant capacity were found to be poorly correlated with changes in muscle redox status.

Paragraph 28 Although the extent of exercise-induced oxidative damage was attenuated in the montmorency cherry juice trial, there was no difference between trials in total antioxidant capacity. This is in contrast to the findings of Howatson et al (13), who found a greater increase in total antioxidant capacity after the race in the cherry juice trial. However in the present study, blood samples were taken from overnight fasted subjects at least 8 h after consumption of the previous dose of montmorency cherry juice concentrate, whereas subjects in the Howatson et al (13) study were not fasted. Consumption of 28 g of sweet Bing cherries has been shown to result in a significant increase in plasma lipophilic ORAC score and reduction in plasma urate that was present up to 5 h post consumption (17, 29). Chronic

consumption of Bing cherries (280 g/d for 28d) has also been shown to reduce some serum markers of inflammation (CRP, NO and RANTES) even in the fasted state (19). Although, the dose response to montmorency cherries has not yet been studied it is likely to be similar in time course if not in magnitude. This may explain the absence of any effect of CherryActive consumption on total antioxidant capacity, since blood samples were taken more than 5h after consumption of CherryActive (at least 8h), and the period of supplementation was only 7 not 28d and may not therefore have been long enough to induce an increase in TAS through the day.

Paragraph 29 The elevated baseline measures of muscle and oxidative damage, which was statistically significant in the case of protein carbonyls, is a potentially confounding factor. This presumably indicates a higher level of background oxidative damage in the CherryActive trial. Although participants were instructed to perform the same training over the 7d of supplementation prior to the controlled intensive exercise bout it appears that for at least 7 of the 10 subjects this was not the case. Despite this we are unaware of any mechanism by which the significantly higher background serum PC would influence the subsequent response to the same exercise protocol. Although as a result, the data should only be generalised with caution.

Paragraph 30 In conclusion, consumption of montmorency cherry juice concentrate for 7 days prior to, the day of and two days after completing a bout of intensive knee extensor resistance enhanced recovery of isometric muscle strength. This improvement in functional recovery was accompanied by a reduction in oxidative stress presumably due to the anti-inflammatory and anti-oxidative effects of the phytochemicals within the montmorency cherries.

Acknowledgments

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Figure Legend

Figure 1: Experimental protocol

Figure 2: MVC force normalised to pre-exercise values. There was a main effect of time ($P < 0.001$) and a significant interaction effect ($P = 0.04$) with enhanced MVC force recovery in the CherryActive[®] trial.

Figure 3: Pressure pain threshold normalised to pre-exercise values was decreased after the exercise intervention ($P < 0.001$) but there was no statistically significant effect of trial or trial by time interaction for the summed response.

Figure 4: Percentage change in serum creatine kinase activity from pre-exercise values. There was a main effect of time ($P = 0.031$).

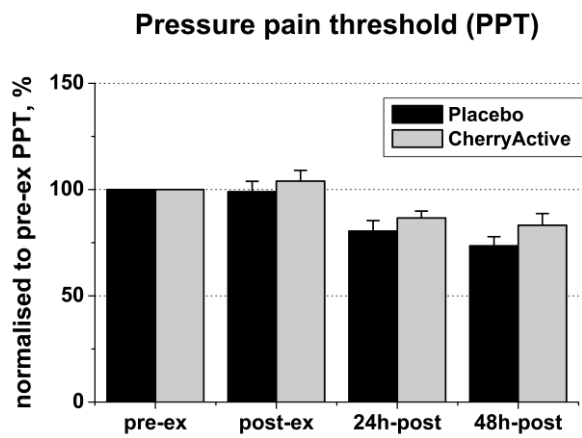
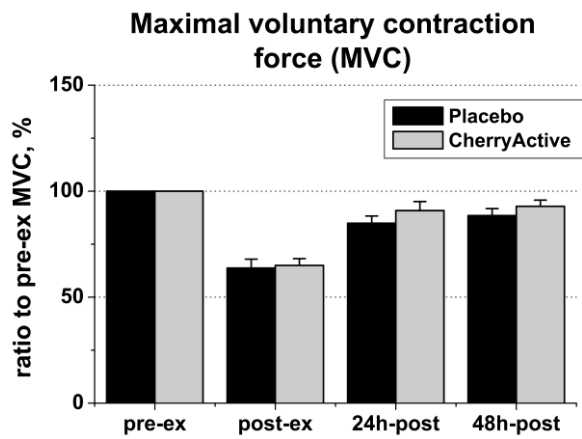
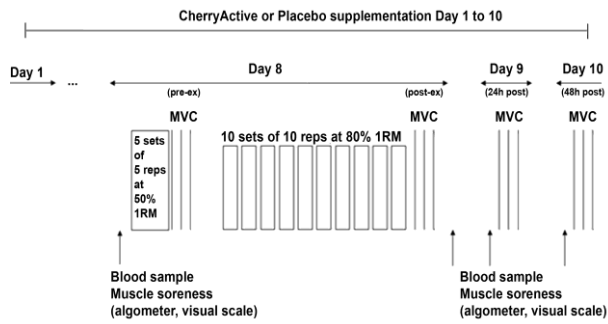
Figure 5: Percentage change in serum protein carbonyls from pre-exercise values. There was a main effect of time ($P = 0.034$) and a significant effect of trial ($P = 0.013$).

Reference List

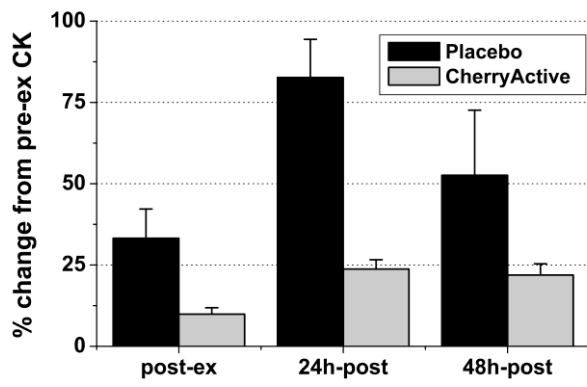
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Creatine kinase activity (CK)



Protein carbonyls (PC)

