International Journal of EXERCISE SCIENCE

Original Research

Montmorency Cherry Juice Consumption does not Improve Muscle Soreness or Inhibit Pro-inflammatory Monocyte Responses Following an Acute Bout of Whole-body Resistance Training

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ABSTRACT

International Journal of Exercise Science 15(6): 686-701, 2022. Montmorency Cherry Juice (MCJ) may improve acute exercise recovery by attenuating inflammation and oxidative stress. However, the anti-inflammatory effects of MCJ on monocyte responses following resistance exercise have not been explored. Seven resistance-trained males (age: 22.9 ± 4.1 yrs; height: 1.8 ± 0.1 m; weight: 81.7 ± 13.2 kg) participated in this study. Participants completed a placebo-controlled crossover design, drinking either MCJ or placebo beverages, 7 days prior to completing an acute bout of unilateral resistance exercise. Statistical significance was assessed using a within-subjects repeated measures ANOVA; alpha level $p \leq 0.05$. Main effects for time were observed for changes in classical and intermediate monocytes ($p \leq 0.05$), but no significant treatment effects were observed for monocyte subtypes $p > 0.05$. Classical monocytes ($CD14^+$ $CD16^-$) increased and peaked 24 hr post-exercise (placebo 1.14 ± 0.04 and MCJ 1.06 ± 0.06-fold). Intermediate monocytes peaked 48 hr post-exercise increasing 1.82 ± 0.41 and 2.01 ± 0.80-fold. Nonclassical monocytes peaked post-exercise (placebo 1.17 ± 0.31 and MCJ 1.02 ± 0.20-fold). Peak pain visual analog scale (VAS) occurred post-exercise for MCJ (3.63 ± 2.01-fold) and 72 hr post-exercise for placebo (4.26 ± 3.46-fold). IL-6 and pressure pain threshold (PPT) peaked 24 hr post-exercise (IL-6 placebo 3.83 ± 1.01- and MCJ 6.43 ± 3.43-fold) and (PPT placebo 86.37 ± 3.95% and MCJ 82.81 ± 2.90% of pressure needed at pre-exercise). Our data suggests MCJ consumption does not decrease muscle soreness, IL-6, or monocyte subset responses following a high-intensity resistance exercise protocol in resistance-trained males.

KEY WORDS: Exercise, rejuvenation, anthocyanins, inflammation

INTRODUCTION

Human skeletal muscle facilitates locomotion and metabolic regulation throughout the lifespan (15). Comprising approximately forty percent of total body mass, this biologically active organ requires a complex array of regulatory signals for maintenance and repair following exercise-induced damage (2, 15, 24, 27). Following a damaging bout of exercise, skeletal muscle secretes factors to signal leukocytes to the site of injury (8). Neutrophils invade first, signaled by factors such as cytokines interleukin-6 (IL-6), interleukin-8 (IL-8) (8), and macrophage-derived CXC-chemokine-ligand 1 (CXCL-1) (33). Moreover, recruited neutrophils produce monocyte chemoattractant protein 1 (CCL-2) (35), and interleukin-17 (IL-17) to attract and activate monocytes to the area of inflammation (34).

Monocyte populations are heterogeneous, and in humans consist of three predominant subsets: classical monocytes ($CD14^+$ $CD16^-$), intermediate monocytes, ($CD14^+$ $CD16^+$), and non-classical monocytes ($CD14^+$ $CD16^{++}$) (7). While debated, intermediate and non-classical monocytes are suggested to be pro-inflammatory, and the classical subset is phagocytic (22). All three subsets possess the ability to differentiate into macrophages (7) and upon entry into damaged skeletal muscle, play key roles in muscle regeneration (33). Interference of monocyte or macrophage kinetics have been shown to negatively affect skeletal muscle regeneration (9). Therefore, it appears critical to maintain proper inflammatory dynamics, particularly at the level of the monocyte, for optimal resistance training adaptations.

While inflammation is essential, the effects can be beneficial or detrimental depending on the context. Exercise-induced inflammation is associated with changes in muscle function such as reduced maximal isometric voluntary contraction strength and pain, and is a consequence of prolonged, unaccustomed, or high-intensity exercise (11, 24). Consequently, treatments to mitigate the deleterious effects of inflammation following exercise have been investigated (16). Montmorency cherry juice (MCJ), also known as tart cherry juice, has been suggested to diminish oxidative stress, inflammation, and muscle soreness in various study populations (18). Specifically, MCJ has been investigated as an ergogenic aid targeting inflammation after endurance exercise (3–5, 13, 17, 19, 21), and resistance training (6, 12, 20). These findings suggest that MCJ may minimize inflammation associated with acute exercise responses (3, 4, 6, 13, 17, 20, 21) through decreasing inflammatory markers such as Interleukin-6 (IL-6) (3,4,17) and attenuating pain sensations (3, 12, 17, 19, 20). The anti-inflammatory effects of MCJ are thought to be due to its high anthocyanin content acting as antioxidants (26), and have been shown to attenuate the cyclooxygenase 2 (COX-2) pathway (28). However, these benefits may be paradoxical in trained subjects as acute inflammation caused by exercise-induced muscle damage promotes hypertrophic muscle adaptations and repair (10).

At this time MCJ has been shown to attenuate non-cellular markers of inflammation, yet responses in circulating immune cell subsets, such as monocytes, have not been investigated. Therefore, the purpose of this study is to further examine the impact of MCJ on the acute inflammatory response to resistance exercise, focusing on systemic monocyte responses. We

hypothesize that MCJ consumption will attenuate the inflammatory monocyte phenotypes (intermediate $CD14^{+}$ $CD16^{+}$ and nonclassical $CD14^{+}$ $CD16^{++}$) acutely following exercise compared to an iso-energetic placebo beverage. Furthermore, we hypothesize that the MCJ group will report less muscle soreness following the resistance exercise bout.

METHODS

Participants

The sample size was determined using an a priori power calculation performed utilizing GPower version 3.1.9.2 (Universitat Kiel, Germany). Sample size estimates were calculated using a within-between interaction design, and data from Howatson et al. 2010 (17). Our primary outcome variable was Interleukin-6 resulting in an effect size f value of 0.69 with an alpha of 0.05 and 95% power. The calculation resulted in n = 8. Thirteen participants from the Central Washington University campus were recruited and enrolled to participate. Participants were self-described as resistance trained (consistent resistance training ≥ three days per week for ≥ one year) and able to demonstrate proficient skills during the exercise familiarization. Exclusion criteria consisted of any diagnosed cardiovascular, metabolic, renal, auto-immune/inflammatory, or pulmonary disease; currently taking medications affecting metabolism; taking any supplemental antioxidant or weight loss products; current smoker or ceased smoking within the last six months. Additionally, those unable to perform a high-intensity resistance exercise protocol or allergic to MCJ were excluded. Following an explanation of the procedures and associated risks, all volunteers were asked to provide written informed Consent. All procedures were approved by Central Washington University's Human Subject Review Council. Furthermore, this research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (23).

Protocol

Following consent, participants completed a single blind placebo-controlled crossover study design consisting of an exercise familiarization, and two ten-day supplement trials separated by a two-week washout. Prior to each visit, participants were instructed to avoid alcohol consumption for 48 hours, fast for 12 hours prior to venipuncture blood draw and abstain from anti-inflammatory medications such as Non-steroidal Anti-inflammatory Drugs (NSAIDs) throughout the study period. During the study's supplement trials, participants consumed 30 mL of either MCJ concentrate (CherryActive: Active Edge, UK) or isoenergetic placebo (Kool Aid; Kraft Foods, USA) twice a day (once in the morning and once in the evening) for ten consecutive days. During each supplement trial, participants consumed a supplemental beverage beginning on day 1 and completed a unilateral resistance exercise protocol on day 8 followed by three days of follow up measurements (24, 48, and 72 hours post-exercise) represented in Figure 1.

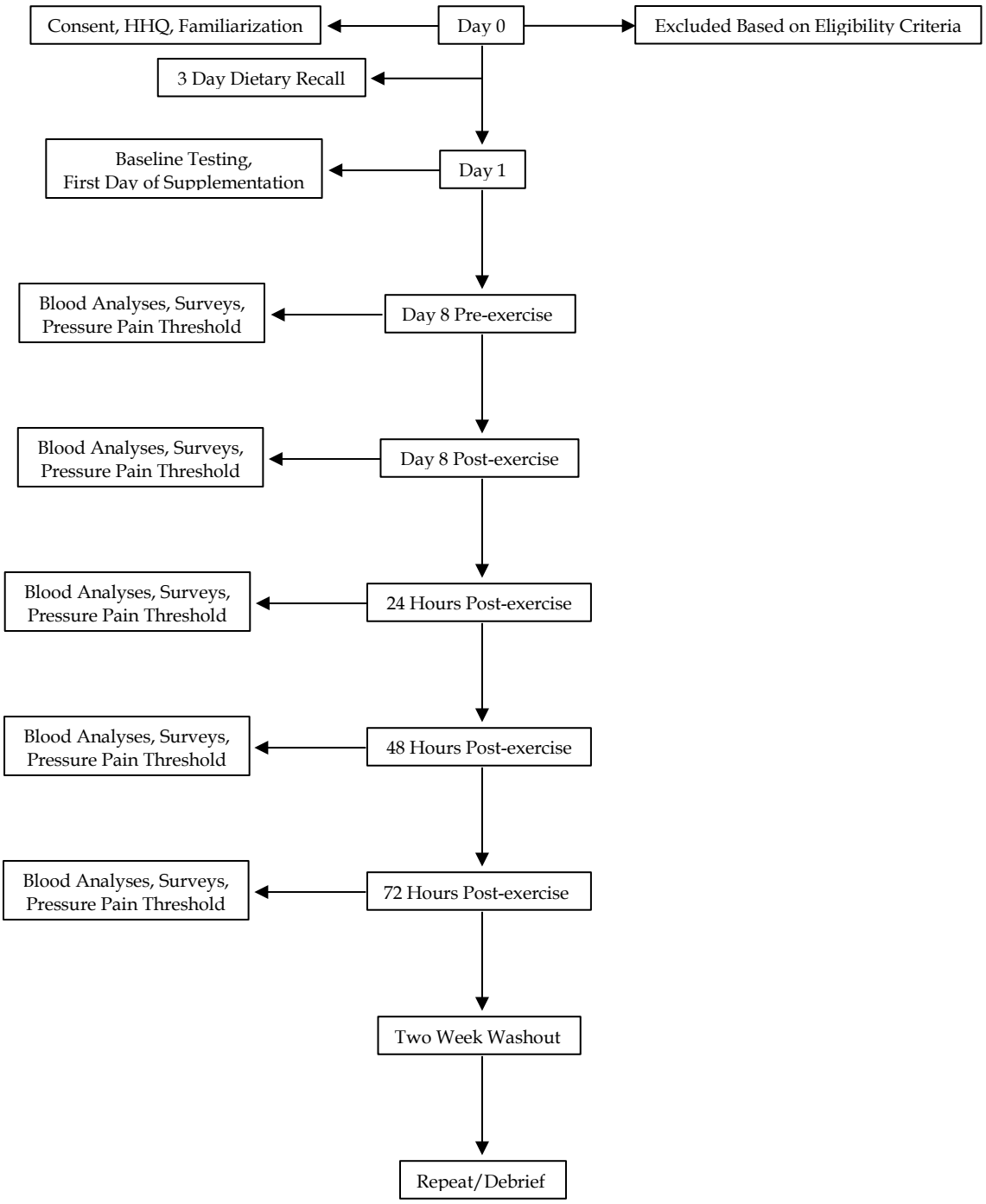

Figure 1. Study design flow diagram. Day 0 consisted of consent, health history, and exercise familiarization (eligible subjects only). Three-day dietary recalls were performed prior to the start of the supplement trial (day 1). At day 1 baseline measures were taken. Post-intervention exercise testing was performed on day 8. On day 8, blood samples were taken pre-exercise, immediately post-exercise, and 24, 48 and 72 hours post-exercise.

Following the first supplement trial the supplement beverage was crossed over, and the exercising limb was switched to eliminate any repeated bout effect. Participants were instructed to continue their habitual diet and exercise, but to refrain from exercise for 48 hours before and

after the unilateral resistance exercise protocol (day 8). Three-day diet records were used to assess dietary compliance. Dietary records were administered (two-week days and one weekend day) during the seven days following the familiarization protocol prior to supplement trial one, and during the final week of washout before supplement trial two (ESHA Food Processor, version 10.6, Salem, OR, USA). Following supplement trial one, all study procedures were repeated with the opposite treatment during supplement trial two.

The familiarization consisted of a five-minute treadmill warm-up at a self-selected walking pace followed by a general warm-up of leg swings and shoulder drills at 20 repetitions each. At the completion of the warm-up the following exercises were described: single-leg goblet step-up, single-leg extension, single-leg curl, single-arm dumbbell chest press, and single-arm dumbbell row. Participants demonstrated that they could proficiently perform each exercise, unloaded, then weights were slowly increased until a three-repetition maximum was determined. Proficiency was determined by the supervising investigator and based on if the participant was able to move through a full range of motion while maintaining control of the weight being lifted. The exercise procedure was adapted from a resistance training protocol previously shown to significantly increase leukocytosis on the exercising limb assessed by radionuclide-labeled leukocytes (25). Prior to the resistance training protocol, participants performed the same warm-up completed prior to the familiarization. Utilizing a ramp method of adding weight, participants started by using their body weight for all exercises and gradually increased weight over four warm-up sets for single-leg goblet step-up and three sets for all other exercises until working weights were achieved for each exercise. The exercise protocol consisted of single-leg goblet step-up, single-leg extension, single-leg curl, single-arm dumbbell press, and single-arm dumbbell row. Participants performed four working sets of their three-repetition maximum for single-leg goblet step up, and four working sets of their six-repetition maximum for all other exercises. If any set required assistance, the following set was decreased in weight by 5%. A rest period of three minutes was used between each set and exercise.

The supplement intervention consisted of 30 mL of MCJ concentrate or placebo consumed twice per day for ten days. Each 30 mL MCJ serving contained 102 kcal, 0 g of fat, 25 g of carbohydrate, 2.6 g of fiber, 1.1 g of protein, and 0 g of salt. The total anthocyanin content is reported as 320 mg per 30 mL serving (CherryActive, UK). Each 30 mL serving was added to 240 mL of water and placed in serving bottles so each bottle had two servings of MCJ. The placebo was prepared from a commercially available flavor packet measured to match the caloric composition of the cherry juice without the anthocyanin content (Kool-aid, Kraft foods, USA). Each beverage was consumed upon waking and prior to bed. Following the washout period, the trial was repeated with the opposite supplement prescription. Participant adherence to beverage supplementation was established through verbal and written confirmation, reminders sent over text, and photos sent to the primary investigator following consumption of each serving.

On day 1 of each supplement trial baseline measurements were assessed prior to the ingestion of the first morning supplement. Upon arrival, participants were asked to sit in a comfortable position for ten minutes then resting heart rate (HR) and blood pressure (BP) were assessed.

Resting blood samples were then obtained from the antecubital vein. When ready, the participant was measured for height and weight to the nearest 0.1 cm and 0.5 kg respectively. Body composition was determined using air displacement plethysmography (BODPOD®; COSMED, Rome, Italy) utilizing standard convention from the manufacturer. Additionally, pressure pain threshold was assessed at the vastus lateralis, vastus medialis, rectus femoris, pectoralis major and latissimus dorsi using an algometer (Wagner Instruments Inc., Greenwich, CT). The final measure consisted of a 100mm linear visual analog scale (VAS) to assess self-perceived muscle soreness.

Fasting blood draws occurred at the following time points: baseline (day 1), pre-exercise and post-exercise (day 8), and the morning of 24, 48, and 72 hours post-exercise of each supplement trial. At each time point ~20 mL of blood was collected from the antecubital space utilizing a 21-gauge needle into commercially available vacutainer tubes. Universal precautions were used at all times, and draws were performed utilizing proper order of draw to prevent additive contamination.

Serum separator tubes (BD #367988; Pulmo Labs, Porter Ranch, CA) containing a silica clotting gel were allowed to clot for approximately one hour then centrifuged at 1000 X gravity for 10 minutes at 4°C. Serum was aliquot into individual vials to be stored at -80° Celsius until analysis by a commercially available Enzyme Linked Immunosorbant Assay (ELISA) kit (Biolegend #430505; San Diego, CA). Blood samples intended for flow cytometry were collected in tubes containing K2 EDTA (BD #367899; Pulmo Labs, Porter Ranch, CA) and immediately prepared for monocyte analysis.

For flow cytometry, Baseline, pre-exercise, and post-exercise blood samples were processed as described below. 12 mL of blood was aliquot and combined with 12 mL of 1X Phosphate Buffered Saline (PBS) then layered on top of Lymphopure (Biolegend #426202; San Diego, CA). Samples were then centrifuged at 1300 RPM for 30 minutes at room temperature (RT) with the brake off. Following centrifugation, peripheral blood mononuclear cells (PBMCs) were collected and re-suspended in 13 mL 1X PBS. Samples were centrifuged at 1300 RPM for 10 minutes at RT with the brake on to create a cell pellet, and then decant. The cell pellet was suspended in 1 mL Cell Staining Buffer (Biolegend #420201; San Diego, CA), counted using a hemocytometer, vortexed, and 100uL (~1 million cells) were pipette into a fluorescence-activated cell sorting (FACS) tube. Antibodies Alexa Fluor 488 anti-human CD14 (Biolegend #367129; San Diego, CA) and PE anti-human CD16 (Biolegend #302007; San Diego, CA) were vortexed prior to each being added to the FACS tube at 1:20 dilution. Three more 100uL tubes were created for only CD14, CD16, or cell staining buffer for single stain compensation controls. All tubes were vortexed then incubated on ice in the dark for 45 minutes. Follow up visits at 24, 48 and 72hours post-exercise were processed as described above; however, the follow-up PBMCs were suspended in 20% DMSO 80% FBS, and slowly frozen for 24 hours in a bath of isopropanol at -80°C. Following the 24 hours, the frozen PBMCs were immediately placed in liquid nitrogen. Upon thawing, the PBMCs were washed twice with PBS, and an additional single stain control of Propidium Iodide at 1:20 dilution was used to gate out nonviable cells.

Flow cytometry was performed on all samples using a Biorad S3 Cell Sorter (Biorad; Hercules, CA). Samples were acquired using excitation lasers at 488 and 561nm. A minimum of 20,000 events were collected and single stain compensation controls were used to correct for fluorochrome spillover each day of analysis. Analysis was performed using FlowJo software (FlowJo LLC; Ashland, OR). Gating procedures were conducted using forward by side scatter assessment previously described (1, 36). Unstained samples for each day were used as the negative control for the CD14 and CD16 subpopulations. The samples were initially gated for monocytes based off of forward scatter area by side scatter area characteristics. Dublets were then removed by creating a thin oblique oval surrounding the population in forward scatter area by forward scatter height. Monocyte subsets were then defined in the FL-1 compensated channel and FL-2 compensated channel view based on refined gating procedures (36).

Pressure pain threshold (PPT) was measured at the vastus medialis, rectus femoris, vastus lateralis, pectoralis major, and latissimus dorsi using a hand-held algometer. Time points for assessment were baseline (day 0), pre and post-exercise (day 8), and follow up assessments (24, 48, and 72 hours post-exercise). The same investigator performed this assessment for all time points. Participants were asked to sit with their arms at their sides and their legs at 90°. The investigator placed the head of the algometer flat against the center of the muscle belly, and slowly increase pressure until the participant indicated reaching a point of discomfort. The point of discomfort was described as a six out of ten on a 0-10 point scale with ten being the greatest pain you have ever felt and zero being no pain at all. All measures were performed in duplicate then accepted and averaged if the two values fell within 1.0 kg. Readings from each muscle were summed to create a composite score.

The visual analog scale (VAS) assessed perceptual muscle soreness. The VAS consists of a 100-mm line anchored on each side with not at all or extremely sore. Participants placed a vertical mark along the 100-mm line where they were best described at that moment. The score was measured as the numerical distance out of 100 mm.

Statistical Analysis

Statistical analysis utilized a two-way repeated measures ANOVA across treatment and time. To determine if the washout period was effective dependent t-tests were performed to assess differences between supplement trial one and supplement trial two on day one. A significant value of ($p \leq 0.05$) would indicate that the washout was ineffective; however, if significance was greater than 0.05 between supplement trials, then the repeated measures ANOVA was used with an alpha set at $p \leq 0.05$. Data were analyzed using GraphPad Prism version 8.0.2 for Mac (GraphPad Software, La Jolla California USA), and represented as mean ± SEM unless described otherwise.

RESULTS

Six participants withdrew or were excluded due to illness, tobacco use, sample loss, or doctor mandated weight loss. Seven participants completed the six-week protocol and consumed all 20 servings of each beverage. Baseline characteristics and dietary recall values are shown in Table 1. There was no significant difference for calories or macronutrients between supplement trial one and supplement trial two (F = 0.21, p > 0.96, η^2 = 0.0008). Additionally, there were no significant differences across all variables observed at baseline for each supplement trial (p > 0.05).

Table 1. Participant characteristics (mean ± SD).

Variable	n = 7	
Age (years)	22.9 ± 4.1	
Height (m)	1.8 ± 0.1	
Body mass (kg)	81.7 ± 13.2	
Body mass index (kg · m^{-2})	26.6 ± 4.3	
Fat (%)	15.3 ± 6.8	
Estimated One-Repetition Single Limb Maxes	Right	Left
Goblet Step Up (kg)	37.3 ± 7.8	37.0 ± 8.1
Single Leg Extension (kg)	56.6 ± 13.4	56.2 ± 13.4
Leg Curl (kg)	30.1 ± 4.4	30.7 ± 3.7
DB Chest Press (kg)	37.0 ± 6.1	36.0 ± 6.1
DB Row (kg)	42.3 ± 7.3	42.3 ± 7.3
Dietary Recall	Supplement Trial 1	Supplement Trial 2
Calories (kcals)	2756.25 ± 393.54	2605.26 ± 733.31
Protein (grams)	178.11 ± 68.12	165.06 ± 101.88
Carbohydrate (grams)	261.32 ± 56.12	255.16 ± 94.75
Fiber (grams)	19.83 ± 11.25	20.39 ± 8.83
Fat (grams)	93.55 ± 32.53	101.82 ± 32.63

Note: SD = standard deviation; m = meters; kg = kilograms; kcals = kilocalories

Five of the seven completed subjects passed quality control for flow cytometry and were analyzed for monocyte characteristics. No significant treatment effects were observed between beverages for monocyte responses (p > 0.05); however, main effects for time were observed for all monocyte populations (classical F = 3.86, p = 0.04, η^2 = 0.13; intermediate F = 3.28, p = 0.03, η^2 = 0.23) except nonclassical (F = 1.99, p = 0.14, η^2 = 0.15) represented in Figure 2 A-F.

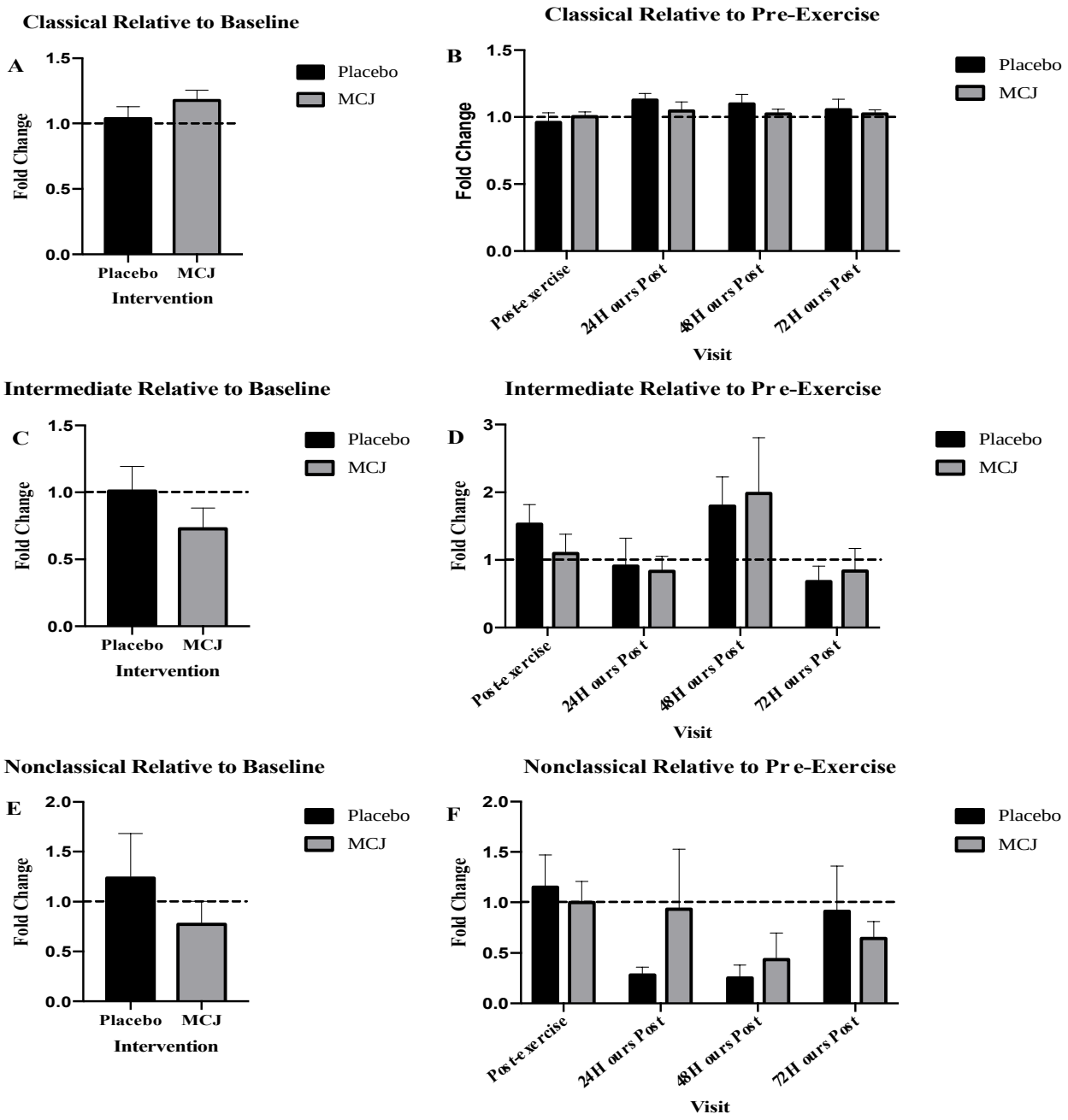

Figure 2. Systemic monocyte response to acute resistance exercise. Data are presented as fold change for cell phenotype and intervention across time represented as mean ± SEM (n = 5). **A:** The effects of 7 days of MCJ supplementation on classical monocytes ($CD14^+$ $CD16^-$) (day 8 pre-exercise relative to baseline (pre-supplementation). **B:** The effects of MCJ supplementation on the classical monocyte response; data normalized to pre-exercise monocyte populations (day 8). **C:** The effects of 7 days of MCJ supplementation on intermediate monocyte ($CD14^+$ $CD16^+$) (day 8 pre-exercise relative to baseline (pre-supplementation). **D:** The effects of MCJ supplementation on the intermediate monocyte response; data are normalized to pre-exercise monocyte populations (day 8). **E:** The effects of 7 days of MCJ supplementation on nonclassical monocytes ($CD14^+$ $CD16^{++}$) (day 8 pre-exercise relative to baseline (pre-supplementation). **F:** The effects of MCJ supplementation on the nonclassical monocyte response to acute exercise; data are normalized to pre-exercise monocyte populations (day 8). The dotted line represents normalization to baseline or pre-exercise measures (**----**).

In response to the supplement interventions, the percentage of classical monocytes, intermediate and non-classical monocyte populations did not significantly change from the start of supplementation to day 8 pre-exercise with placebo or MCJ. In response to exercise, peak classical monocyte increase occurred 24 hours post-exercise placebo 1.14 ± 0.04 and MCJ 1.06 ± 0.06-fold. Intermediate monocytes peaked at 48 hours post-exercise increasing 1.82 ± 0.41 and 2.01 ± 0.80 fold compared to pre-exercise for placebo and MCJ, respectively. Furthermore, nonclassical monocyte peak increase occurred immediately post-exercise increasing 1.17 ± 0.31 and 1.02 ± 0.20-fold for placebo and MCJ, respectively.

No significant treatment effects were observed in IL-6 levels (F = 0.17, p = 0.95, η^2 = 0.009); however, main effects for time were observed (F = 3.70, p = 0.009, η^2 = 0.19 Figure 3 A and B). Pre to post-exercise levels of IL-6 marginally increased 0.66 ± 0.29 and 0.54 ± 0.29-fold compared to pre-exercise. Both treatments illustrated a peak circulating IL-6 level 24 hours post-exercise increasing 3.83 ± 1.01 and 6.43 ± 3.43-fold relative to pre-exercise for placebo and MCJ, respectively.

Changes in pressure pain threshold from baseline to day 8 pre-exercise are illustrated in Figure 4. No significant treatment effects were observed (F = 0.35, p = 0.79, η^2 = 0.02), or time effects were observed (F = 2.20, p = 0.10, η^2 = 0.12). PPT indicated increased sensitivity from pre to post-exercise with the greatest decrease in pressure needed occurring 24 hours post-exercise 86.37 ± 3.95% and 82.81 ± 2.90% compared to pre-exercise for placebo and MCJ respectively.

Fold change in self-perceived muscle soreness are represented in Figure 5. No significant treatment (F = 1.30, p = 0.28, η^2 = 0.07), or time effects were observed (F = 0.81, p = 0.49, η^2 = 0.04). VAS increased by 3.88 ± 2.62 and 5.56 ± 3.40-fold from baseline to pre-exercise for placebo and MCJ, respectively. Following exercise, the greatest self-perceived muscle soreness increase occurred on day 8 post-exercise for MCJ (3.63 ± 2.01-fold) and 72 hours post-exercise for placebo (4.26 ± 3.46-fold).

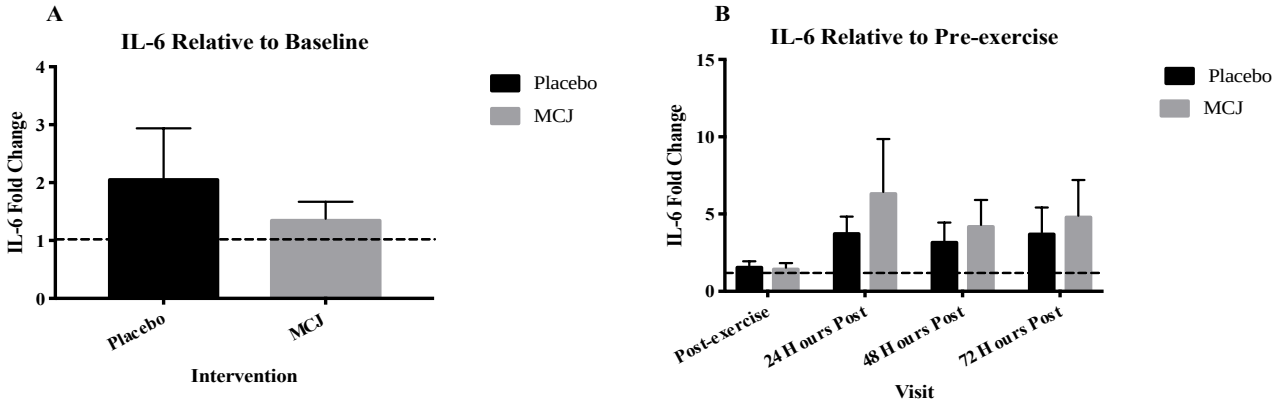

Figure 3. The effects of MCJ supplementation on serum IL-6. **A:** Assessment of the effects of 7 days of MCJ supplementation on serum IL-6. **B:** Assessment of the effects of MCJ supplementation on the IL-6 response to acute exercise; data are normalized to pre-exercise IL-6 levels (day 8). The dotted line represents normalization to baseline or pre-exercise measures (**----**).

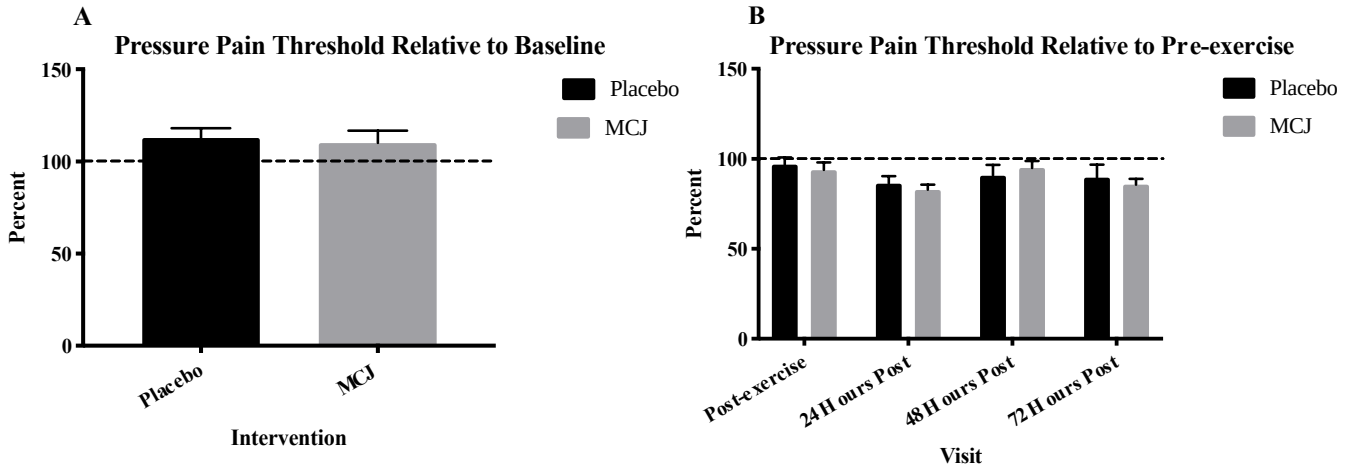

Figure 4. The effects of MCJ supplementation on pressure pain threshold. Pressure pain threshold (PPT) percentage was summed for major muscle groups (vastus lateralis, vastus medialis, rectus femoris, pectorialis major, and latissimus dorsi). **A:** Changes in PPT following 7 days of MCJ supplementation. **B:** The effects of MCJ supplementation on post-exercise PPT. Dotted line is normalized to baseline or pre-exercise measures (**----**).

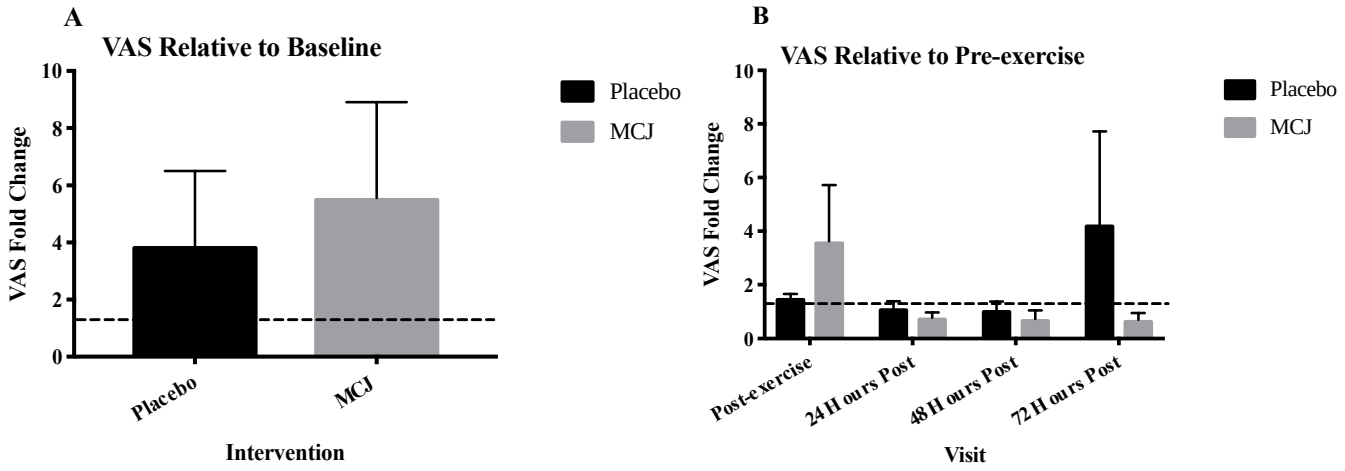

Figure 5. The effects of MCJ supplementation on self-perceived muscle soreness. Muscle soreness was assessed using a visual analog scale. **A:** The effects of MCJ supplementation on muscle soreness. **B:** The effects of MCJ supplementation on post-exercise muscle soreness. Dotted line is normalized to baseline or pre-exercise measures **(----).**

DISCUSSION

MCJ has been touted for its high anthocyanin content and anti-inflammatory properties (18, 28). In the context of exercise, circulating IL-6 levels change dramatically and have been previously shown to diminish following MCJ consumption. Howatson et al. 2010 illustrated that MCJ attenuated post-marathon IL-6 levels to 41.8 pg·mL^{-1} compared to placebo at 82.1 pg·mL^{-1} (17). Additionally, Bell et al. 2014 showed that the MCJ condition resulted in ~50% of the serum IL-6 observed in the placebo group following a simulated cycling race (4). While these data confirm that the substantial increase in IL-6 is conserved following high-intensity resistance exercise,

there does not appear to be a significant difference between MCJ and placebo. This may be due to differential activation of IL-6 during various exercise modalities. Howatson et al., 2010 as well as Bell et al., 2014 utilized endurance exercise over an extended period of time such as marathon running or cycling (4, 17). This is important as the acute rise in IL-6 with exercise is suggested to be proportional to glycogen utilization, which is greater during prolonged endurance exercise (31). While our resistance exercise protocol utilizes glycogen stores, the magnitude of this usage is presumably much less than an extended endurance bout. Such differences in exercise modality may explain why we did not observe a significant change in IL-6 with MCJ supplementation.

Another indicator of skeletal muscle inflammation is perceptual muscle soreness. Previous literature has shown a minimization of pain while consuming MCJ following an exercise bout (3, 12, 17, 19, 20) providing support for MCJ as an ergogenic aid in sports with minimal rest between competitive bouts. While our data appears to illustrate a non-significant increase in perceived soreness following the exercise bout there is no observed difference between beverages. The discrepancy between the data may be due to the type and duration of exercise chosen. Howatson et al. 2010 and Kuehl et al., 2010 used a running protocol covering approximately 26.2km, while Bell et al. 2016 used the Loughborough Intermittent Shuttle Test consisting of six 15-minute shuttle sessions separated by three minutes of rest to mimic a soccer game (3, 17, 19). The high volume of work may induce greater pain sensations than a typical training bout through increased muscle damage. Additionally, Connolly et al. 2006 and Levers et al. 2015 utilized protocols specifically designed to elicit muscle damage through multiple bouts of eccentric exercise, or high-volume squats, respectively (12, 20). Each study determined that MCJ conferred pain resilience to their damaging protocol (12, 20). However, a similar study using 10 sets of 10 for leg extension did not show any difference between MCJ and placebo (6). Incidentally, 10 sets of 10 one leg extensions may not have induced enough damage, as compared to a marathon, to see an effect of MCJ consumption on pain. Results of the present study are similar to Bowtell et al. 2011 as no significant differences in pain were shown between intervention beverages (6). This may be due to the protocol not intentionally inducing a large aggregation of muscle damage. Contrarily, our adapted exercise protocol is based on a similar procedure used to illustrate immune cell dynamics (25). Furthermore, it has been hypothesized that measurable muscle damage may not be needed to confer resistance training adaptations necessitating research into protocols not specifically intended to cause large sums of damage (14).

In opposition to the proposed ergogenic benefits of MCJ and the anti-inflammatory properties it conveys, it has been speculated that consumption may lead to diminished exercise-induced skeletal muscle adaptations. The premise being the disruption of monocytes have been shown to cause states of muscle wasting or disrepair (32) suggesting that an attenuation of monocytes following exercise through MCJ consumption may limit optimal skeletal muscle recovery. However, our data suggest that in comparison to placebo, monocyte activity is unchanged implying their role in muscle damage resolution is equivalent following MCJ usage. This may be in-part due to the resistance trained cohort we studied, since resistance training has been

shown to bolster trained individuals' ability to mitigate inflammatory burden (30). Furthermore, exercise training increases monocyte mobilization (29) which may lead to more efficient recovery following our acute resistance exercise bout which could be masking differences that would otherwise be present in a more susceptible cohort.

To our knowledge this study is the first to investigate the effects of MCJ on monocyte phenotypes. It appears that MCJ does not inhibit the monocyte mobilization response in comparison to placebo. Moreover, perceived exercise soreness through algometer and visual analog scales illustrated no change associated with MCJ consumption. Contrary to the initial hypothesis, these data support the notion that MCJ may not affect the acute systemic inflammatory response following resistance exercise in resistance-trained males. Specifically, the monocyte response to exercise appears equivalent to placebo, which may preserve monocyte mobilization to acute resistance exercise thereby promoting skeletal muscle rejuvenation. However, it should be noted that only seven male participants completed this study with five being analyzed for monocyte characterization. Therefore, these results may differ with a more expansive sample size, or in female participants. Taken together, these data suggest that MCJ may not influence the inflammatory response following a whole-body high-intensity resistance training bout in resistance trained men. This is positive, as acute inflammation appears to be essential for optimal skeletal muscle rejuvenation.

This study utilizes a novel approach to determine the effect of MCJ on inflammation. The data suggests MCJ consumption does not dampen the inflammatory response (IL-6 and monocyte mobilization) to an acute bout of resistance exercise. This may be beneficial for individuals wanting to consume an antioxidant rich Montmorency cherry product throughout their training cycles without concern for deleterious effects upon training adaptations.

ACKNOWLEDGEMENTS

This project was funded by the American College of Sports Medicine's Northwest Chapter Student Research Award, and Central Washington University's Department of Health Sciences. The authors declare no conflicts of interest. All experiments complied with the current laws of the United States of America. The authors declare no conflicts of interest.

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