The effects of betalain-rich concentrate supplementation in attenuating muscle damage following eccentric exercise

STEVEN VITTI¹, MICHAEL BRUNEAU JR.², KYLE LEYSHON³, SUSAN SOTIR⁴, SAMUEL HEADLEY⁴, ELIZABETH O'NEILL⁴

¹Department of Allied Health, University of Illinois, Springfield, United States of America
²Department of Health Sciences, Drexel University, Philadelphia, United States of America
³Department of Human Performance, Concordia University, Chicago, United States of America
⁴Department of Exercise Science and Sport Studies, Springfield College, Springfield, United States of America

ABSTRACT

Betalains are bioactive pigments that have been shown to reduce muscle damage and enhance recovery from exercise. However, to date, studies have examined the effects of betalains on aerobic exercise alone, and thus, their possible benefits for muscle damage recovery following eccentric exercise is unknown. We, therefore, aimed to examine the effects of a betalain-rich concentrate (BRC) on indices of muscle damage following eccentric exercise. Eleven healthy, recreationally active males were randomly assigned into a treatment group (50 mg of BRC, containing 12.5 mg of betalains, 3 times per day for 3 days) or a control group and performed 30 maximal eccentric contractions of the elbow flexors. Maximal voluntary contraction (MVC), arm circumference (AC), muscle soreness (MS), and range of motion (ROM) were measured before, immediately after, and 24, 48, and 72 hr following eccentric exercise. Creatine kinase (CK) was measured before, 24, 48, and 72 hr following the eccentric exercise. No significant differences or interactions were observed for any of the variables (p = > .05); however, a non-significant trend with a large effect size (p = .07, \( \eta_p^2 = .28 \)) was found for the main effect for MVC. Although we failed to identify any statistically significant differences in any of the variables measured, the large effect size observed for MVC may have practical benefits in the enhancement of skeletal muscle recovery following eccentric exercise.

Keywords: Exercise recovery; Antioxidants; Beetroot.

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INTRODUCTION

Eccentric exercise induces ultrastructural damage to skeletal muscle tissue. The extent of tissue damage in response to eccentric exercise depends upon factors such as the novelty of the eccentric exercise, the number of muscle groups involved (i.e., single joint vs. multi-joint exercises), and the body parts involved (i.e., upper body vs. lower body; Peake et al., 2017). Prolonged decrements in skeletal muscle strength is considered the most reliable indirect index of muscle damage (Clarkson & Hubal, 2002); though, other proxies of muscle damage, albeit less reliable, include reductions in range of motion, increases in circulating muscle enzymes, delayed onset muscle soreness, and localized edema (Paulsen et al., 2012).

Following eccentric exercise, leukocytes, specifically neutrophils and pro-inflammatory macrophages (M1), accumulate in the injured tissue and initiate structural remodelling by clearing damaged tissue via the release of reactive oxygen species (ROS) and proteolytic enzymes (Peake et al., 2017). Approximately 24 hr after the initial damage, the transition from M1 macrophages to anti-inflammatory macrophages (M2) initiates the remodelling process by increasing myoblast proliferation and satellite cell activity, which promote skeletal muscle repair (Chazaud, 2016). Although the time course of the M1 to M2 remodelling process may vary, prolonged disturbances in redox homeostasis and pro-inflammatory states can lead to secondary muscle damage, thereby delaying skeletal muscle repair (Peake et al., 2017).

Betalains, a natural bioactive pigment contained in vegetables such as beetroots, have been shown to reduce inflammation in a dose-dependent manner (Pietrzkowski et al., 2010), and exhibit antioxidant (Albano et al., 2015) and anti-nitrosative properties (Sakihama et al., 2012). The findings of recent studies suggest that betalains could potentially provide ergogenic benefits and enhance skeletal muscle recovery following exercise. Van Hoorebeke et al. (2016) reported improvements in 5 km time trial performance following 7 days of a betalain-rich concentrate (BRC) supplementation. Moreover, attenuated increases in lactate dehydrogenase, a marker of muscle damage, was observed immediately, and 30 min following the time trial. Employing a similar dosing strategy, Montenegro et al. (2016) observed improvements in running and recovery performance, along with attenuated increases in creatine kinase (CK) immediately following exercise. These findings suggest that BRC may be useful in mitigating muscle damage and hastening recovery. While the mechanisms responsible for these observed differences remain speculative, betalains have been shown to downregulate pro-inflammatory pathways (El Gamal et al., 2014), and inhibit lipid oxidation (Kanner et al., 2001), which independently or collectively could preserve the cellular integrity of local myocytes and attenuate skeletal muscle damage.

Peak plasma concentrations of betalains occur approximately 3 hr following ingestion, with complete disappearance from plasma in 12 hr (Tesoriere et al., 2004). The aforementioned studies using BRC supplementation followed a 7-day loading scheme whereby the final doses were administered on the day of testing (2 hr prior to, and immediately following exercise). Considering the pharmacokinetics of betalains, it is likely that the final doses rather than the preloading period provided the ergogenic benefit and mitigated muscle damage. We, therefore, aimed to examine the effects of 3 days of BRC supplementation on markers of muscle damage following eccentric muscle damage. We hypothesized that BRC supplementation would enhance skeletal muscle recovery, attenuate increases in CK, and improve indices of muscle damage.
METHODS

Participants
Eleven healthy, active males age 20-29 were recruited from Springfield College. All participants provided written informed consent and completed a health screening prior to enrolment (Visit 1). Individuals were excluded if they: 1) had any musculoskeletal injuries of the upper body, 2) had medical issues that would be a contraindication to vigorous exercise, 3) failed to meet the activity guidelines, and 4) were taking any medication that would interfere with the interpretation of our results (e.g., anti-inflammatory drugs). All remaining and eligible participants were instructed to avoid strenuous activity and anti-inflammatory drugs for the entirety of the study. Participants reported to the laboratory for a total of nine visits over the course of two non-consecutive weeks and were asked to adhere to a similar diet while enrolled in the study. Each participant was asked to complete a dietary food log for 3 days beginning on the initial testing day (Visit 2). All procedures were approved by the Springfield College Institutional Review Board prior to data collection.

Experimental design
This study employed a randomized crossover design. During Visit 1, participants were fitted into the isokinetic dynamometer (Biodex System 2, Biodex Medical Systems, Inc. Shirley, New York, USA) and all adjustment values were recorded for consistent placement during each visit. Prior to leaving the laboratory, participants were randomly assigned to either Condition 1, BRC supplementation, or Condition 2, the control. Those assigned the BRC were instructed to consume 50 mg of beetroot extract containing a standardized 12.5 mg of betalains three times per day, every 6 hr on the day of, 24 hr and 48 hr following the muscle damage protocol (Figure 1). Participants did not receive a placebo for the control and were instructed to report to the laboratory during their designated time. Lastly, participants were randomly assigned to perform the first eccentric exercise protocol using their dominant or non-dominant arm.

All testing procedures began on Visit 2. Participants arrived at the laboratory following an overnight fast. Those assigned the BRC were asked to consume the supplement 2.5 hr prior to arriving. Upon arrival, baseline measures of muscle soreness (MS), range of motion (ROM) and arm circumference (AC) were collected, and blood draws were obtained. The participants were then fitted into the isokinetic dynamometer for MVC assessment and the eccentric exercise protocol. Upon completion of the exercise protocol, all measures, with the exception of the blood, were obtained. Participants returned to the laboratory for 3 days after at their designated times (Visits 3, 4 and 5; 24 hr, 48 hr, and 72 hr, respectively) and all measures were collected. Participants were scheduled to complete the second testing session 2 weeks after the initial testing session on the same day and time using the contralateral arm. The procedures for Visits 6-9 mirrored those of Visits 2-5.

Figure 1. Schematic representing BRC supplementation timeline.
Maximal Voluntary Contraction and Eccentric Exercise Protocol
To assess MVC, the participants were situated in the isokinetic dynamometer with a resting elbow position of 90°. Once positioned, they were instructed to exert maximal force of the elbow flexors for 3 sec for a total of three sets. Thirty seconds of rest was provided between each MVC, and the largest value was recorded for analysis. MVC is represented as the percentage change from baseline.

Once MVC was collected, the elbow was positioned at a starting joint angle of 60° with a terminal joint angle of 180°. The eccentric exercise protocol consisted of 30 maximal eccentric contractions at an angular velocity of 60°/s. The arm was returned to resting position passively at a velocity of 60°/s, giving approximately 2 s between each contraction. During both the MVC and exercise protocol, each participant was verbally encouraged to perform each repetition with maximal effort.

Range of Motion
ROM was measured in degrees at the elbow joint. The bony landmarks include the lateral epicondyle of the humerus, the radial styloid, and the acromion process. Three measurements of resting and flexed arm angles were obtained. The average of the three were recorded to the nearest degree. ROM is presented as the delta between the average resting angle and the flexed angle.

Arm Circumference
AC was measured using standard measuring tape. Participants were asked to relax their arms at their sides, and measurements were taken mid arm, measured halfway between the acromion and olecranon process. Three measurements were taken, and the average of the three was recorded to the nearest cm. AC is reported as the delta change from baseline.

Perceived muscle soreness
Perceived soreness was determined using a visual analogue scale (VAS). The VAS consists of a 100-mm line with “no pain” and “unbearable pain” on polar sides. Participants were asked to mark the area between the polar ends that indicated the level of perceived soreness while maximally flexing the at the elbow. VAS values were recorded in mm relative to the specific area chosen by the participants.

Blood analyses
All blood samples were centrifuged for 14 min at 1000 RCF at 5 °C and serum was stored at -80 °C until analysis. All blood was processed at the University of Massachusetts Amherst Life Sciences Laboratory using the Piccolo® XpressTM Chemistry Analyzer (Abaxis, Inc., Union City, CA). Piccolo® Metlyte Plus reagent discs (Abaxis, Union City, CA) was used to analyse CK.

Statistical analyses
Descriptive statistics were calculated for participant characteristics. Preliminary data analyses were performed to ensure the assumptions of each analysis were met. Mauchly’s Test of Sphericity was used to indicate if the assumption of sphericity was met. Greenhouse-Geisser values were reported for all variables and factors that violated the assumption of sphericity. The Shapiro-Wilk test was used to assess the normality of the data distributions. Data that violated the assumption of normality were transformed and subsequently retested for normality. If the data failed to meet the assumption of normality after transformation, we yielded to non-parametric statistics and compared the values to the parametric statistics. No differences in the model statistics were observed, so the raw values were used in our interpretation herein. A listwise deletion was carried out for a single participant with several missing CK values.
Four 2 (Condition) X 5 (Time) repeated measure ANOVAs were used to determine mean differences between MVC, ROM, AC, and MS for each condition over five time points. One 2 (Condition) X 4 (Time) repeated measure ANOVA was used to determine mean differences in CK over four time points. Paired sample t-tests were performed to detect significant differences in macronutrient distribution between conditions. All statistical analyses were performed with the IBM Statistical Package for the Social Sciences (SPSS), version 25.0. Alpha level was set at .05.

RESULTS

A summary of the participant characteristics is provided in Table 1. Analysis of the food diary information revealed no significant differences in energy consumption or macronutrient distribution ($p > .05$). Results for all measurements are presented in Table 2.

Table 1. Mean ± Standard Deviations for participant characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.73 ± 3.35</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 ± 0.06</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.46 ± 13.84</td>
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</tbody>
</table>

Table 2. Indices of Muscle Damage Following Maximal Eccentric Exercise.

<table>
<thead>
<tr>
<th>Variable (Δ%)</th>
<th>Baseline</th>
<th>Post</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>p-value (I)</th>
<th>p-value (C)</th>
<th>p-value (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.35</td>
<td>.07</td>
<td>.00*</td>
</tr>
<tr>
<td>BRC</td>
<td>100</td>
<td>75.4 ± 9.2</td>
<td>83.0 ± 17.0</td>
<td>91.3 ± 15.9</td>
<td>95.5 ± 20.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>100</td>
<td>69.8 ± 12.6</td>
<td>77.0 ± 16.4</td>
<td>82.6 ± 18.1</td>
<td>87.6 ± 18.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROM (Δ°)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.64</td>
<td>.71</td>
<td>.00*</td>
</tr>
<tr>
<td>BRC</td>
<td>0.00</td>
<td>27.2 ± 11.7</td>
<td>11.1 ± 13.4</td>
<td>9.5 ± 15.9</td>
<td>10.1 ± 15.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>0.00</td>
<td>21.5 ± 6.8</td>
<td>13.0 ± 11.4</td>
<td>11.8 ± 9.8</td>
<td>13.2 ± 14.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC (Δ cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.67</td>
<td>.65</td>
<td>.00*</td>
</tr>
<tr>
<td>BRC</td>
<td>0.00</td>
<td>1.11 ± 0.6</td>
<td>0.2 ± 0.6</td>
<td>0.3 ± 0.6</td>
<td>0.6 ± 0.8</td>
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<tr>
<td>CON</td>
<td>0.00</td>
<td>0.9 ± 0.7</td>
<td>0.2 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>0.4 ± 1.0</td>
<td></td>
<td></td>
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<tr>
<td>MS (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.85</td>
<td>.33</td>
<td>.00*</td>
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<tr>
<td>BRC</td>
<td>0.00</td>
<td>36.4 ± 17.5</td>
<td>41.8 ± 14.7</td>
<td>43.6 ± 10.3</td>
<td>33.6 ± 15.7</td>
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<tr>
<td>CON</td>
<td>0.00</td>
<td>40.0 ± 26.1</td>
<td>44.6 ± 18.1</td>
<td>45.5 ± 18.6</td>
<td>40.0 ± 24.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK (I/U)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.84</td>
<td>.67</td>
<td>.02*</td>
</tr>
<tr>
<td>BRC</td>
<td>218.1 ± 75.9</td>
<td>--</td>
<td>357.6 ± 250.4</td>
<td>861.5 ± 1481.5</td>
<td>1536.0 ± 1888.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>255.1 ± 147.6</td>
<td>--</td>
<td>265.7 ± 134.8</td>
<td>662.4 ± 779.8</td>
<td>1298.9 ± 1575.2</td>
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</tbody>
</table>

*Significant difference ($p < .05$).

The data herein is expressed as Means ± Standard Deviations. MVC = maximal voluntary contraction; ROM = range of motion; AC = arm circumference; MS = muscle soreness; CK = creatine kinase; I = interaction; C = condition; T = treatment.

Maximal Voluntary Contraction

A significant difference for the main effect of time was observed ($p = .00$). No significant Condition X Time interaction was observed ($p = .11$). No significant difference for the main effect of Condition was observed; however, there was a trend towards statistical significance with a corresponding large effect size ($p = .07$, $\eta^2_p = .28$).
Creatine Kinase
A significant main effect for Time was observed for CK \((p = .02)\) following the muscle damage protocol. No significant interactions \((p = .81)\) or differences for the main effect of Condition \((p = .68)\) were observed, demonstrating no differences between the BRC and control.

Arm Circumference
A significant main effect for Time was observed for AC \((p = .00)\), indicating the presence of significant localized swelling immediately, and for the 3 days following the eccentric exercise protocol. No significant interactions \((p = .66)\) or differences for the main effect of Condition \((p = .64)\) were observed.

Range of Motion
A significant main effect for Time was observed for ROM \((p = .00)\) immediately following the eccentric exercise protocol. No significant interactions \((p = .64)\) or differences for the main effect of Condition \((p = .71)\) were observed.

Perceived muscle soreness
A significant main effect for time was observed for MS \((p = .00)\) immediately following the eccentric exercise protocol and persisted for the subsequent 3 days. No significant interactions \((p = .91)\) or differences for the main effect of Condition \((p = .32)\) were observed.

DISCUSSION
Our study is the first to examine the possible mediating effects of BRC on skeletal muscle recovery following eccentric exercise. The primary aim of our investigation was to examine the effects of 3 days of BRC on commonly measured indices of muscle damage following an exercise-induced muscle damage protocol. We found that 3 days of BRC had no significant effect on indices of muscle damage.

Previous work by Montenegro et al. (2016) and Van Hoorebecke et al. (2016) found enhanced aerobic performance and blunted markers of muscle damage following BRC supplementation. Although the exact mechanisms responsible remain unknown, the researchers hypothesize that the anti-inflammatory and antioxidant properties of betalains reduced the extent of muscle damage during exercise, thus preserving the force-generating capacities of the exercised muscle. While we failed to find a significant difference in force-generating capacities in our study, the trend toward significance and corresponding large effect size between treatments for MVC may be practically meaningful. We observed a percentage difference of approximately 9% and 8% between the two conditions at 48 hr and 72 hr, respectively. Though these differences reflect recovery of a single muscle group, it is worth mentioning that even minute increases in skeletal muscle recovery are often considered to have meaningful value, specifically for athletes looking for a competitive edge (Cintineo et al., 2018).

We also failed to observe any difference in CK between conditions. Though not significantly different, the changes were greater in the BRC across all time points. Similar findings were reported by Bowtell et al. (2011), who found improvements in muscle force recovery following Montmorency cherry juice consumption with no differences in CK between the conditions. The researchers did, however, observed attenuated increases in protein carbonyls (PC), a marker of oxidative stress. While PCs were not assessed in our study, it is plausible to suggest that the large effect observed could be attributed to differences in local muscle redox status. Calcium sensitivity of the contractile unit in fast twitch fibres is extremely sensitive to free radical species. Large disturbances of redox homeostasis could depress force production by altering calcium...
handling (Place et al., 2015) and/or decreasing calcium sensitivity to troponin (Lamb & Westerblad, 2011). Because eccentric contractions preferentially damage fast-twitch muscle fibres (Brockett et al., 2002), the free radical scavenging properties of betalains could theoretically increase force production independent of muscle damage. A recent study by Kamandulis et al. (2017) reported prolonged reductions in MVC independent of increases in ROS immediately and 24 hr following 100 drop jumps. However, ROS production has been found to increase in the days following acute eccentric muscle damage. Close et al. (2004) reported peak elevated ROS production 72 hr following exercise-induced muscle damage. Therefore, we postulate that the BRC supplement administered in the current investigation may have preserved skeletal muscle function by scavenging local ROS generation within the damaged muscle.

To date, only one study has examined the effects of high doses of betalains on skeletal muscle recovery following exercise-induced muscle damage. Clifford et al. (2016) reported no improvements in MVC of the knee extensors following multiple servings of beetroot juice (BRJ) containing 194 mg of betacyanins, a class of betalains that gives beetroot its red appearance. It is worth noting that the MVC and CK values reported by Clifford et al. (2016) are indicative of mild muscle damage (Paulsen et al., 2012). Therefore, the exercise protocol may have been insufficient to observe the efficacy of the betalains contained within the BRJ as leucocytes infiltration is minimal following mild muscle damage (Paulsen et al., 2012). Furthermore, consideration must be made for the source of betalains. Research regarding the absorption, stability, and subsequent bioavailability of betalains is lacking. The bioaccessibility of betalains, specifically betacyanin, appears to be restricted by the presence of a food matrix (Tesoriere et al., 2004). Thus, the efficacy of the betalains contained within the BRJ administered by Clifford et al. may have been restricted by other components contained within BRJ. The supplement used in our study consisted of a concentrate that was standardized to contain 12.5 mg of betalains separated from nitrates and sugars. Therefore, the absence of sugar, in addition to encapsulation, might have enhanced the absorption and bioavailability of the betalains (Miguel, 2018; Tesoriere et al., 2004), which could explain some of the observed discrepancies in our study. It is important to point out that our study examined muscle damage of the elbow flexors, not the knee extensors. Therefore, it might not be appropriate to compare CK values as a qualitative assessment of muscle damage.

Our study is not without limitation. First, it is important to acknowledge that there was no placebo assigned to the control group. However, despite this obvious limitation, we identified no differences in any of the indices of muscle damage between conditions, suggesting that the lack of placebo likely had little influence on the outcomes. Second, plasma betalains were not measured. Consequently, it cannot be stated with certainty that the large effect observed was attributed to the BRC supplementation. Thus, one should exercise caution when interpreting our findings.

CONCLUSION

Our eccentric exercise protocol was sufficient in inducing moderate muscle damage as indicated by the decrements in muscle force, increases in CK, muscle soreness, and local swelling. We found that 3 days of BRC had no significant impact on indices of muscle damage following eccentric exercise; however, we did observe a large effect size between conditions. Therefore, despite our study’s limitations, we believe that BRC could perhaps provide a practical strategy for enhancing muscle recovery. Future research efforts should aim to replicate our study using a placebo-control design, and a larger sample. In addition, future research should consider assessing markers of oxidative damage (e.g., protein carbonyls), acute-phase markers of inflammation, and reactive oxygen and nitrogen species. The information gained from such investigations would help determine whether any observed differences might be attributed to (a) attenuated
muscle damage per se, or (b) modifications in the force-generating capacity of the exercised muscle independent of muscle damage.

**AUTHOR CONTRIBUTIONS**

This study was designed by SV and KL; the data was collected and analysed by SV and MB; data interpretation and manuscript preparation were undertaken by SV, EO, SS, SH, and MB. All authors approved of the final version of the paper.

**SUPPORTING AGENCIES**

No funding agencies were reported by the authors.

**DISCLOSURE STATEMENT**

No potential conflict of interest was reported by the authors.

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**REFERENCES**


