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Recovery facilitation with Montmorency cherries following high-intensity, metabolically challenging exercise

Running Title: Montmorency cherries and exercise recovery

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Abstract

The impact of Montmorency tart cherry concentrate [MC] on physiological indices and functional performance was examined following a bout of high intensity stochastic cycling. Trained cyclists (n = 16) were equally divided into 2 groups (MC or isoenergetic placebo [PLA]) and consumed 30 mL of supplement, twice per day for eight consecutive days. On the fifth day of supplementation, participants completed a 109 minute cycling trial designed to replicate road race demands. Functional performance (maximum voluntary isometric contraction [MVIC], cycling efficiency, 6-second peak cycling power) and delayed onset muscle soreness [DOMS] were assessed at baseline, 24, 48 and 72 h post-trial. Blood samples collected at baseline, immediately pre and post-trial, and 1, 3, 5, 24, 48 and 72 h post-trial were analysed for indices of inflammation (IL-1-β, IL-6, IL-8, TNF-α, hsCRP), oxidative stress (lipid hydroperoxides) and muscle damage (creatine kinase). MVIC ($P < 0.05$) did not decline in the MC group (vs. PLA) across the 72 h post trial period and economy ($P < 0.05$) was improved in the MC group at 24 h. IL-6 ($P < 0.001$) and hsCRP ($P < 0.05$) responses to the trial were attenuated with MC (vs. PLA). No other blood markers were significantly different between MC and PLA groups. The results of the study suggest that Montmorency cherry concentrate can be an efficacious functional food for accelerating recovery and reducing exercise-induced inflammation following strenuous cycling exercise.

KEY WORDS: CYCLING, FUNCTIONAL PERFORMANCE, INFLAMMATION, MUSCLE FUNCTION

Introduction

Athletic training and competition causes physiological stress that is followed by a period of recovery (Leeder et al. 2012). A high-priority for athletes is the ability to accelerate recovery in order to allow subsequent training or competition to be attained at the requisite intensity and as such, numerous recovery interventions have been investigated (Barnett 2006; Howatson et al. 2008). Recently, antioxidant supplementation has received a great deal of attention (Urso et al. 2003; Powers et al. 2008; Howatson et al. 2010; McAnulty et al. 2011) due to the purported ability to reduce inflammation (Bell et al. 2013; Kelley et al. 2013) and oxidative stress (Mastaloudis et al. 2006; Goldfarb et al. 2011; Peternelj et al. 2011; Bell et al. 2013) that manifest during and following intense exercise. Optimum recovery is of great importance to athletes in numerous sporting scenarios where repeated days performance might be required (Bell et al. 2013). Consequently, antioxidant supplements may have a role to play in optimising recovery following strenuous exercise.

Montmorency tart cherries have been proposed as a recovery supplement due to their high concentrations of phytochemicals, and in particular, the flavanoids anthocyanins (Howatson et al. 2010; McCune et al. 2011; Bell et al. 2013). Anthocyanins have been shown to reduce oxidative stress and inhibit the activity of the inflammatory mediator cyclooxygenase (COX) to a similar extent as non-steroidal anti-inflammatory drugs (NSAIDs) (Wang et al. 1999; Seeram et al. 2001). These findings have led to a series of studies investigating the use of Montmorency cherries influencing recovery (Connolly et al. 2006; Ducharme et al. 2009; Howatson et al. 2010; Kuehl et al. 2010; Bowtell et al. 2011; Bell et al. 2014b). Connolly et al (2006) was the first to demonstrate accelerated muscle function recovery with Montmorency cherry supplementation in the days following damaging high intensity eccentric exercise, (Connolly et al. 2006). Using a similar study design, Bowtell et al (2011) also found that following eccentric-induced muscle damaging exercise, knee extensor force recovered more rapidly with Montmorency cherries compared to an isoenergetic

placebo (Bowtell et al. 2011). Unfortunately, Connolly et al (2006) did not measure markers of inflammation or oxidative stress and although Bowtell et al (2011) reported that a trend towards lower protein oxidation (protein carbonyls) with Montmorency cherry supplementation, there were no differences in indices of inflammation.

An attenuated inflammatory and oxidative stress response to exercise and more rapid recovery of muscle performance has also been reported using a different model of exercise when supplementing with Montmorency cherries (Howatson et al. 2010). Howatson et al (2010) showed that following marathon running, where there is high mechanical and metabolic stress, inflammation (interleukin-6 [IL-6], high-sensitivity C-reactive protein [hsCRP]) and oxidative stress (thiobarbituric acid reactive substances [TBARS]) were lower in the cherry supplemented group versus a placebo. Additionally, in the 48 h post-race, recovery of maximal force during a voluntary isometric contraction (MVIC) of the knee extensors was improved in the Montmorency cherry group compared to the placebo group. In contrast to Bowtell et al. (2011), the marathon running used in this study induced systemic inflammation sufficiently to demonstrate changes in the inflammatory response between groups. Both studies, however, reported reduced oxidative stress responses to the exercise protocol; Howatson et al (2010) reported attenuated lipid peroxidation, whilst Bowtell et al. (2011) reported reduced protein oxidation. These discrepancies might be explained by the differences in exercise mode used to induce the stress response. Bowtell et al (2011) used a protocol designed exclusively to induce a mechanical stress with high force eccentric muscle actions. Conversely, the marathon running used by Howatson et al (2010), placed a high degree of both mechanical and metabolic stress due to the eccentric muscle actions and prolonged high energy expenditure involved in long distance running (Howatson et al. 2010). Collectively,, it is conceivable that Montmorency cherries could also be suited to aiding recovery by reducing inflammation associated with exercise involving a high metabolic component.

Consequently, exercise posing a challenge that is predominantly metabolic in nature, provides a highly appropriate model to investigate the impact of Montmorency cherries on recovery and may provide insight into the relationship between inflammation, oxidative stress and muscle performance. Cycling is a sport that requires little or no eccentric muscle actions, but requires prolonged high metabolic activity, that can cause perturbations in inflammation, oxidative stress and muscle function (Bell et al. 2014b). Additionally, given the evidence from previous research regarding improved strength recovery with Montmorency cherries (Connolly et al. 2006; Howatson et al. 2010; Bowtell et al. 2011), the assessment of muscle function following an exclusively metabolic challenge, such as cycling, may translate to other exercise paradigms where a metabolic (as opposed to mechanical) physiological stress is imposed.

In this study, the primary objective was to identify the impact of Montmorency cherry supplementation on recovery of muscle function following an exercise stress induced through a metabolic challenge (high-intensity stochastic cycling). It was hypothesised that supplementation with Montmorency cherries would accelerate recovery of muscle function and this would be accompanied by attenuation in the exercise-induced inflammation and oxidative stress responses following simulated road race cycling.

Materials and Methods

Participants

Sixteen healthy, male trained cyclists (mean ± SD age, height, mass, VO_{2peak} was 30 ± 8 yrs; 181.1 ± 6.7 cm, 76.5 ± 9.2 kg, 61.6 ± 10.4 $mL.kg^{-1}.min^{-1}$, respectively) were recruited to take part in the study. Training and health status were assessed through the completion of a cycling training history and health screening questionnaire, respectively. For inclusion, participants must have cycle trained for >5 hours per week over the preceding 24 months. Additionally, participants agreed to withdraw from any other exercise throughout the duration of this study. Exclusion criteria for the study

included; >45 years of age, female, allergy to specific fruit products, currently taking any nutritional supplements or medication, history of gastrointestinal, renal or cardiovascular disease. Following institutional ethical clearance, written, informed consent was collected from all participants after both verbal and written briefings on the requirements of the study.

Study Design

The study utilised a double blind, counterbalanced, placebo controlled independent groups design in order to identify the effects of Montmorency tart cherry concentrate (MC) on recovery from a metabolic challenge (prolonged, high-intensity, stochastic cycling). The protocol required participants to complete 6 visits to the laboratory across a period of up to 20 days (**Figure 1)**. Briefly, on visit 1, participants completed preliminary aerobic profiling ($\dot{V}O_{2peak,}$ W_{max}) and baseline measures of functional performance (lower limb active muscles soreness assessment [DOMS], cycling economy [CE], 6-second peak cycling power and maximum isometric voluntary contraction of the quadriceps [MIVC]). Participants returned to the laboratory within 2-4 days and completed familiarisation of the exercise protocol only. Following this, participants were subject to stratified randomisation based on VO_{2peak} and then allocated to either MC or Placebo (PLA) groups (63.1 ± 11.0 vs. 60.2 ± 10.2 $mL.kg.min^{-1}$, respectively). Participants completed a 4 day supplement loading phase leading to visit 3, which began a minimum of 7 days following the familiarisation trial. Visit 3 consisted of a prolonged, high-intensity, stochastic cycling trial lasting 109 minutes (**Figure 2)** performed on an electromagnetically braked, cycle ergometer (Velotron RacerMate, Seattle, WA). Visits 4-6 took place 24, 48 and 72 h post-trial, during which participants repeated the baseline measures, additionally, each visit was conducted at 7.45am following an overnight fast to avoid diurnal variation and ensure consistent intervals between supplementation and exercise. Venous blood samples were collected at baseline (prior to 4 the day loading phase), immediately pre-trial, immediately post-trial and 1, 3, 5, 24, 48 and 72 h post-trial for markers of inflammation, oxidative stress and muscle damage.

Supplementation and dietary control

Following group allocation, participants were provided with MC or placebo (PLA) supplementation and instructed to consume 30 mL of the supplement twice per day (8 am and 6 pm) for 8 consecutive days (4 days pre-, on the day of, and 3 days post trial). On the visits involving exercise (visits 3-6) supplementation was consumed 15 minutes following venous blood sampling and 10 minutes prior to performance. Previous research (Bitsch et al. 2004; Kurilich et al. 2005) has demonstrated that systemic anthocyanin bioavailability increases to a peak between 1-2 hours post-ingestion, which coincides with the completion of the exercise tasks in this study. The concentrate was consumed with 100 mL of water and this supplementation strategy has previously been used in previous work demonstrating accelerated recovery (Bowtell et al. 2011; Bell et al. 2014b). According to manufacturer's specification (Cherry Active Ltd, Hanworth, UK), each 30 mL dose of MC contained ~90-110 Montmorency tart cherries; independent laboratory analysis shows the juice to provide 9.2 $mg.mL^{-1}$ of anthocyanins and 669.4 $mg.mL^{-1}$ of carbohydrate (Atlas Biosciences, Tuscon, AZ). The PLA was a commercially available mixed berry cordial (less than 5% fruit in concentrate form), mixed with 100 mL water and maltodextrin (MyProtein Ltd, Northwich, UK) until matched for carbohydrate content of the MC (20.07 g). All supplements were prepared in opaque bottles by an independent member of the department in order to maintain the double blind design of the study.

During the dosing period (4 days pre-trial to 3 days post trial), participants were required to adhere to a low-polyphenolic diet. More specifically, fruits, vegetables, tea, coffee, alcohol, chocolate, cereals, wholemeal bread and grains were prohibited and in order to assess for dietary compliance, food diaries were completed for the duration of study, which has been used successfully in previous work (Howatson et al. 2012).

Pre-trial Assessments

During the first visit to the laboratory, participants completed two cycling tests; a submaximal exercise test and an incremental ramp exercise test to exhaustion to elucidate maximal aerobic power (W_{max}), VO_{2peak} and gas exchange threshold (GET); both tests were completed using the aforementioned electro-magnetically braked cycle ergometer. The submaximal test required participants to begin cycling at 100 W, which increased by 25 W every 4 minutes. Heart rate and capillary blood samples were taken from the earlobe in the last 30 s of each stage and immediately analysed for blood lactate concentration using a Biosen C-Line analyser (EKF Diagnostics, Cardiff, UK). Cycling was terminated when the lactate turn-point had been reached (Davis et al. 1983). Throughout the submaximal test, pulmonary gas exchange was continually monitored through an online system (Oxycon Pro, CareFusion, San Diego, CA). Data were averaged over the final 30 s of each stage in order to analyse for relative $\dot{V}O_2$ cost. Following the completion of the submaximal test, participants were given 10 minutes rest prior to completing the incremental ramp test. The ramp test consisted of 3 minutes of cycling at 100 W, followed by an increase in work rate of 1 W every 3 seconds (20 $W.min^{-1}$) until the participant reached volitional exhaustion or cadence dropped below 70 rpm. Breath-by-breath pulmonary gas exchange data was collected throughout the ramp protocol and $\dot{V}O_{2peak}$ was calculated as the highest 30-second average (Bailey et al. 2009a). Using the regression equation calculated from the submaximal $\dot{V}O_2$ data, and the $\dot{V}O_{2peak}$, it was then possible to identify the power output achieved at $\dot{V}O_{2peak}$ (W_{max}). GET was determined using either the first disproportionate increase in CO_2 production ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$ or an increase in expired ventilation ($\dot{V}_E/\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$) (Bailey et al. 2009a). Saddle, handlebar height and fore/aft position was recorded and replicated throughout all further cycling tests in the study for each participant.

Pre-Trial Functional Performance Assessment

Prior to entering into the supplementation and trial period, participants completed a battery of physical tests in order to gain baseline measures of performance capability. The battery consisted of

active muscles soreness assessment (DOMS), cycling economy (CE), 6-second peak cycling power and maximum voluntary isometric contraction of the quadriceps (MVIC). DOMS was assessed using a 0-200 mm visual analogue scale, with the phrases 'No pain' at one end and 'Pain/Soreness as bad as it could be' at the other (Howatson et al. 2010). Participants completed a squat to approximately a 90° knee flexion before standing and immediately marked upon the scale to indicate their level of soreness (Howatson et al. 2010). The cycling economy test consisted of a 10 minute cycle at the power output corresponding to 90% of the GET (Bailey et al. 2009a). Breath-by-breath pulmonary gas exchange was monitored throughout and $\dot{V}O_2$ data was averaged across the final 4 minutes of the test to give the relative O_2 cost for that intensity. Following the CE test, 6-second peak power was determined. A 5 minute standardised warm-up, was followed by the completion of a series of 6-second maximal sprints through a range of gears, the gear which elicited the highest power output was subsequently used for all further 6 second peak power tests A minimum of 3 minutes between sprints was enforced to allow for recovery. MIVC of the dominant knee extensors was determined using a strain gauge (MIE Medical Research Ltd., Leeds, UK). Participants were seated on a platform and the strain gauge was attached to the dominant ankle at an internal joint angle of 80° (verified by a goniometer). Three submaximal trials were completed at approximately 50%, 70% and 90% of participants' perceived maximum, followed by three maximal trials, each separated by 1 min. Participants were given standardised verbal encouragement for the duration of each 3 second contraction and the peak force was recorded as the baseline measure of MIVC. Previous work from our lab has demonstrated the technical error of measurement to be 26 Newtons or 3.7% for this measure of muscle function (Leeder 2013).

Fatigue Inducing Protocol

Participants completed a 10 minute, self-selected warm-up on the cycle ergometer, which included 3 x 3 second sprints at perceived efforts of 70%, 80% and 90%, occurring at 7, 7.5 and 8 minutes, respectively. The main exercise task (**Figure 2)** was designed to replicate the demands of a road

cycling race and has previously been used in other cycling studies (Vaile et al. 2008; Bell et al. 2014b). The task consisted of 66 sprints lasting 5, 10 or 15 seconds with a work (W) to recovery (R) ratio of 1:6, 1:3 or 1:1. Sprints were divided into nine sets, with an active recovery (ACT) period lasting 5 minutes between sets 1-2, 2-3, 4-5, 5-6, 7-8 and 8-9. All R and ACT periods were completed at a power of 40-50% of W_{max}. An additional 9 minutes of sustained effort was incorporated through the completion of three time trials (TT) lasting 2 minutes, 2 minutes and 9 minutes, respectively. Participants were instructed to complete as much work as possible during all TT periods and received verbal encouragement throughout. Power output was collected throughout all trials at a frequency of 3 Hz and subsequently transformed into work done (kJ). Water was available for participants to drink *ad libitum* and strong verbal encouragement was provided by the same researcher throughout the duration of the trial.

Blood Sampling

Blood sampling was conducted using venepuncture and following collection, tubes were immediately centrifuged at 2400 x *g*, 4°C for 15 minutes before having the supernatant removed and stored in aliquots. Aliquots were then immediately stored at -80°C and subsequently analysed for indices of inflammation, oxidative stress and muscle damage.

Muscle Damage Indices

Serum creatine kinase (CK) was analysed using kinetic UV test (Olympus Analyser, Olympus Diagnostica GmbH, Hamburg) using the method based upon the recommendations of the International Federation of Clinical Chemistry (IFCC). Inter- and intra-assay coefficients of variation were both 1.5%.

Inflammatory Indices

Plasma tumour necrosis factor alpha (TNF-α), interleukin-1-beta (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8) were analysed using a multiplex electrochemiluminescence assay (Sector Imager 2400, Meso Scale Discovery, Rockville, MD). Intra and inter plate coefficients of variation were; 4.64 and 11.5% (TNFα), 6.8% and 9.5% (IL-1- β), 4.9% and 8.8% (IL-6), 3.41% and 8.5% (IL-8) and respectively. Serum high sensitivity C-reactive protein (hsCRP) was analysed through an immunoturbidimetric assay (Roche Modular P, Roche Diagnostics Ltd, Burgess Hill, UK). Respective inter and intra assay coefficients of variation were 0.7% and 4.7%.

Oxidative Stress Indices

Plasma aqueous phase lipid hydroperoxides (LOOH) were assessed using the ferrous oxidation of xylenol orange method (FOX 1), using a modification of the methods by Wolff (1994) and Nouroozzadeh et al. (1994). Briefly, this ferrous iron/xylenol orange (FOX) assay quantifies the susceptibility to iron-induced lipid hydroperoxide formation in blood. The presence of iron ions in the assay protocol might therefore, yield slightly higher lipid hydroperoxide values compared with other methods. However, all samples were quantified in duplicate as a single batch analysis therefore any artifactual increase as a result of iron ion contamination would be consistent across all biological samples. Using a spectrophotometer (U-2001, Hitachi, England) absorbance was read at 560 nm against a linear standard curve (range 0–5 $\mu mol.L^{-1}$). Inter- and intra-assay coefficients of variation were <4% and <2%, respectively.

Statistical Analysis

All data analyses were conducted using IBM SPSS Statistics 20 for Windows (Surrey, UK) and are reported as mean ± standard deviation. Differences between blood marker variables were analysed by using a condition (MC v PLA) by time-point (Pre-supplement, Pre-trial, 1, 3, 5, 24, 48 and 72 h) mixed model analysis of variance (ANOVA). Functional performance measures were analysed using the same model however with four fewer levels (Pre-supplement, Pre-trial, 24, 48 and 72 h). Where

significant group baseline differences were apparent, results were normalised to baseline values. As a result, post-exercise MVIC, hsCRP and cycling efficiency results were represented as a percentage of their baseline value, prior to subsequent statistical analysis. Mauchley's Test of Sphericity was used to assess homogeneity of data and where violations were present, corrections were made using Greenhouse-Geiser adjustment. Where necessary, interaction effects were assessed using LSD *post-hoc* analysis. Prior to all analyses, a significance level of $P < 0.05$ was set.

Results

Baseline measures of MVIC were 634 ± 115 Newtons for MC and 713 ± 131 Newtons in the PLA group. A group effect demonstrated that MVIC decline was significantly attenuated in the MC group ($F_{(1,2)}$ = 7,913, P = 0.014) versus PLA (**Figure 3)** with between group differences equating to 10, 12 and 21% at 24, 48 and 72 h respectively, although there was no interaction effect present. MVIC values in the MC group did not drop below baseline scores, whilst the PLA group scores remained depressed throughout the 72 h measurement period.

No group effect was found for cycling efficiency following baselines measures of 41.1 ± 7.9 mL.kg.min^{-1} for the MC group and 38.3 ± 10.6 mL.kg.min^{-1} in the PLA group. However, a significant interaction effect ($F_{(1,4)}$ = 4.336, P = 0.009) was observed. Post-hoc testing identified a significant difference at 24 h (P = 0.015) where $\dot{V}O_2$ was 4% lower in the MC than the PLA group when normalised to baseline values. DOMS demonstrated a significant main effect for time ($F_{(1,4)}$ = 4.902, P = 0.005) with increases in DOMS ratings reported in both groups; there were no group or interaction effects. Baseline 6-second sprint power results were 785 ± 205 W and 834 ± 205 W for MC and PLA respectively. Analysis demonstrated a significant effect for time ($F_{(1,4)}$ = 5.921, P = 0.002), with power returning towards baseline values in both groups across the 72 hour post-trial period, but there were no group or interaction effects. A summary of these measures are presented in **Table 1**.

309

310 Evaluation of plasma revealed baseline IL-6 values of 0.83 ± 0.43 and 1.15 ± 0.45 pg.mL^{-1} for MC and
311 PLA groups respectively. Data analysis showed a group effect ($F_{(1,2)}$ = 39.992, P < 0.001)
312 demonstrating that the IL-6 response was significantly attenuated in the MC (vs. PLA) group across
313 the protocol. The peak group difference of 1.36 pg.mL^{-1}, occurred immediately post-trial (**Figure 4**).
314 Following measures taken at baseline (MC 1.14 ± 1.18 and PLA 0.55 ± 0.53 pg.mL^{-1}), hsCRP remained
315 lower in the MC group versus PLA group at all time points except 24 h (**Figure 5**). Conversely in the
316 PLA group, hsCRP values remained above baseline across the course of the study period. Analysis of
317 hsCRP data demonstrated significant differences between groups ($F_{(1,2)}$ = 2.431, P < 0.05) The peak
318 difference between groups (76%) occurred at 24 h post trial before a trend for both groups to return
319 towards pre-exercise levels by 72 h. Further markers of inflammation; IL-8 ($F_{(1,9)}$ = 25.364, P < 0.001)
320 and TNF-α ($F_{(1,9)}$ = 4.665, P < 0.001), muscle damage, CK ($F_{(1,9)}$ = 2.049, P < 0.05), and oxidative stress,
321 LOOH ($F_{(1,9)}$ = 4.969, P < 0.001), all demonstrated main effects for time. Specifically, IL-8 was
322 increased at post-trial and 1, 24 and 48 h (P < 0.05), CK was increased at post-trial, 3 and 5 h (P <
323 0.05) and LOOH was significantly raised from pre-trial at 3 h (P < 0.05). No group or interaction
324 effects were found for any of these measures. Lastly, IL-1-β did not show any group, time or
325 interaction effects in response to the trial (**Table 1**).

326

327 The total work done (kJ) across the exercise protocol was 155.1 ± 23.6 kJ and 151.3 ± 27.9 kJ for MC
328 and PLA groups, respectively, which were not different between groups. Finally, food consumption
329 data was not subjected to detailed scrutiny with regards to macro and micronutrient intake;
330 however food diaries were reviewed for compliance with dietary restrictions. Analysis of diaries
331 revealed 100% adherence to the imposed dietary restrictions.

332

333 **Discussion**

It was hypothesised that consumption of Montmorency cherry concentrate would both decrease the inflammatory and oxidative stress responses following a simulated cycling road race and accelerate recovery of functional performance. In support of the hypothesis, there was no post-trial decline in MVIC performance with MC consumption indicating a protective effect on muscle function. No other functional performance measures were different between groups over time. With regards to inflammation, both IL-6 and hsCRP were attenuated in the MC group. Conversely, IL-1-β, IL-8 and TNF-α did not differ between groups. Contrary to our hypothesis, the oxidative stress response (LOOH) did not differ between groups. Lastly, there were no differences in CK between groups at any point following the exercise task.

With regards to MVIC, significant declines in performance were not apparent for the 72 h post-exercise assessment period with MC supplementation. On the contrary, PLA group performance of MVIC remained depressed below baseline at 72 h, with a 14% mean difference between groups. This finding suggests that MC consumption may preserve muscle function which normally declines in association with the post-exercise stress response. Initial cellular disruption from the exercise stress is followed by a secondary inflammatory response which can further exacerbate the perturbations in homeostasis (Howatson and van Someren 2008). Although there were no differences in the CK response between groups, there was reduced IL-6 and hsCRP found in the MC group, suggesting that the acute inflammatory stress response was dampened. This may have resulted in a reduction in proteolytic and lipolytic cascades that are associated with inflammation via the cyclooxygenase, prostaglandin, IL-6 pathway (Trappe et al. 2013). Whilst this is not the first study to demonstrate attenuated decline in MVIC performance with MC supplementation (Connolly et al. 2006; Howatson et al. 2010; Bowtell et al. 2011), it is the first to do so following exercise that did not include a mechanically damaging component through eccentric muscle actions and as a result, the stress responses caused can be attributed to metabolic and not a mechanical challenge. The small increases in CK and DOMS observed in the current study also suggest that there was minimal muscle

cell membrane permeability which is associated with mechanical damage, and that the inflammatory responses can be attributed to metabolic processes. As a result the preservation of MVIC in the MC group may be attributed to attenuated inflammation, and consequently may play a key role in recovery, independent of cellular disruption.

The continued decline of MVIC performance after 48 h in the PLA group is a further interesting finding. Recovery of MVIC has been shown to return towards baseline 24-72 hours post-exercise previously (Connolly et al. 2006; Howatson et al. 2010). However, the time-course of muscle function decline in the current study is not unique; previous literature has also demonstrated continued decline in muscle function (peak isokinetic torque) across 72 hours following eccentrically induced muscle damage (Cockburn et al. 2010). Such contrasting responses may be attributed to differences in exercise mode, participant cohort and methods used to assess muscle function. Importantly however in regards to the current study, the maintenance of MVIC across the 72 h post-exercise, indicates a protective effect of MC upon an aspect of muscle function.

Although no interaction effects were found, a significant group difference showed hsCRP was elevated in the PLA group throughout the study. To illustrate this, mean responses between baseline and pre-trial (loading phase) suggest MC provided a modest anti-inflammatory action prior to exercise which is in agreement with recent work demonstrating the acute anti-inflammatory effects of MC (Bell et al. 2014a). Conversely, the removal of foods containing antioxidants in the PLA group diet appeared to cause a modest rise in inflammatory status (**Figure 5)** and as a result it may be surmised that habitual diets have an impact on daily inflammatory state. Despite this, mean absolute hsCRP and IL-6 values were almost identical between groups' immediately pre-trial, suggesting similar inflammatory states prior to exercise. Regardless, the hsCRP response to exercise was consistent with previous work (Weight et al. 1991; Howatson et al. 2010); both groups peaked at 24 h before returning towards pre-exercise levels at 48 h. Notably, the MC group demonstrated

386 an attenuated rise versus PLA at 24 h (16% vs 96% increase from baseline). With regards to IL-6,
387 cycling has previously been shown to increase plasma levels in the acute phase following
388 performance (Robson-Ansley et al. 2009) and furthermore, prolonged exercise involving significant
389 muscle mass has been suggested to produce a marked increase in systemic levels regardless of the
390 mode of exercise (Fischer 2006). The suppressed IL-6 response with MC supplementation in the
391 immediate hours following exercise in the present study is in agreement with previous work
392 (Howatson et al. 2010) and thus provides further support to the anti-inflammatory properties of MC.
393 Additionally, no differences between groups were found with regards to work done, suggesting that
394 the metabolic cost of the task was the same and that subsequent inflammatory processes had been
395 induced by a similar stimulus in both groups. With regards to application of these findings,
396 reductions in inflammation may be beneficial to physical performance. Indeed, administration of
397 recombinant IL-6 reduced 10 km running time-trial performance in trained male runners (Robson-
398 Ansley et al. 2004).
399
400 In contrast to previous work (Connolly et al. 2006; Howatson et al. 2010), this investigation ensured
401 the PLA supplement contained equal CHO content. This is an important control measure as acute
402 CHO ingestion has previously been reported to influence the appearance of IL-6 in plasma following
403 strenuous exercise (Febbraio et al. 2003; Robson-Ansley et al. 2011). Resultantly, the attenuation
404 in inflammatory responses cannot be associated with pre-exercise carbohydrate (CHO) intake,
405 although it should be noted that in applied scenarios, cyclists would be encouraged to ingest CHO
406 throughout the exercise, possibly reducing the inflammatory response and limiting the
407 generalizability of these results. A more likely explanation for the anti-inflammatory actions of MC,
408 however, may be that it inhibited the actions of prostaglandin enzymes responsible for the
409 conversion of arachadonic acid to prostaglandins which play an important role in inflammatory
410 pathways (Trappe and Liu 2013). Future work might consider examination of these molecules to
411 gain further understanding of the inflammatory cascade. Wang et al. (1999) demonstrated *in vitro,*

412 that cyanidin, the prominent anthocyanin within MC, was similarly effective in reducing the activity
413 of prostaglandin endoperoxide H synthase-1 and -2 to the non-steroidal anti-inflammatory drugs
414 ibuprofen and naproxen. Additionally, previous work has reported anthocyanin metabolites to be
415 present in the circulation up to 48 h post-consumption (Czank et al. 2013) and as such may provide a
416 potential mechanism for the results observed here.
417
418 In contrast to our hypothesis, the oxidative stress (LOOH) responses to the trial were not different
419 between groups despite showing elevations post-exercise versus pre-exercise measures. Previous
420 literature has demonstrated MC supplementation attenuated measures of lipid peroxidation
421 (thiobarbituric acid reactive species [TBARS]) and a trend for reduced protein oxidation (protein
422 carbonyls [PC]) in response to marathon running (Howatson et al. 2010) and maximal eccentric
423 contractions of the knee extensors (Bowtell et al. 2011), respectively. However, the use of both
424 TBARS and PC as measures of oxidative stress in human studies have been criticised due to their lack
425 of specificity (Urso and Clarkson 2003; Bell et al. 2013). The TBARS assay has been suggested to also
426 react with non-functional aldehydes, carbohydrates and prostaglandins (Alessio 2000), whereas
427 carbonyl groups can also be formed from aldehyde groups formed during lipid peroxidation (Dalle-
428 Donne et al. 2003) thereby creating difficulty in discriminating between lipid and protein oxidation
429 with the PC assay. Arguably, however this could be viewed as a strength when measuring global
430 oxidative stress. The current study results suggest that the MC dose provided was not effective in
431 reducing lipid peroxidation following the single bout of exercise performed, although conceivably
432 the sample time points might miss peaks and modulation of these variables.
433
434 The improved cycling efficiency at 24 h is an interesting observation; the MC group provided VO_2
435 values that were ~4% lower than those presented by the PLA group when results were normalised to
436 baseline values. This is not the first report of a functional food improving exercise efficiency;
437 beetroot juice has previously been reported to lower the oxygen cost of submaximal exercise, which

438 has been attributed to increased dietary nitrate impacting upon ATP expenditure in the maintenance
439 of sarcoplasmic Ca^{2+} homeostasis (Bailey et al. 2009b; Vanhatalo et al. 2010). Additionally, nitrate
440 supplementation has been shown to reduce blood pressure in normotensive humans (Larsen et al.
441 2006) suggesting an impact upon vascular function. In relation to the current study, MC contains a
442 high volume of polyphenols which have also been suggested to influence vascular function (Yung et
443 al. 2008). Although somewhat speculative, it is conceivable that the polyphenolic content of MC
444 may have influenced endothelial function allowing improved blood flow and thereby contribute to
445 improved exercise efficiency, but this needs to be confirmed and examined in a paradigm specifically
446 designed to address this question.
447
448 In contrast to the maintenance of MVIC with MC supplementation, it was surprising to notice no
449 differences between groups in 6-second maximal power, which was measured to assess a cycle-
450 specific task. In fact, surprisingly a main effect for time demonstrated that both groups actually
451 improved this performance across the 72 h post-trial period. In comparison with MVIC, the muscular
452 movement and skill associated with cycle sprinting is far more complex. As a result, it is possible
453 that a learning effect may have influenced the improvement in performance, particularly given that
454 the participants were endurance trained and were likely to be much less experienced to this type of
455 maximal power test. Future studies using such a measure in this population should consider a
456 substantial familiarisation period prior to assessing 6-second maximal power.
457
458 Interventions aimed at reducing the inflammatory and oxidative stress responses to exercise have
459 received criticism with regards to their influence upon subsequent physiological adaptation; the
460 suggestion is that attenuating inflammation and oxidative stress, could reduce protein synthesis
461 (Trappe et al. 2002; Mikkelsen et al. 2009; Urso 2013) and dampen cell signalling (Gomez-Cabrera et
462 al. 2012) and thereby inhibit adaptation (Paulsen et al. 2014). Conversely, anti-inflammatory
463 interventions have been suggested to have no effect on protein gene expression (Mikkelsen et al.

2011) or repeated bout effect adaptation (Paulsen et al. 2010) and have recently been suggested to be beneficial in increasing muscle hypertrophy in older adults (Trappe et al. 2011). Additionally, no blunting effects on adaptation have been demonstrated with any functional food supplementation, although there is certainly a growing need for research in this area. Regardless, many scenarios exist where accelerated recovery of performance is more important than physiological adaptation such as cycling tours and tournament based competitions; as a result, MC offers an efficacious strategy for physical recovery following strenuous exercise.

Although functional performance decline following the trial was attenuated, a greater battery of sport specific performance measures, such as a time-trial, would have proved a useful inclusion. In addition, the dosing strategy used in the study incorporated both pre-, on the day of, and post-trial supplementation; as a result it is difficult to ascertain the time at which MC exerts its positive effects, or whether there is a cumulative effect, whereby multiple doses result in an increased capacity to combat anti-inflammatory pathways or perhaps increase tissue bioavailability of the functional compounds. Furthermore, the adherence to a low polyphenolic diet throughout the course of this study, limits the generalizability of the results. Clearly, athletes would be encouraged to maintain a diet high in vegetables containing essential vitamins and minerals and the removal of these from the diet may have influenced the results of this study. Future work might incorporate habitual diets to investigate if MC has an additive influence on the measures identified in this study. With regards to real-life application of this study's results, it is important to note that most competitive cyclists will train or compete daily. As a result, multiple days of exercise may have provided a more applied scenario where the increased stress responses are more representative of cyclists' physical demands.

In summary, and in support of the hypothesis, the main finding of the study is that Montmorency cherry concentrate supplementation maintained muscle function (as determined by MVIC),

following an exercise stress induced exclusively through a metabolic challenge. This was accompanied by the attenuation of inflammatory responses providing a possible mechanistic link to the performance benefits demonstrated. This study provides new information which adds to the growing body of literature providing evidence for the use of MC supplementation in recovery from both metabolically and mechanically challenging exercise. Given the findings in the current study and previous literature, future work should explore other sport specific applications for MC supplementation, such as field and court invasion sports, where the physiological stress is simultaneously challenging from a metabolic and mechanical perspective, which have both been shown in isolation to benefit from MC supplementation.

Conflicts of Interest

The cherry marketing institute (a not for profit organisation) provided financial support for the analysis of inflammatory indices. All other elements of the study were funded by Northumbria University, and the University of Ulster, UK. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare no conflict of interest.

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Study design was devised by PGB and GH; data collection and analysis was conducted by PGB, IHW and GWD; data interpretation and manuscript preparation were undertaken by PGB, GH, ES, GWD and IHW. All authors read and approved the final version of the paper.

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654

655 **Table 1:** All dependant variables without group differences.

	Baseline		Pre-trial		Post-trial		1 h		3 h		5 h		24 h		48 h		72 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CE (VO2 [mL.kg.min^{-1}])																		
MC	41.0	7.9											40.8$^{\$}$	7.6	41.2	8.1	40.5	8.4
PLA	38.3	10.5											39.6$^{\$}$	10.8	38.0	11.4	36.6	9.6
DOMS (VAS [mm])*																		
MC	1.68	3.10											20.67	27.60	14.92	22.51	10.79	15.48
PLA	0.25	0.32											25.00	19.70	21.80	18.72	24.42	28.14
6-sec Max Power (W)*																		
MC	785	206											865	256	908	293	911	272
PLA	834	205											826	191	858	199	858	184
IL-1-β (pg.mL^{-1})																		
MC	0.09	0.15	0.09	0.17	0.12	0.19	0.11	0.15	0.13	0.16	0.22	0.32	0.10	0.15	0.12	0.17	0.11	0.20
PLA	0.27	0.47	0.15	0.20	0.23	0.26	0.10	0.09	0.12	0.20	0.22	0.26	0.20	0.19	0.11	0.15	0.10	0.12
IL-8 (pg.mL-1)*																		
MC	2.34	0.96	2.86	0.96	4.50	1.39	3.96	0.37	2.90	0.79	2.71	0.40	2.38	0.63	2.21	0.94	2.68	1.26
PLA	3.21	1.30	2.92	1.55	5.43	1.84	4.67	1.64	3.21	1.23	2.57	0.92	2.39	1.16	2.48	0.79	2.96	1.32
TNF-α (pg.mL-1)*																		
MC	1.66	0.52	1.64	0.29	1.78	0.41	1.69	0.40	1.50	0.30	1.53	0.52	1.54	0.41	1.39	0.46	1.39	0.53
PLA	1.87	0.85	1.72	0.77	1.89	0.70	1.73	0.74	1.53	0.65	1.47	0.69	1.50	0.66	1.43	0.65	1.66	0.74
LOOH (mmol.mL^{-1})*																		
MC	1.33	0.28	1.26	0.12	1.49	0.22	1.31	0.12	1.44	0.27	1.37	0.22	1.23	0.13	1.30	0.28	1.15	0.15
PLA	1.30	0.12	1.31	0.13	1.31	0.09	1.35	0.15	1.49	0.21	1.52	0.20	1.22	0.20	1.24	0.11	1.26	0.21
CK (IU.L^{-1})*																		
MC	202	97	302	185	255	120	225	166	244	98	139	51	190	100	277	221	199	112
PLA	166	83	133	65	135	48	185	94	219	114	312	333	173	105	165	122	150	156
hsCRP (pg.mL^{-1})†																		
MC	1.14	1.18	0.69	0.53	0.69	0.55	0.68	0.57	0.68	0.57	0.74	0.63	1.11	0.97	0.74	0.57	0.56	0.39
PLA	0.55	0.53	0.70	0.69	0.70	0.61	0.75	0.65	0.78	0.64	0.71	0.64	0.95	0.74	0.66	0.48	0.55	0.33

*Significant main effect for time ($P < 0.05$). $^{\$}$Significant interaction effect ($P < 0.05$). CE, Cycling Efficiency; DOMS, Delayed onset muscle soreness; IL-1-β, Interleukin-1-beta; IL-8, Interleukin-8; TNF-α, Tumour Necrosis Factor-Alpha; LOOH, Lipid Hydroperoxides; CK, Creatine Kinase; hsCRP, high-sensitivity C-reactive protein. † For illustrative purposes, statistical analysis performed on percentage of baseline values.

656

Figure Captions

Figure 1. Schematic of testing protocol. All visits (excl. Visit 1) were conducted at 8am following an overnight fast.

Blood sampling (↕ + performance measures).

━ ➞ Supplementation period – 2 x 30 mL per day (8am and 6pm) taken with 100 mL water.

━ ▪ ▶ Dietary restrictions – Following blood sampling at 96 h pre-trial to post-visit 6.

Figure 2: Simulated road cycling race protocol (Vaile *et al,* 2008). Work - Maximal effort sprint. Recovery and ACT - Power at 40-50% W_{max}. TT (Time Trial) - Sustained maximal effort.

Figure 3: Maximum Voluntary Isometric Contraction responses (% Change from baseline) to Montmorency cherry concentrate (MC) and isoenergetic placebo (PLA). *Significant group effect ($P < 0.05$), values are mean ± SD.

Figure 4: Interleukin-6 responses to Montmorency cherry concentrate (MC) and isoenergetic placebo (PLA). *Significant group effect ($P < 0.05$), values are mean ± SD.

Figure 5: high-sensitivity C-Reactive Protein responses (% of baseline) to Montmorency cherry concentrate (MC) and isoenergetic placebo (PLA). *Significant group effect ($P < 0.05$), values are mean ± SD.

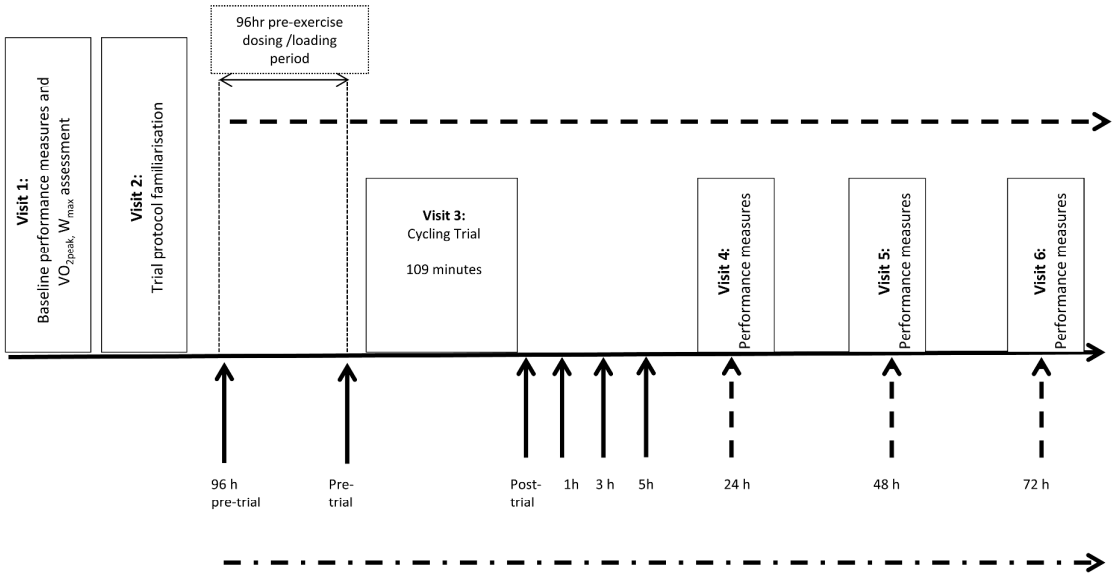

Figure 1.

10 minute warm up (Self-selected pace)

Set Number	Sprint Frequency/Duration	Work to Recovery Ratio
Set 1	12 x 5 s	1:6 (Work:Recovery)
Set 2	12 x 5 s	1:3 (Work:Recovery)
Set 3	12 x 5 s	1:1 (Work:Recovery)
4 min ACT - ***2 min TT*** - 4 min ACT		
Set 4	6 x 10 s	1:6 (Work:Recovery)
Set 5	6 x 10 s	1:3 (Work:Recovery)
Set 6	6 x 10 s	1:1 (Work:Recovery)
4 min ACT - ***2 min TT*** - 4 min ACT		
Set 7	4 x 15 s	1:6 (Work:Recovery)
Set 8	4 x 15 s	1:3 (Work:Recovery)
Set 9	4 x 15 s	1:1 (Work:Recovery)
5 min ACT - ***5 min TT*** - 5 min ACT		

Figure 2.

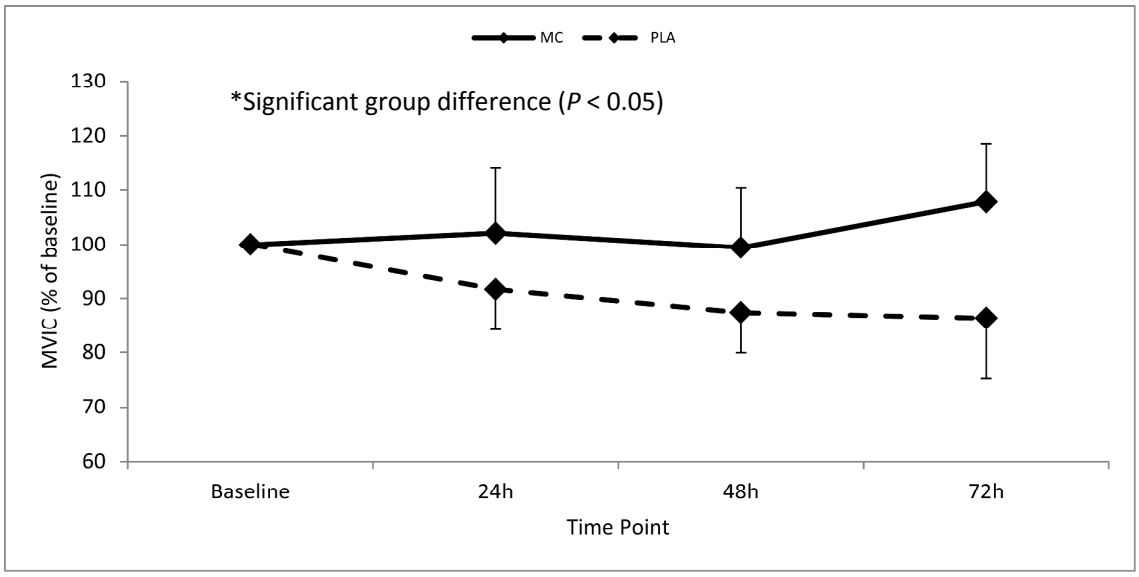

Figure 3.

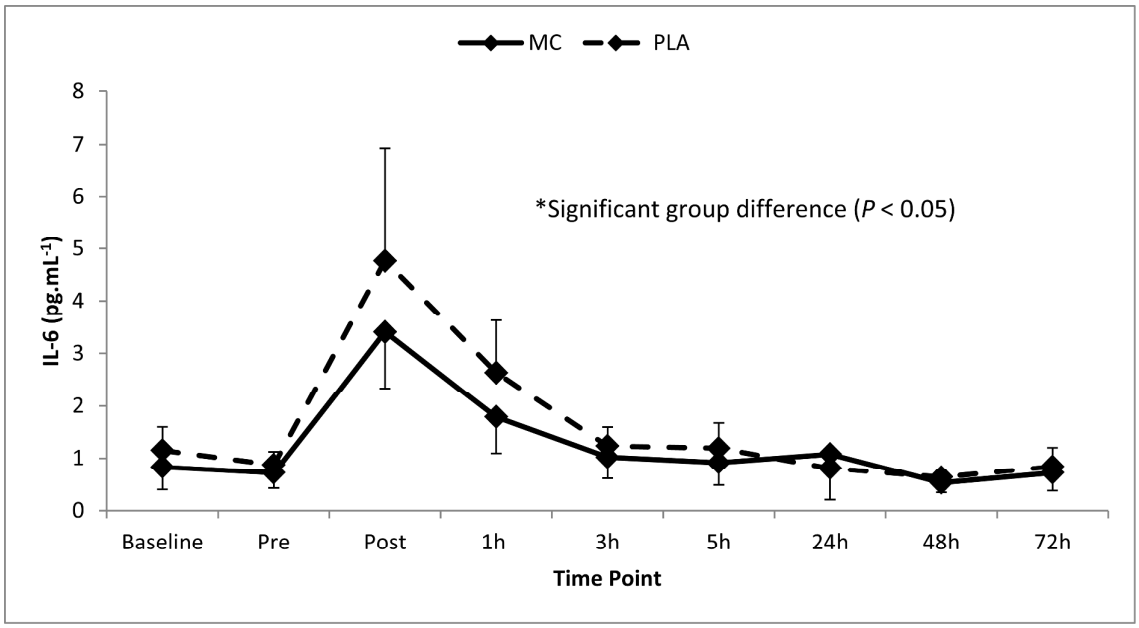

Figure 4.

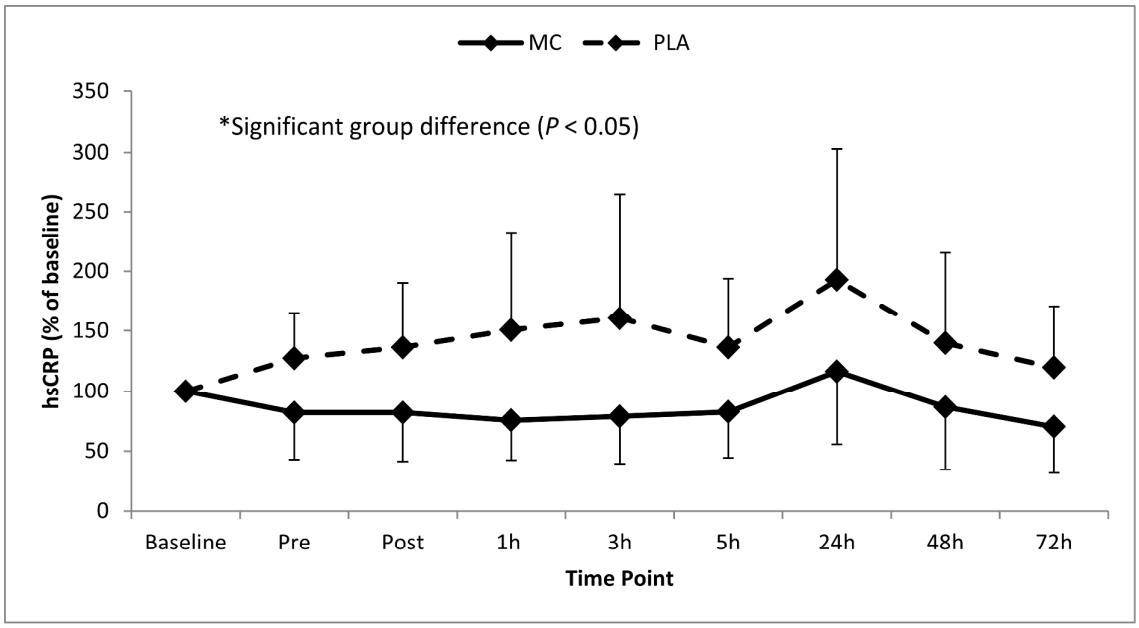

Figure 5.