Antioxidant-rich beetroot juice does not adversely affect acute neuromuscular adaptation following eccentric exercise

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Abstract

This study examined the effects of beetroot juice on the repeated bout effect (RBE) to eccentric exercise. Twenty nine recreationally active males performed two bouts of 100-drop jumps, separated by 14-21 days. Using a double-blind, independent groups design, participants consumed either a higher dose beetroot juice (H-BT; 250 ml, *n* =10), a lower dose beetroot juice (LBT; 125 ml, *n* = 9), or an isocaloric placebo (PLA; 250 ml, *n* = 10) for 3 days after bout 1; no drinks were consumed after bout 2. Maximal isometric voluntary contraction (MIVC), countermovement jump (CMJ), pressure-pain threshold (PPT) and creatine kinase (CK) were measured pre, post, 24, 48 and 72 h following both bouts. In bout 2, CMJ and MIVC recovered quicker and CK activity was attenuated (vs. bout 1) (P <0.05) in all groups, demonstrating an RBE. At 24 h post bout 1, MIVC was 84.1±16.1, 83.6±11.6, 79.7±15.1% relative to baseline values in the H-BT, L-BT and PLA groups, respectively; at 24 h post bout 2, MIVC recovered to 90.7±13.7, 92.9±6.9, 87.8±6.9, in the H-BT, L-BT and PLA groups, respectively. These findings suggest that supplementation with antioxidant-rich beetroot juice does not adversely affect acute adaptations to a bout of eccentric exercise.

Introduction

Antioxidant supplementation is purported as a strategy to attenuate the signs and symptoms of muscle damage (i.e., soreness, loss of muscle function, inflammation) that result from exercise involving strenuous exercise, particularly that has an eccentric contraction component (Bloomer, 2007; Peake and Suzuki 2004). The suggested benefits of consuming antioxidant supplements is to protect cells against contraction-induced increases in reactive oxygen species (ROS), which have the potential to exacerbate the muscle damage response (Toumi and Best, 2003). Blunting inflammation-induced ROS production has been shown to attenuate myofibre damage following lengthening muscle contractions (Brickson et al., 2003; Zerba, Komorowski and Faulkner, 1990; Pizza Peterson, Baas and Koh, 2005) and in some instances, attenuating inflammatory processes has reduced decrements in muscle function (Pizza et al., 2005; Zerba et al., 1990). However, this is not a consistent finding with studies also showing that attenuating ROS production and inflammation had no effect on exercise-induced muscle damage (EIMD) (Goldfarb, Garten, Cho, Chee, and Chambers, 2011; Zembron-Lacny et al., 2009).

Although an excess production of ROS and the associated inflammatory events might initially harm the muscle cell, they are also fundamental to the regenerative process, and are now widely considered to be a necessary stimulus for both acute and chronic cellular adaptations to exercise (Close et al., 2006; Michailidis et al., 2013; Paulsen et al., 2014). For instance, following an acute bout of eccentric exercise, immune cells (i.e., phagocytes) produce ROS and a host of messenger proteins known as cytokines, which have been proposed to act as signalling molecules for molecular changes that reinforce cell defences (McHugh, 2003; Pizza, Koh, McGregor and Brooks, 2002; Xin, Hyldahl, Chipkin and Clarkson, 2014; Hubal, Chen, Thompson and Clarkson, 2008; Hyldahl et al., 2015). Such adaptations could make cells more resistant to damage during similar exercise bouts in the future. This acute adaptive response to eccentric, muscle-damaging exercise is classically

74 illustrated by the repeated bout effect (RBE), in which the magnitude of muscle damage (i.e.,
75 force deficits) evoked by a single exercise damaging stimulus is attenuated in a subsequent
76 bout performed many weeks later (McHugh, 2003; Nosaka and Clarkson, 1995).
77
78 Evidence that post-exercise inflammation and ROS production might afford beneficial effects
79 for physiological adaptation has sparked some debate within the literature (Gomez-Cabrera,
80 Ristow and Viña, 2012). Specifically, the question arises whether prophylactic use of
81 antioxidant supplements to blunt oxidative stress and inflammation could have detrimental
82 effects in the regeneration and adaptive responses that might translate to muscle function
83 and performance enhancement, both in the short and long-term (Gomez-Cabrera et al.,
84 2012; Paulsen et al., 2014; Close et al., 2006). The majority of studies so far have focused
85 on the potential negative effects of antioxidants on chronic adaptations to eccentric-heavy
86 exercise, such as changes in muscle strength and hypertrophy over a number of weeks in
87 either young (Paulsen et al., 2014; Theodorou et al., 2011) or elderly men (Bjørnsen et al.,
88 2015). In comparison, very little attention has been given as to the effect of antioxidants on
89 acute adaptive effects associated with eccentric exercise. However, the fact that the
90 adaptive response from a just single bout of eccentric exercise results from (at least in part)
91 cellular changes, it would be anticipated that antioxidant supplementation might attenuate
92 the magnitude of the RBE. To date, this possibility has only been addressed by one recent
93 study, which showed that 2 weeks of supplementation with a high antioxidant dose of vitamin
94 C (1000 $mg \cdot d \cdot^{-1}$) and E (400 $I \cdot U^{-1}$) did not blunt the RBE in response to a bout of downhill
95 running (He, Hockemeyer and Sedlock, 2015). This was evidenced by the similar
96 attenuations in muscle soreness and plasma creatine kinase (CK) in bout 2 compared to
97 bout 1 for both the supplementation and placebo group. However, these findings are limited
98 by the absence of any measures of muscle function, which are widely considered the best
99 indicators of skeletal muscle damage (Paulsen et al., 2012; Warren, Lowe and Armstrong,
100 1999).
101

Furthermore, reports regarding the deleterious effects of antioxidant supplementation on adaptations, particularly following eccentric exercise, are presently limited to studies that administered very high doses of vitamin C (Close et al., 2006) or a combination of vitamins C and E (Paulsen et al., 2014). In recent years however, there has been increased focus on the physiological effects of antioxidant-rich functional foods, such as cherry juice (Howatson et al., 2010; Bowtell et al., 2011), blueberry juice (McLeay et al., 2011) and pomegranate juice (Trombold, Barnes, Critchley and Coyle, 2010), which have shown promise as recovery aids following strenuous or damaging exercise. Nevertheless, no studies have examined the influence of an antioxidant-rich food supplement on the RBE to a bout of eccentrically biased exercise.

In a recent study, consuming beetroot juice (BTJ), a functional food that is rich in antioxidants (Wootton-Beard and Ryan 2011), attenuated some indices of muscle damage after a bout of eccentric exercise (Clifford, Bell, West, Howatson, Stevenson, 2015) suggesting that BTJ may hold promise as a recovery aid. As a follow up to this study, a sub-set of the participants repeated the same bout of eccentric exercise 3 weeks later, without supplementation. The aim of this follow up study was to examine the effects of BTJ supplementation on the acute adaptive response (represented by the RBE) after muscle damaging exercise. It is important to make the distinction that in this we specifically wanted to test whether BTJ would adversely affect the adaptive response to a single bout of exercise, not a series of exercise bouts, and therefore we were primarily interested in the acute adaptive responses typically seen after a single bout of exercise (i.e., improved muscle recovery, lowered muscle soreness), as opposed to more chronic adaptations (increased hypertrophy and strength). As a secondary aim of this study, we wanted to establish whether a higher or lower dose of BTJ would differentially affect the RBE, given that previous reports have suggested that although higher antioxidant doses might be harmful (i.e., vitamin C ≥1000 mg·day^{-1}) (Close et al, 2006; Paulsen et al, 2014), more moderate doses are not (500 mg mg·day^{-1}) (Yfanti et al., 2010; 2011). We hypothesised that

130 those participants that consumed BTJ after the initial bout would have a blunted RBE
131 compared to those who consumed a placebo beverage, and the magnitude of these
132 responses might be exacerbated after a higher dose of BTJ.

133 **Materials and Methods**

134 ***Participants***

135 A power calculation was conducted to determine an adequate sample size. Using the
136 findings of previous studies that examined group differences in isometric strength (Bell et al.,
137 2014; Howatson et al., 2010), it was estimated that a ≥10% group difference (SD: 7.5%,
138 based on % change from baseline data) in our primary outcome variable, maximal isometric
139 voluntary contractions (MIVC), would be required to detect significant changes. With a power
140 of 0.80 and two tailed α level of 0.05, the estimated number of participants required was n =
141 9 per group. Therefore, we initially recruited 30 volunteers for this experiment to account for
142 potential drop outs. As anticipated, one participant was unable to complete the second part
143 of the study due to an injury unrelated to the study procedures. As such, only twenty nine
144 participants completed all study procedures. All participants were physically active, healthy
145 males (age 21 ± 3 years; height 1.77 ± 0.80 m; body mass 75.6 ± 8.8 kg) who had no prior
146 experience with the bout of muscle damaging exercise. Participants were excluded if they
147 had a known food allergy, had recently (within 1 month of participation) used antioxidant or
148 anti-inflammatory supplements (i.e., non-steroidal inflammatory drugs, vitamin C and E),
149 were suffering from a musculoskeletal injury, or had previous history of renal, gastrointestinal
150 or cardiovascular complications. Participants were instructed to refrain from exercise 2 days
151 prior to and for the duration of data collection for both exercise bouts. Participants were also
152 instructed to avoid foods with a high phytochemical, nitrate or nitrite content (i.e., vegetables,
153 cured meats, fruits and its equivalents (i.e., juices), whole grains, caffeinated beverages)
154 throughout the data collection period. Participants recorded their food intake 2 days before

each exercise bout and continued until 72 hr post. The protocol received institutional ethical approval. All participants gave written informed consent prior to participation.

Experimental overview

The study employed a randomised, double blind, placebo controlled, independent groups design. Excluding familiarisation, participants were required to attend the laboratory on 8 occasions. Participants were stratified into three supplement groups according to their baseline MIVC scores: higher dose of beetroot juice (H-BT; *n* = 10), low dose of beetroot of juice (L-BT; *n* = 9) or a placebo (PLA; *n* = 10). Supplements were consumed immediately, 2 h post-exercise, and at set times 24 and 48 h following a bout of muscle damaging exercise (see *Supplementation* below). Muscle function, muscle soreness and CK were measured pre, post, 24, 48 and 72 h post exercise.14-21 days after bout 1 the same protocol was repeated but no supplements were provided. All testing was conducted in the morning following an overnight fast at the same time of day (within participants). Participants were familiarised with all equipment and study procedures prior to testing (see Figure 1 for schematic of study procedures).

Muscle damaging exercise

Muscle damage was induced using a drop jump protocol previously described by (Howatson Goodall, van Someren, 2009). 100 Jumps were performed 10 seconds apart with a 2 minute rest period provided every 20 jumps. Each jump was performed from a 0.6 m high box; upon landing, participants were instructed to descend to a 90º knee angle before performing a maximal effort vertical jump. Participants were given verbal encouragement throughout to ensure maximal effort was maintained.

Supplementation

The H-BT supplement was a commercially available beetroot juice concentrate drink (Love Beets Super Tasty Beet Juice, Gs Fresh Ltd, Cambridgeshire, UK). Each bottle provided

approximately 401.72 ± 37.72 mg (expressed as Gallic acid equivalents) of phenolic compounds and had an antioxidant capacity of 2.85 ± 0.05 $m \cdot mol^{-1}$ Trolox equivalents. The L-BT was equivalent to half the dose of beetroot provided by the H-BT i.e., 125 ml and made up to the same volume using water. The phytonutrient content of the L-BT was not subjected to in-depth analysis, but estimating from the above data, it was anticipated that it would have approximately half the polyphenol content and antioxidant capacity of the H-BT. The PLA was flavoured with a low calorie fruit squash (Kia Ora, Coca Cola Enterprises, Uxbridge, UK) that had a comparatively lower phytochemical content (43 ± 3.2 mg Gallic acid equivalents) and antioxidant capacity (0.02 ± 0.01 $m \cdot mol^{-1}$ Trolox equivalents) compared to the beetroot juice treatments. The L-BT and the PLA contained maltodextrin (Myprotein, Manchester, UK) and flavourless protein powder (Arla Foods, Amba, Denmark) to match H-BT for macro nutrient content (See table 1.). After the drop jumps, one bottle (250 ml per serving) was consumed immediately post, another 2 h post, and one with an evening meal (3 in total). At 24 and 48 h post, one serving was consumed immediately after completing the dependent variables (Figure 1) and one with an evening meal (2 per day) equating to 7 servings over 3 days. As this was the first study to investigate BTJ in this manner, we had to rely on similarly designed studies to determine the most appropriate dosing strategy. We decided to provide two daily servings of BTJ in the days post-exercise because other antioxidant rich-juices (cherry and pomegranate) at this dose exhibited physiological effects that resulted in improved functional recovery (Howatson et al. 2010; Bell et al. 2014; Trombold et al. 2010). We provided an extra serving on the day immediately post-exercise however, because muscle damage (i.e., decreased muscle function) and the accompanying inflammatory response has been shown to be greater 24 h after performing an analogous muscle-damaging protocol (Chatzinikolaou et al., 2010; Howatson et al., 2009). We therefore reasoned that a slightly higher dose might be needed to help expedite recovery in the more immediate hours (<24) after exercise.

206 To comply with the double-blind design, each bottle was provided in identically masked
207 bottles, only distinguished by a single letter code, which was kept by a member of the
208 department not involved in data collection. As detailed in a previous study, due to the distinct
209 taste of the BTJ we were unable to match the PLA for taste and texture. In an attempt to
210 overcome this, the participants are not informed of what the specific drinks under
211 examination are; just that they will receive an antioxidant-containing drink to assess its
212 impact on recovery. This ensured that the participants did not know the overall aim of the
213 study, eliminating any bias based on pre-conceptions regarding BTJs potential ergogenic
214 effects.

215 ***Muscle soreness***

216 Muscle site-specific soreness was assessed as pressure pain threshold (PPT) with a
217 handheld algometer (N^2). Measurements were taken with participants lying supine; pressure
218 was applied continuously at a rate of 10 N $cm^{-2} \cdot s^{-1}$ to a pre-marked site on the muscle belly
219 until participants verbally signified pain or discomfort. All measures were performed by the
220 same examiner and the average of two readings was used for analysis. Muscle sites were
221 vastus laterialis: mid-way between the superior aspect of the greater trochanter and head of
222 the tibia, rectus femoris: mid-way between the anterior patella and inguinal fold, and
223 gastrocnemius: most medial aspect of the calf at relaxed maximum girth.

224 ***Maximal isometric voluntary contraction***

225 Maximal isometric voluntary contractions (MIVCs) were performed using a portable strain
226 gauge (MIE Medical Research Ltd., Leeds, UK), as in previous studies (Howatson et al.
227 2009). Participants sat upright with a gauze attached to their right ankle just above the
228 malleoli, and were instructed to extend their leg with maximal force, holding the contraction
229 for 3 seconds. Force was recorded in Newtons (N) and at a 90° knee joint angle, as
230 determined by a goniometer. The peak value from 3 maximal contractions (separated by 60

seconds) was used for analysis. This measure was calculated to have good reliability (coefficient variability (CV); 1.1%).

Counter movement jump height

Counter movement jumps (CMJ) were recorded with an optical system (Optojump next, Bolzano, Italy) to measure jump height in cm. With hands on their hips, participants descended into a 90° squat and jumped vertically with maximal effort. The average of 3 maximal jumps (separated by 30 seconds) was used for analysis. CV for this procedure was 2.1%.

Blood sample collection and analysis

Creatine kinase (CK) concentrations were determined from venous blood samples (10 ml) collected via venepuncture into EDTA vacutainers. Samples were immediately centrifuged at 3000 x g, 4°C for 15 minutes to separate plasma and subsequently stored in eppendorf's at -80°C. Plasma was analysed for CK spectrophotometrically using an automated system (Roche Modular, Roche Diagnostics, UK).

Statistical analysis

Data were analysed using IBM SPSS statistics version 21 and expressed as mean ± standard deviation (SD). A mixed model analysis of variance (ANOVA) with 3 treatment levels (H-BT vs. L-BT vs. PLA), x 2 bouts (bout 1 vs. bout 2), x 5 time points (pre, post, 24, 48 and 72 h post exercise) was used to test for significant differences between the dependent variables. Data analysis for MIVC, CMJ and PPT were conducted using percentage change from baseline values to account for individual variability. Group differences in height, weight, age, and baseline MIVC scores were analysed using one-way independent group ANOVAs. If significant group and interaction effects were observed, Fisher LSD *post hoc* tests were performed to locate where the differences occurred. Significance was set at $P < 0.05$ prior to analyses.

Results

There were no differences in participant characteristics (age, height, mass) and baseline MIVC between groups ($P > 0.05$). Results presented herein represent a subset of a larger study, and therefore data relating to group differences after bout 1 will not be discussed, but can be found elsewhere (Clifford et al. 2015). Main effects for time were observed for all dependent variables (CMJ, MIVC, CK and PPT; $P < 0.05$) following bout 1, indicating that the drop jumps effectively induced muscle damage. The decrement in MIVC was less in bout 2 compared to bout 1 ($F_{(1,26)} = 4.497$; $P = 0.04$; Figure 2), providing evidence of an RBE. The percentage decrease in MIVC in bout 1 (average across groups) was 16.1%, 12.2% and 17.3% at 24, 48 and 72 h post exercise, respectively, whereas after bout 2, decrements were attenuated to 9.5%, 6.2%, and 2.3% at 24, 48 and 72 h post exercise, respectively. There were no bout x group interactions ($F_{(2,26)} = 0.206$; $P = 0.815$), indicating that strength loss was similarly attenuated in all groups. At 72 h post bout 2, MIVC had recovered to 98.0 ± 9.0, 97.8 ± 6.0 and 97.1 ± 7.0% of baseline values in the H-BT, L-BT and PLA groups, respectively. The decrease in CMJ height was also attenuated in bout 2 compared to bout 1 (bout effect; $F_{(1,26)} = 25.430$; $P <0.001$) and there were no differences between the groups (bout x group interaction; $F_{(2,26)} = 0.709$; $P = 0.501$). 72 h post bout 1 CMJ height recovered in the H-BT, L-BT and PLA groups to 91.7 ± 9.7, 93.2 ± 7.1 and 87.4 ± 7.3% of baseline values, respectively, whereas 72 h following bout 2, CMJ height recovered to 101.4 ± 5.9, 96.9 ± 7.4 and 96.9 ± 4.8% of baseline values, respectively (Figure 3). PPT did not show an overall bout effect ($F_{(1,84)} = 1.683$; $P = 0.198$); but a bout x group interaction was observed ($F_{(2,84)} = 4.003$; $P = 0.022$), with post hoc analysis revealing that PPT was improved in bout 1 compared to bout 2 in PLA only (Table 2; $P = 0.001$). A main effect for bout showed that plasma CK was lower in bout 2 compared to bout 1 ($F_{(1,24)} = 15.200$; $P = 0.001$; Figure 4); there were no differences between the 3 groups (bout x group interaction; $F_{(2,21)} = 2.422$; $P = 0.113$).

Discussion

283 We have previously shown that BTJ attenuated some indices of muscle damage following a
284 single bout of eccentric exercise (Clifford et al., 2015). The aim of the current study was to
285 follow up these results and examine whether the beneficial effects demonstrated by BTJ in
286 that study would negatively impact the RBE, as illustrated by improved functional recovery in
287 a subsequent exercise bout. The principal finding of the current study was that 3 days of BTJ
288 supplementation, at either a higher (250 ml per serving) or lower (125 ml per serving) dose,
289 did not interfere with the RBE, therefore the acute adaptive response to a single bout of
290 eccentric exercise, despite showing evidence of attenuating muscle damage (data not
291 discussed, see Clifford et al. 2015). We observed significant reductions in MIVC and CMJ
292 height in all three groups after bout 1; however, consistent with a RBE, these reductions
293 were significantly attenuated in bout 2, irrespective of supplementation and dose. These
294 findings are in agreement with previous studies that observed similar attenuations in strength
295 loss when a series of drop jumps were repeated 14-21 days after an initial bout (Howatson
296 et al., 2009). CK activity was also significantly lower following bout 2 than bout 1, an effect
297 typical of the RBE, and which has been observed in previous investigations using repeated
298 bouts of eccentric exercise (Paulsen et al., 2010; Stupka, Tarnopolsky, Yardley and Phillips,
299 2001). The fact that there were no group differences in MIVC, CMJ or CK activity after bout
300 2, would suggest that contrary to our hypothesis, BTJ supplementation did not interfere with
301 the cellular mechanisms postulated to underpin the RBE to a single bout of exercise. While
302 the precise cellular mechanisms that contribute to the RBE are not clear, they are postulated
303 to include an increased expression of inflammatory related genes (Xin et al., 2013; Hubal et
304 al., 2008), a blunted inflammatory and oxidative stress response (Pizza et al., 2002; Pizza,
305 Baylies and Mitchell, 2001; Nikolaidis et al., 2007; Hirose et al., 2004) that together drive
306 extensive cytoskeletal remodelling (Hubal et al., 2008; Hyldahl et al., 2015) to protect the
307 muscle from damage when exposed to a similar stimulus in the future (McHugh, 2003).
308 Although the aforementioned mechanisms were not directly measured in the present study,
309 it is highly likely that they are at least partly responsible for the RBE we observed (i.e., faster
310 resolution of force deficits, reduced CK efflux). Therefore, the magnitude of the RBE would

have intuitively been altered if this cascade of events had been negatively affected by BTJ. Furthermore, and again counter to our hypothesis, the similar responses to bout 2 in both the LBT and HBT groups suggests that no dose-response effects were evident in terms of the magnitude of the RBE experienced. Notwithstanding these findings, it is important to acknowledge that the effects of BTJ (and at different doses) on longer term adaptive responses remains to be elucidated.

In contrast, only in the PLA group was the decrement in PPT, used as a measure of muscle soreness, attenuated in bout 2 versus bout 1. However, PPT did not differ between the PLA and BTJ groups in the 72 h period following bout 2. This discrepancy is therefore likely explained by the fact that PPT was significantly improved with BTJ supplementation after bout 1 (Table 3), but not PLA. Thus, after bout 2, while no further improvements in PPT were evident in the BTJ groups (probably because no BTJ was provided on this occasion), there was a significant improvement in the PLA group, as expected with an RBE.

The most pertinent new question posed in this study is the examination of exercise-induced adaptation after supplementation with an antioxidant-rich food (BTJ), whereas previous studies have predominately focused on the potential effects of high dose antioxidants vitamin C and E (Nikolaidis, Kerksick, Lamprecht and McAnulty, 2012; Sousa, Teixeira, Soares, 2014). Furthermore, studies examining the effects of antioxidants on adaptation to a single bout of eccentrically biased exercise, where muscle damage is principally induced via mechanical stress, are scarce. Nevertheless, our findings are in agreement with those of a recent study, in which 2 weeks of vitamin C and E supplementation did not have any adverse effects on adaptation to repeated bouts of downhill running (He et al., 2015). Furthermore, they also concur with the study of Theodorou et al., (2011) who although not measuring the RBE *per se*, reported that 11 weeks of supplementation with vitamin C and E had no effect (positive or negative) on the recovery of muscle function following an acute exercise bout performed after 4 weeks of eccentric-exercise training. Nevertheless, a few studies have suggested that functional measures of exercise-induced adaptations might be

blunted by antioxidant supplementation and these cannot be ignored. For instance, Close et al., (2006) reported that vitamin C supplementation (1000 mg·day^{-1}) for 14 days following a bout of 30 minutes of downhill running impaired the acute regeneration (within 2 weeks) of isokinetic muscle strength compared to a placebo. Deleterious effects with antioxidant supplementation were also demonstrated in a long term trial, where combined ingestion of vitamin C (1000 mg·day^{-1}) and E (235 mg·day^{-1}) for 12 weeks impaired resistance training-induced gains in muscle strength and lean muscle (Bjørnsen et al., 2015).

The fact that the aforementioned studies showing negative effects were not designed to specifically assess the RBE precludes any direct comparisons to the current study. Nevertheless, it is important to highlight that unlike previous work, the present study investigated acute adaptive responses after consuming a phytochemical rich food in BTJ, not highly concentrated doses of vitamin C antioxidants. It has been proposed that antioxidant molecules derived from plant sources, such as polyphenols, are likely to elicit distinct physiological effects to nutritional antioxidant supplements, which are typically formulated in highly concentrated doses (Nikolaidis et al., 2012). This is possibly due to the fact that 1) antioxidant-rich functional foods are less likely to provide isolated molecules in excessive doses (Sousa et al., 2013), which is perhaps due to differences in bioavailability, and; 2) many contain a diverse range of molecules, each of which might possess additional biochemical effects beyond just antioxidant (Nikolaidis et al., 2012). For instance, the BTJ used in the present study contains a range of bioactive molecules, such as nitrate, phenolics and betalains, which, in addition to being antioxidants, have demonstrated anti-inflammatory, anti-proliferative, and chemo-preventive effects (El Gamal et al., 2014; Jadert et al., 2012; Justice et al., 2015; Lechner et al., 2010). This data would suggest that BTJ might possess distinct biochemical effects to concentrated antioxidant sources such as vitamin C and E, and this may result in different physiological outcomes in terms of functional recovery and the RBE. Further research is needed to clarify the potential differing effects of these two supplements on acute adaptive responses to eccentric exercise.

The main limitation of this study is the inability to ascertain the inflammatory and oxidative stress response to both bouts of exercise. Thus, it cannot be ruled out that the present findings are due to the fact that BTJ had little, if any, influence on the level of inflammation and oxidative stress following exercise, and that other mechanisms were responsible for the enhanced functional recovery observed with BTJ (Clifford et al., 2015). Future work is required to elucidate the potential mechanisms that might be involved. Nevertheless, given that the potential implications of antioxidant supplementation on exercise performance are of most concern for athletes and practitioners, our findings are limited to the acute changes in functional recovery markers. Furthermore, although our priori power calculation indicated that we had sufficient power to reduce the probability of a type II error, we acknowledge that the study may still have been underpowered for detecting small but potentially meaningful changes between the supplement groups (<10% changes). We therefore stress that based on the present data alone, it cannot be conclusively ruled out that BTJ does not influence the RBE. But we hope these initial findings will serve to stimulate further interest in this topic and encourage researchers to perform future studies with these data in mind.

Despite these limitations, this is the first study to suggest that acute supplementation with an antioxidant-rich BTJ does not adversely affect the RBE to a single bout of exercise. These preliminary findings suggest that athletes seeking strategies to increase their antioxidant intake might favour the use of BTJ or possibly other antioxidant-rich functional foods over high doses of vitamin C and E supplements that might interfere with exercise-induced adaptations. Nonetheless, future work with higher participant numbers is needed to not only corroborate these conclusions but to also examine the chronic use of BTJ in the adaptive process to ascertain its influence in longer-term adaptive training responses.

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394 **Conflict of interest**

395 This study was funded as part of a doctoral degree that receives financial support from Gs
396 Fresh Ltd. The funders supplied the supplements used in this study but had no role in the
397 conception of the study, its design, preparation, analysis, writing and publication of the
398 manuscript; therefore, the authors declare no conflict of interest.

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585 **Figure legends**

586 **Figure 1**: Schematic outline of study procedures. Dependent variables measured
587 were countermovemt jump (CMJ), maximal isometric voluntary contractions (MIVC),
588 pressure pain threshold (PPT) and creatine kinase (CK).
589 **Figure 2**: Changes (% from baseline) in maximal isometric voluntary contractions
590 pre and up to 72 h after exercise bout 1 and bout 2. *Attenuation of muscle force in
591 bout 2 compared to bout 1(P = 0.040); n = 29.
592 **Figure 3**: Changes (% from baseline) in counter movement jump height pre and up
593 to 72 h after exercise bout 1 and bout 2. *Attenuation of jump height in bout 2
594 compared to bout 1(P = 0.001); n = 29.

595 **Figure 4**: Plasma creatine kinase concentrations pre and up to 72 h after exercise
596 bout 1 and bout 2. *Decrease in CK efflux in bout 2 compared to bout 1(P = 0.001); n
597 = 29.

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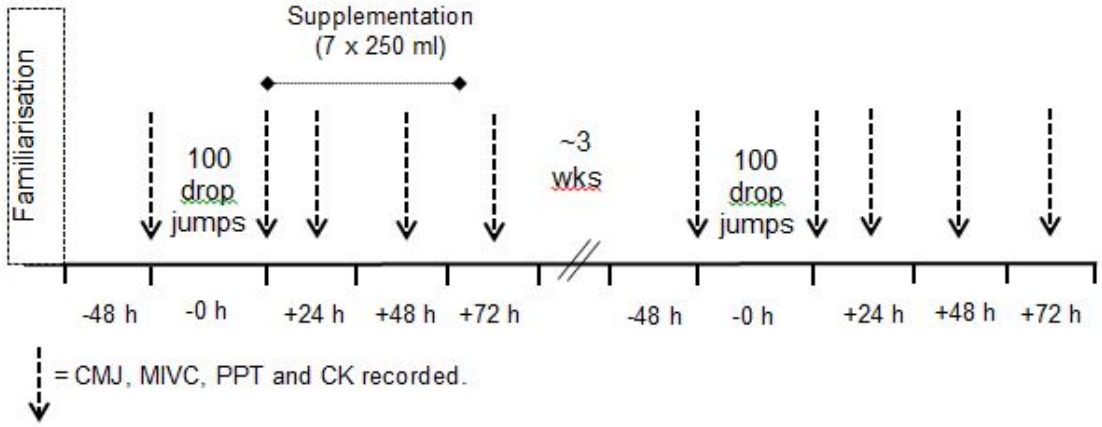
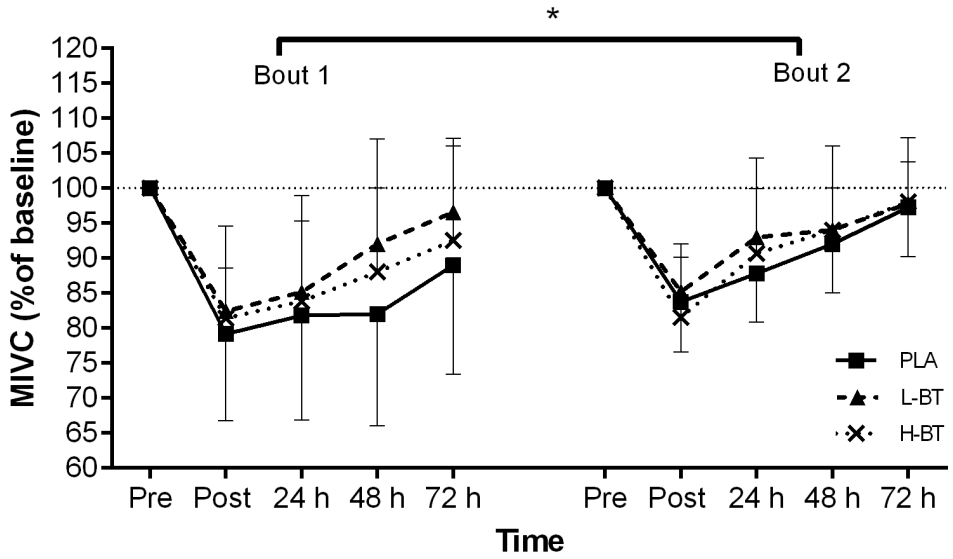
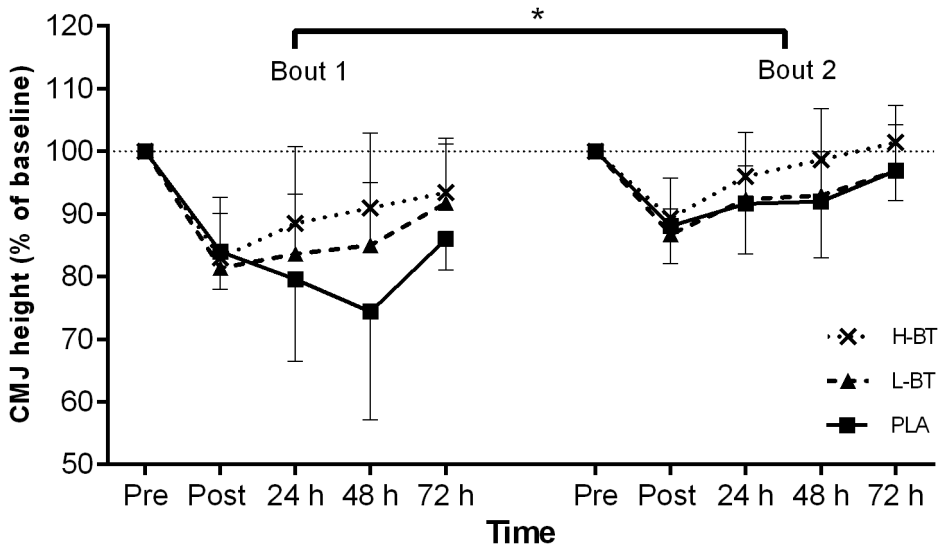

Familiarisation
Supplementation
(7 x 250 ml)
100
drop
jumps
~3
wks
100
drop
jumps
-48 h
-0 h
+24 h
+48 h
+72 h
-48 h
-0 h
+24 h
+48 h
+72 h
= CMJ, MIVC, PPT and CK recorded.


*
Bout 1
Bout 2
MIVC (%of baseline)
120
115
110
105
100
95
90
85
80
75
70
65
60
Pre
Post
24 h
48 h
72 h
Pre
Post
24 h
48 h
72 h
Time
PLA
L-BT
H-BT


*
Bout 1
Bout 2
CMJ height (% of baseline)
120
110
100
90
80
70
60
50
Pre
Post
24 h
48 h
72 h
Pre
Post
24 h
48 h
72 h
Time
H-BT
L-BT
PLA

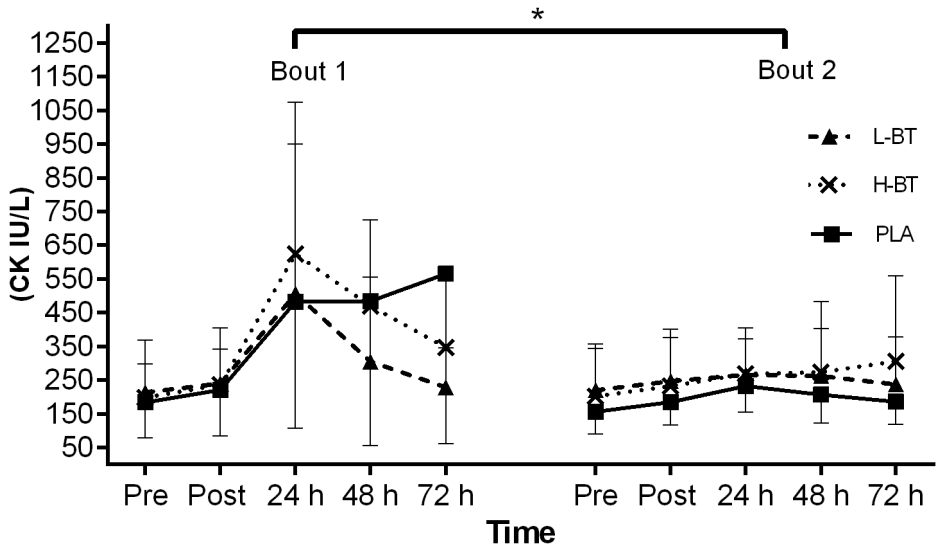

*
Bout 1
Bout 2
L-BT
H-BT
PLA
(CK IU/L)
1250
1150
1050
950
850
750
650
550
450
350
250
150
50
Pre
Post
24 h
48 h
72 h
Pre
Post
24 h
48 h
72 h
Time

Table 1: Macronutrient composition of the higher beetroot juice (H-BT), low beetroot juice (LBT) and placebo (PLA) supplements.

Supplement	H-BT	L-BT	PLA
Energy (Kcal)	81.0	78.6	76.8
Volume (ml)	250	250	250
Carbohydrate (g)	16.4	16.4	16.4
Protein (g)	2.8	2.8	2.8
Fat (g)	0.4	0.2	Trace
Nitrate (mg)	~250	~125	Trace

706 **Table 2:** Percentage change from baseline in pressure pain threshold (PPT) for the
707 3 supplement groups.

			Time Post exercise				
			Pre	**Post**	**24 h**	**48 h**	**72 h**
PPT	**HBT**	1	100 ± 0	84.7 ± 14.9	88.1 ± 20.5	91.1 ± 22.0	103.5 ± 23.2
		2	100 ± 0	90.6 ± 11.3	86.0 ± 16.8	93.2 ± 16.1	95.7 ± 12.9
	L-BT	1	100 ± 0	93.2 ± 21.4	87.1 ± 19.7	92.4 ± 24.0	104.5 ± 20.1
		2	100 ± 0	90.3 ± 10.7	86.6 ± 12.5	91.2 ± 13.7	99.8 ± 12.4
	PLA	1	100 ± 0	85.01 ± 18.8	67.4 ± 20.8	61.7 ± 20.8	80.0 ± 28.9
		2	100 ± 0	87.5 ± 14.4	79.5 ± 15.1*	81.5 ± 14.9*	90.5 ± 17.9*

708 *significantly different from bout 1. *n* = 29.
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