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

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24 **Abstract**

25 This study examined the effects of beetroot juice on the repeated bout effect (RBE) to  
26 eccentric exercise. Twenty nine recreationally active males performed two bouts of 100-drop  
27 jumps, separated by 14-21 days. Using a double-blind, independent groups design,  
28 participants consumed either a higher dose beetroot juice (H-BT; 250 ml,  $n = 10$ ), a lower  
29 dose beetroot juice (LBT; 125 ml,  $n = 9$ ), or an isocaloric placebo (PLA; 250 ml,  $n = 10$ ) for 3  
30 days after bout 1; no drinks were consumed after bout 2. Maximal isometric voluntary  
31 contraction (MIVC), countermovement jump (CMJ), pressure-pain threshold (PPT) and  
32 creatine kinase (CK) were measured pre, post, 24, 48 and 72 h following both bouts. In bout  
33 2, CMJ and MIVC recovered quicker and CK activity was attenuated (vs. bout 1) ( $P < 0.05$ ) in  
34 all groups, demonstrating an RBE. At 24 h post bout 1, MIVC was  $84.1 \pm 16.1$ ,  $83.6 \pm 11.6$ ,  
35  $79.7 \pm 15.1\%$  relative to baseline values in the H-BT, L-BT and PLA groups, respectively; at  
36 24 h post bout 2, MIVC recovered to  $90.7 \pm 13.7$ ,  $92.9 \pm 6.9$ ,  $87.8 \pm 6.9$ , in the H-BT, L-BT and  
37 PLA groups, respectively. These findings suggest that supplementation with antioxidant-rich  
38 beetroot juice does not adversely affect acute adaptations to a bout of eccentric exercise.

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## 47 **Introduction**

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

62

63 Although an excess production of ROS and the associated inflammatory events might  
64 initially harm the muscle cell, they are also fundamental to the regenerative process, and are  
65 now widely considered to be a necessary stimulus for both acute and chronic cellular  
66 adaptations to exercise (Close et al., 2006; Michailidis et al., 2013; Paulsen et al., 2014). For  
67 instance, following an acute bout of eccentric exercise, immune cells (i.e., phagocytes)  
68 produce ROS and a host of messenger proteins known as cytokines, which have been  
69 proposed to act as signalling molecules for molecular changes that reinforce cell defences  
70 (McHugh, 2003; Pizza, Koh, McGregor and Brooks, 2002; Xin, Hyldahl, Chipkin and  
71 Clarkson, 2014; Hubal, Chen, Thompson and Clarkson, 2008; Hyldahl et al., 2015). Such  
72 adaptations could make cells more resistant to damage during similar exercise bouts in the  
73 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 ( $1000 \text{ mg}\cdot\text{d}^{-1}$ ) and E ( $400 \text{ I}\cdot\text{U}^{-1}$ ) 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).

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

112

113 In a recent study, consuming beetroot juice (BTJ), a functional food that is rich in  
114 antioxidants (Wootton-Beard and Ryan 2011), attenuated some indices of muscle damage  
115 after a bout of eccentric exercise (Clifford, Bell, West, Howatson, Stevenson, 2015)  
116 suggesting that BTJ may hold promise as a recovery aid. As a follow up to this study, a sub-  
117 set of the participants repeated the same bout of eccentric exercise 3 weeks later, without  
118 supplementation. The aim of this follow up study was to examine the effects of BTJ  
119 supplementation on the acute adaptive response (represented by the RBE) after muscle  
120 damaging exercise. It is important to make the distinction that in this we specifically wanted  
121 to test whether BTJ would adversely affect the adaptive response to a single bout of  
122 exercise, not a series of exercise bouts, and therefore we were primarily interested in the  
123 acute adaptive responses typically seen after a single bout of exercise (i.e., improved  
124 muscle recovery, lowered muscle soreness), as opposed to more chronic adaptations  
125 (increased hypertrophy and strength). As a secondary aim of this study, we wanted to  
126 establish whether a higher or lower dose of BTJ would differentially affect the RBE, given  
127 that previous reports have suggested that although higher antioxidant doses might be  
128 harmful (i.e., vitamin C  $\geq 1000 \text{ mg}\cdot\text{day}^{-1}$ ) (Close et al, 2006; Paulsen et al, 2014), more  
129 moderate doses are not ( $500 \text{ mg}\cdot\text{day}^{-1}$ ) (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  $\geq 10\%$  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  $\alpha$  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  $21 \pm 3$  years; height  $1.77 \pm 0.80$  m; body mass  $75.6 \pm 8.8$  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

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

### 157 ***Experimental overview***

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

### 170 ***Muscle damaging exercise***

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

### 177 ***Supplementation***

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



180 approximately  $401.72 \pm 37.72$  mg (expressed as Gallic acid equivalents) of phenolic  
181 compounds and had an antioxidant capacity of  $2.85 \pm 0.05$  m·mol<sup>-1</sup> Trolox equivalents. The  
182 L-BT was equivalent to half the dose of beetroot provided by the H-BT i.e., 125 ml and made  
183 up to the same volume using water. The phytonutrient content of the L-BT was not subjected  
184 to in-depth analysis, but estimating from the above data, it was anticipated that it would have  
185 approximately half the polyphenol content and antioxidant capacity of the H-BT. The PLA  
186 was flavoured with a low calorie fruit squash (Kia Ora, Coca Cola Enterprises, Uxbridge, UK)  
187 that had a comparatively lower phytochemical content ( $43 \pm 3.2$  mg Gallic acid equivalents)  
188 and antioxidant capacity ( $0.02 \pm 0.01$  m·mol<sup>-1</sup> Trolox equivalents) compared to the beetroot  
189 juice treatments. The L-BT and the PLA contained maltodextrin (Myprotein, Manchester, UK)  
190 and flavourless protein powder (Arla Foods, Amba, Denmark) to match H-BT for macro  
191 nutrient content (See table 1.). After the drop jumps, one bottle (250 ml per serving) was  
192 consumed immediately post, another 2 h post, and one with an evening meal (3 in total). At  
193 24 and 48 h post, one serving was consumed immediately after completing the dependent  
194 variables (Figure 1) and one with an evening meal (2 per day) equating to 7 servings over 3  
195 days. As this was the first study to investigate BTJ in this manner, we had to rely on similarly  
196 designed studies to determine the most appropriate dosing strategy. We decided to provide  
197 two daily servings of BTJ in the days post-exercise because other antioxidant rich-juices  
198 (cherry and pomegranate) at this dose exhibited physiological effects that resulted in  
199 improved functional recovery (Howatson et al. 2010; Bell et al. 2014; Trombold et al. 2010).  
200 We provided an extra serving on the day immediately post-exercise however, because  
201 muscle damage (i.e., decreased muscle function) and the accompanying inflammatory  
202 response has been shown to be greater 24 h after performing an analogous muscle-  
203 damaging protocol (Chatzinikolaou et al., 2010; Howatson et al., 2009). We therefore  
204 reasoned that a slightly higher dose might be needed to help expedite recovery in the more  
205 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  $10\text{ N cm}^{-2}\cdot\text{s}^{-1}$  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 lateralis: 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 gauge 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  $90^\circ$  knee joint angle, as  
230 determined by a goniometer. The peak value from 3 maximal contractions (separated by 60

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

### 233 ***Counter movement jump height***

234 Counter movement jumps (CMJ) were recorded with an optical system (Optojump next,  
235 Bolzano, Italy) to measure jump height in cm. With hands on their hips, participants  
236 descended into a 90° squat and jumped vertically with maximal effort. The average of 3  
237 maximal jumps (separated by 30 seconds) was used for analysis. CV for this procedure was  
238 2.1%.

### 239 ***Blood sample collection and analysis***

240 Creatine kinase (CK) concentrations were determined from venous blood samples (10 ml)  
241 collected via venepuncture into EDTA vacutainers. Samples were immediately centrifuged at  
242 3000 x g, 4°C for 15 minutes to separate plasma and subsequently stored in eppendorf's at -  
243 80°C. Plasma was analysed for CK spectrophotometrically using an automated system  
244 (Roche Modular, Roche Diagnostics, UK).

### 245 ***Statistical analysis***

246 Data were analysed using IBM SPSS statistics version 21 and expressed as mean ±  
247 standard deviation (SD). A mixed model analysis of variance (ANOVA) with 3 treatment  
248 levels (H-BT vs. L-BT vs. PLA), x 2 bouts (bout 1 vs. bout 2), x 5 time points (pre, post, 24,  
249 48 and 72 h post exercise) was used to test for significant differences between the  
250 dependent variables. Data analysis for MIVC, CMJ and PPT were conducted using  
251 percentage change from baseline values to account for individual variability. Group  
252 differences in height, weight, age, and baseline MIVC scores were analysed using one-way  
253 independent group ANOVAs. If significant group and interaction effects were observed,  
254 Fisher LSD *post hoc* tests were performed to locate where the differences occurred.  
255 Significance was set at  $P < 0.05$  prior to analyses.

## 256 **Results**

257 There were no differences in participant characteristics (age, height, mass) and baseline  
258 MIVC between groups ( $P > 0.05$ ). Results presented herein represent a subset of a larger  
259 study, and therefore data relating to group differences after bout 1 will not be discussed, but  
260 can be found elsewhere (Clifford et al. 2015). Main effects for time were observed for all  
261 dependent variables (CMJ, MIVC, CK and PPT;  $P < 0.05$ ) following bout 1, indicating that  
262 the drop jumps effectively induced muscle damage. The decrement in MIVC was less in bout  
263 2 compared to bout 1 ( $F_{(1,26)} = 4.497$ ;  $P = 0.04$ ; Figure 2), providing evidence of an RBE.  
264 The percentage decrease in MIVC in bout 1 (average across groups) was 16.1%, 12.2% and  
265 17.3% at 24, 48 and 72 h post exercise, respectively, whereas after bout 2, decrements  
266 were attenuated to 9.5%, 6.2%, and 2.3% at 24, 48 and 72 h post exercise, respectively.  
267 There were no bout x group interactions ( $F_{(2,26)} = 0.206$ ;  $P = 0.815$ ), indicating that strength  
268 loss was similarly attenuated in all groups. At 72 h post bout 2, MIVC had recovered to  $98.0$   
269  $\pm 9.0$ ,  $97.8 \pm 6.0$  and  $97.1 \pm 7.0\%$  of baseline values in the H-BT, L-BT and PLA groups,  
270 respectively. The decrease in CMJ height was also attenuated in bout 2 compared to bout 1  
271 (bout effect;  $F_{(1,26)} = 25.430$ ;  $P < 0.001$ ) and there were no differences between the groups  
272 (bout x group interaction;  $F_{(2,26)} = 0.709$ ;  $P = 0.501$ ). 72 h post bout 1 CMJ height recovered  
273 in the H-BT, L-BT and PLA groups to  $91.7 \pm 9.7$ ,  $93.2 \pm 7.1$  and  $87.4 \pm 7.3\%$  of baseline  
274 values, respectively, whereas 72 h following bout 2, CMJ height recovered to  $101.4 \pm 5.9$ ,  
275  $96.9 \pm 7.4$  and  $96.9 \pm 4.8\%$  of baseline values, respectively (Figure 3). PPT did not show an  
276 overall bout effect ( $F_{(1,84)} = 1.683$ ;  $P = 0.198$ ); but a bout x group interaction was observed  
277 ( $F_{(2,84)} = 4.003$ ;  $P = 0.022$ ), with post hoc analysis revealing that PPT was improved in bout 1  
278 compared to bout 2 in PLA only (Table 2;  $P = 0.001$ ). A main effect for bout showed that  
279 plasma CK was lower in bout 2 compared to bout 1 ( $F_{(1,24)} = 15.200$ ;  $P = 0.001$ ; Figure 4);  
280 there were no differences between the 3 groups (bout x group interaction;  $F_{(2,21)} = 2.422$ ;  $P =$   
281  $0.113$ ).

## 282 **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

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

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

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

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

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

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

380 Despite these limitations, this is the first study to suggest that acute supplementation with an  
381 antioxidant-rich BTJ does not adversely affect the RBE to a single bout of exercise. These  
382 preliminary findings suggest that athletes seeking strategies to increase their antioxidant  
383 intake might favour the use of BTJ or possibly other antioxidant-rich functional foods over  
384 high doses of vitamin C and E supplements that might interfere with exercise-induced  
385 adaptations. Nonetheless, future work with higher participant numbers is needed to not only  
386 corroborate these conclusions but to also examine the chronic use of BTJ in the adaptive  
387 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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412 **References**

- 413 Bell, P. G., Walshe, I. H., Davison, G. W., Stevenson, E. J., and Howatson, G. (2014).  
414 Recovery facilitation with Montmorency cherries following high-intensity, metabolically  
415 challenging exercise. *Applied Physiology, Nutrition, and Metabolism*, 40(4), 414-423.
- 416 Bjørnsen, T., Salvesen, S., Berntsen, S., Hetlelid, K. J., Stea, T. H., Lohne-Seiler, H., Rohde,  
417 G., Haraldstad, K., Raastad, T. Kopp, U., Haugeberg, G., Mansoor, M, A., Bastani, N,E.,  
418 Blomhoff, R. Stølevik, Seynnes, O, R. and Paulsen, G. (2015). Vitamin C and E  
419 supplementation blunts increases in total lean body mass in elderly men after strength  
420 training. *Scandinavian Journal of Medicine and Science in Sports and exercise*, DOI:  
421 10.1111/sms.12506.
- 422 Bowtell, J, L., Sumners, D, P., Dyer, A., Fox, P., Milev, K, N. (2011). Montmorency cherry  
423 juice reduces muscle damage caused by intensive strength exercise. *Medicine and Science*  
424 *in Sports and Exercise*, 43(8), 1544-1551.
- 425 Bloomer, R. J. (2007). The role of nutritional supplements in the prevention and treatment of  
426 resistance exercise-induced skeletal muscle injury. *Sports Medicine*, 37(6), 519-532.
- 427 Brickson, S., Ji, L. L., Schell, K., Olabisi, R., Schneider, B. S. P., and Best, T. M. (2003).  
428 M1/70 attenuates blood-borne neutrophil oxidants, activation, and myofiber damage  
429 following stretch injury. *Journal of Applied Physiology*. 95(3), 969-976.
- 430 Chatzinikolaou, A., Fatouros, I.G., Gourgoulis, V., Avloniti, A., Jamurtas, A.Z., Nikolaidis,  
431 M.G., Douroudos, I., Michailidis, Y., Beneka, A., Malliou, P. and Tofas, T., (2010). Time  
432 course of changes in performance and inflammatory responses after acute plyometric  
433 exercise. *The Journal of Strength & Conditioning Research*, 24(5): 1389-1398.
- 434 Chen, T.C., Tseng, W.C., Huang, G.L., Chen, H.L., Tseng, K.W. and Nosaka, K., (2013a).  
435 Low-intensity eccentric contractions attenuate muscle damage induced by subsequent

- 436 maximal eccentric exercise of the knee extensors in the elderly. *European Journal of Applied*  
437 *Physiology*, 113(4): 1005-1015.
- 438 Chen, T.C., Chen, H.L., Lin, M.J., Chen, C.H., Pearce, A.J. and Nosaka, K., (2013b). Effect  
439 of two maximal isometric contractions on eccentric exercise-induced muscle damage of the  
440 elbow flexors. *European Journal of Applied Physiology*, 113(6): 1545-1554.
- 441 Clifford, T., Bell, O., West, D. J., Howatson, G., and Stevenson, E. J. (2015). The effects of  
442 beetroot juice supplementation on indices of muscle damage following eccentric exercise.  
443 *European Journal of Applied Physiology*, 1-10.
- 444 Close, G. L., Ashton, T., Cable, T., Doran, D., Holloway, C., McArdle, F., and MacLaren, D.  
445 P. (2006). Ascorbic acid supplementation does not attenuate post-exercise muscle soreness  
446 following muscle-damaging exercise but may delay the recovery process. *British Journal of*  
447 *Nutrition*, 95(5), 976-981.
- 448 El Gamal, A. A., AlSaid, M. S., Raish, M., Al-Sohaibani, M., Al-Massarani, S. M., Ahmad, A.,  
449 Hefnawy, M., Al-Yahya, M., Basoudan, O, A., and Rafatullah, S. (2014). Beetroot (Beta  
450 vulgaris L.) extract ameliorates gentamicin-induced nephrotoxicity associated oxidative  
451 stress, inflammation, and apoptosis in rodent model. *Mediators of Inflammation*, 2014.
- 452 Goldfarb, A. H., Garten, R. S., Cho, C., Chee, P. D., & Chambers, L. A. (2011). Effects of a  
453 fruit/berry/vegetable supplement on muscle function and oxidative stress. *Medicine and*  
454 *Science in Sports and Exercise*, 43(3), 501-508.
- 455 Gomez-Cabrera, M. C., Ristow, M., & Viña, J. (2012). Antioxidant supplements in exercise:  
456 worse than useless?. *American Journal of Physiology-Endocrinology and Metabolism*,  
457 302(4), E476-E477.
- 458 He, F., Hockemeyer, J. A., & Sedlock, D. (2015). Does Combined Antioxidant Vitamin  
459 Supplementation Blunt Repeated Bout Effect? *International Journal of Sports Medicine*, 36,  
460 407-413.

- 461 Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J. and Suzuki, K., (2004).  
462 Changes in inflammatory mediators following eccentric exercise of the elbow flexors.  
463 *Exercise Immunology Review*, 10(75-90): 20-30.
- 464 Howatson, G., Goodall, S., & van Someren, K. A. (2009). The influence of cold water  
465 immersions on adaptation following a single bout of damaging exercise. *European Journal of*  
466 *Applied Physiology*, 105(4), 615-621.
- 467 Howatson, G., Hoad, M., Goodall, S., Tallent, J., Bell, P. G., and French, D. N. (2012).  
468 Exercise-induced muscle damage is reduced in resistance-trained males by branched chain  
469 amino acids: a randomized, double-blind, placebo controlled study. *Journal of the*  
470 *International Society of Sports Nutrition*, 9: 20.
- 471 Howatson, G., McHugh, M.P., Hill, J.A., Brouner, J., Jewell, A.P., Van Someren, K.A.,  
472 Shave, R.E. and Howatson, S.A., (2010). Influence of tart cherry juice on indices of recovery  
473 following marathon running. *Scandinavian Journal of Medicine & Science in Sports*, 20(6),  
474 843-852.
- 475 Howatson, G., McHugh, M, P., Hill, J, A., Brouner, J. Jewell, A, P., van Someren, K, A.,  
476 Shave, R, E., and Howatson, S, A. (2010). Influence of tart cherry juice on indices of  
477 recovery following marathon running. *Scandinavian Journal of Medicine and Science in*  
478 *Sports and Exercise*, 20(6), 843-852.
- 479 Hubal, M. J., Chen, T. C., Thompson, P. D., and Clarkson, P. M. (2008). Inflammatory gene  
480 changes associated with the repeated-bout effect. *American Journal of Physiology-*  
481 *Regulatory, Integrative and Comparative Physiology*, 294(5), 1628-1637.
- 482 Hyldahl, R.D., Nelson, B., Xin, L., Welling, T., Groscost, L., Hubal, M.J., Chipkin, S.,  
483 Clarkson, P.M. and Parcell, A.C., (2015). Extracellular matrix remodeling and its contribution  
484 to protective adaptation following lengthening contractions in human muscle. *The FASEB*  
485 *Journal*, 29(7), 2894-2904.

- 486 Jadert, C., Petersson, J., Massena, S., Ahl, D., Grapensparr, L., Holm, L., Lundberg, J, L.,  
487 and Phillipson, M. (2012). Decreased leukocyte recruitment by inorganic nitrate and nitrite in  
488 microvascular inflammation and NSAID-induced intestinal injury. *Free Radical Biology and*  
489 *Medicine*, 52(3), 683-692.
- 490 Justice, J, N., Gioscia-Ryan, R, A., Johnson, L, C., Battson, M, L., de Picciotto, N, E., Beck,  
491 H, J., Jiang, H., Sindler, A, L., Bryan, N, S., Enoka, R, M., and Seals, D, R. (2015). Sodium  
492 nitrite supplementation improves motor function and skeletal muscle inflammatory profile in  
493 old male mice. *Journal of Applied Physiology*, 118(2):163-169.
- 494 Lapointe, B. M., Frenette, J., & Côté, C. H. (2002). Lengthening contraction-induced  
495 inflammation is linked to secondary damage but devoid of neutrophil invasion. *Journal of*  
496 *Applied Physiology*, 92(5): 1995-2004.
- 497 Lechner, J. F., Wang, L. S., Rocha, C. M., Larue, B., Henry, C., McIntyre, C. M., Riedl, K. M.,  
498 Schwartz, S, J., and Stoner, G. D. (2010). Drinking water with red beetroot food color  
499 antagonizes esophageal carcinogenesis in N-nitrosomethylbenzylamine-treated rats. *Journal*  
500 *of Medicinal Food*, 13(3): 733-739.
- 501 Lynn, R. and Morgan, D.L., (1994). Decline running produces more sarcomeres in rat vastus  
502 intermedius muscle fibers than does incline running. *Journal of Applied Physiology*, 77(3):  
503 1439-1444.
- 504 McHugh, M. P. (2003). Recent advances in the understanding of the repeated bout effect:  
505 the protective effect against muscle damage from a single bout of eccentric exercise.  
506 *Scandinavian Journal of Medicine and Science in Sports*, 13(2): 88-97.
- 507 McLeay, Y., Barnes, M, J., Mundel, T., Hurst, S, M., Hurst, R, D., and Stannard, R, R.  
508 (2012). Effect of New Zealand blueberry consumption on recovery from eccentric exercise-  
509 induced muscle damage. *Journal of the International Society of Sports Nutrition*, 9(1):19.

- 510 Michailidis, Y., Karagounis, L. G., Terzis, G., Jamurtas, A. Z., Spengos, K., Tsoukas, D.,  
511 Chatzinikolaou, A., Mandalidis, D., Stefanetti, R, J., Papassotiriou, I., Athanasopoulos, S.,  
512 Hawley, J, A., Russell, A, P., and Fatouros, I, G. (2013). Thiol-based antioxidant  
513 supplementation alters human skeletal muscle signaling and attenuates its inflammatory  
514 response and recovery after intense eccentric exercise. *American Journal of Clinical*  
515 *Nutrition*, 98(1): 233-245.
- 516 Nikolaidis, M. G., Paschalis, V., Giakas, G., Fatouros, I. G., Koutedakis, Y., Kouretas, D.,  
517 and Jamurtas, A. Z. (2007). Decreased blood oxidative stress after repeated muscle-  
518 damaging exercise. *Medicine and Science in Sports and Exercise*, 39(7): 1080-1089.
- 519 Nikolaidis, M. G., Kerkick, C. M., Lamprecht, M., and McAnulty, S. R. (2012). Does vitamin  
520 C and E supplementation impair the favorable adaptations of regular exercise? *Oxidative*  
521 *Medicine and Cellular Longevity*, 2012.
- 522 Nosaka, K., & Clarkson, P. M. (1995). Muscle damage following repeated bouts of high force  
523 eccentric exercise. *Medicine and Science in Sports and Exercise*, 27(9): 1263-1269.
- 524 Paulsen, G., Egner, I. M., Drange, M., Langberg, H., Benestad, H. B., Fjeld, J. G., Halle, J.,  
525 and Raastad, T. (2010). A COX-2 inhibitor reduces muscle soreness, but does not influence  
526 recovery and adaptation after eccentric exercise. *Scandinavian Journal of Medicine and*  
527 *Science in Sports and Exercise*, 20(1): 195-207.
- 528 Paulsen, G., Hamarsland, H., Cumming, K. T., Johansen, R. E., Hulmi, J. J., Borsheim, E.,  
529 Wiig, H., Garthe, I and Raastad, T. (2014). Vitamin C and E supplementation alters protein  
530 signalling after a strength training session, but not muscle growth during 10 weeks of  
531 training. *Journal of Physiology*, 592, 5391-5408.
- 532 Paulsen, G., Mikkelsen, U. R., Raastad, T., and Peake, J. M. (2012). Leucocytes, cytokines  
533 and satellite cells: what role do they play in muscle damage and regeneration following  
534 eccentric exercise? *Exercise Immunology Review*, 18, 42-97.

- 535 Peake, J., and Suzuki, K. (2004). Neutrophil activation, antioxidant supplements and  
536 exercise-induced oxidative stress. *Exercise Immunology Review*, 10, 129-141.
- 537 Pizza, F.X., Baylies, H. and Mitchell, J.B., (2001). Adaptation to eccentric exercise:  
538 neutrophils and E-selectin during early recovery. *Canadian Journal of Applied Physiology*,  
539 26(3): 245-253.
- 540 Pizza, F. X., Koh, T. J., McGregor, S. J., and Brooks, S. V. (2002). Muscle inflammatory cells  
541 after passive stretches, isometric contractions, and lengthening contractions. *Journal of*  
542 *Applied Physiology*, 92(5): 1873-1878.
- 543 Pizza, F. X., Peterson, J. M., Baas, J. H., & Koh, T. J. (2005). Neutrophils contribute to  
544 muscle injury and impair its resolution after lengthening contractions in mice. *Journal of*  
545 *Physiology*, 562(3): 899-913.
- 546 Sousa, M., Teixeira, V. H., and Soares, J. (2014). Dietary strategies to recover from  
547 exercise-induced muscle damage. *International Journal of Food Science and Nutrition*,  
548 65(2): 151-163.
- 549 Stupka, N., Tarnopolsky, M. A., Yardley, N. J., and Phillips, S. M. (2001). Cellular adaptation  
550 to repeated eccentric exercise-induced muscle damage. *Journal of Applied Physiology*,  
551 91(4): 1669-1678.
- 552 Theodorou, A. A., Nikolaidis, M. G., Paschalis, V., Koutsias, S., Panayiotou, G., Fatouros, I.  
553 G., Koutedakis, V., and Jamurtas, A. Z. (2011). No effect of antioxidant supplementation on  
554 muscle performance and blood redox status adaptations to eccentric training. *American*  
555 *Journal of Clinical Nutrition*, 93(6), 1373-1383.
- 556 Toumi, H., and Best, T. M. (2003). The inflammatory response: friend or enemy for muscle  
557 injury?. *British Journal of Sports Medicine*, 37(4): 284-286.

- 558 Trombold J, R., Barnes, J, N., Critchley, L., and Coyle, E, F. (2010). Ellagitannin  
559 consumption improves strength recovery 2-3 d after eccentric exercise. *Medicine and*  
560 *Science in Sports and Exercise*, 42(3), 493-498.
- 561 Warren, G. L., Lowe, D. A., and Armstrong, R. B. (1999). Measurement tools used in the  
562 study of eccentric contraction-induced injury. *Sports Medicine*, 27(1): 43-59.
- 563 Wootton-Beard, P. C., and Ryan, L. (2011). A beetroot juice shot is a significant and  
564 convenient source of bioaccessible antioxidants. *Journal of Functional Foods*, 3(4): 329-334.
- 565 Xin, L., Hyldahl, R. D., Chipkin, S. R., and Clarkson, P. M. (2014). A contralateral repeated  
566 bout effect attenuates induction of NF-kappaB DNA binding following eccentric exercise.  
567 *Journal of Applied Physiology*, 116(11): 1473-1480.
- 568 Yfanti, C., Akerström, T., Nielsen, S., Nielsen, A.R., Mounier, R., Mortensen, O.H.,  
569 Lykkesfeldt, J., Rose, A.J., Fischer, C.P. and Pedersen, B.K., (2010). Antioxidant  
570 supplementation does not alter endurance training adaptation. *Medicine and Science in*  
571 *Sports and Exercise*, 42(7): 1388-1395.
- 572 Yfanti, C., Nielsen, A.R., Åkerström, T., Nielsen, S., Rose, A.J., Richter, E.A., Lykkesfeldt, J.,  
573 Fischer, C.P. and Pedersen, B.K., (2011). Effect of antioxidant supplementation on insulin  
574 sensitivity in response to endurance exercise training. *American Journal of Physiology-*  
575 *Endocrinology and Metabolism*, 300(5): 761-770.
- 576 Zerba, E., Komorowski, T. E., and Faulkner, J. A. (1990). Free radical injury to skeletal  
577 muscles of young, adult, and old mice. *American Journal of Physiology-Cell Physiology*,  
578 258(3), 429-435.
- 579 Zembron-Lacny, A., Slowinska-Lisowska, M., Szygula, Z., Witkowski, K., Stefaniak, T., and  
580 Dziubek, W. (2009). Assessment of the antioxidant effectiveness of alpha-lipoic acid in  
581 healthy men exposed to muscle-damaging exercise. *Journal of Physiology and*  
582 *Pharmacology*, 60(2): 139-143.



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585 **Figure legends**

586 **Figure 1:** Schematic outline of study procedures. Dependent variables measured  
587 were countermovement 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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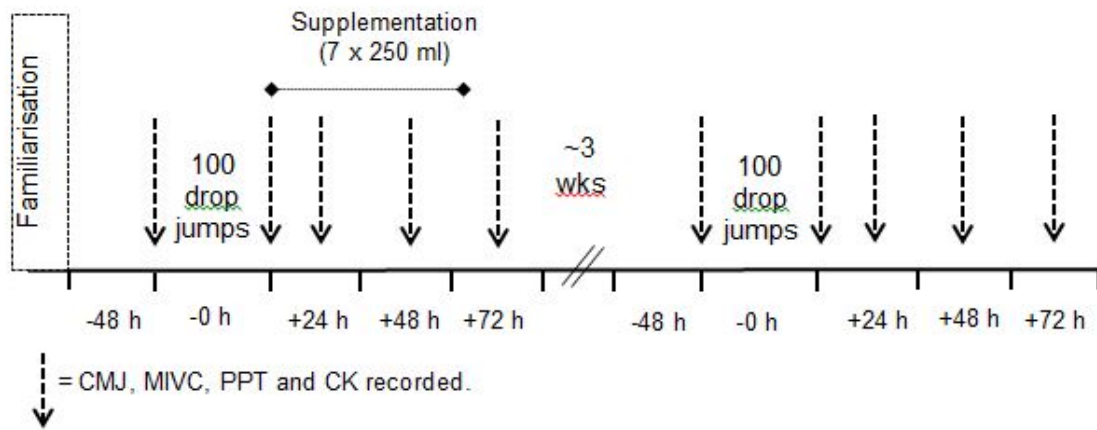
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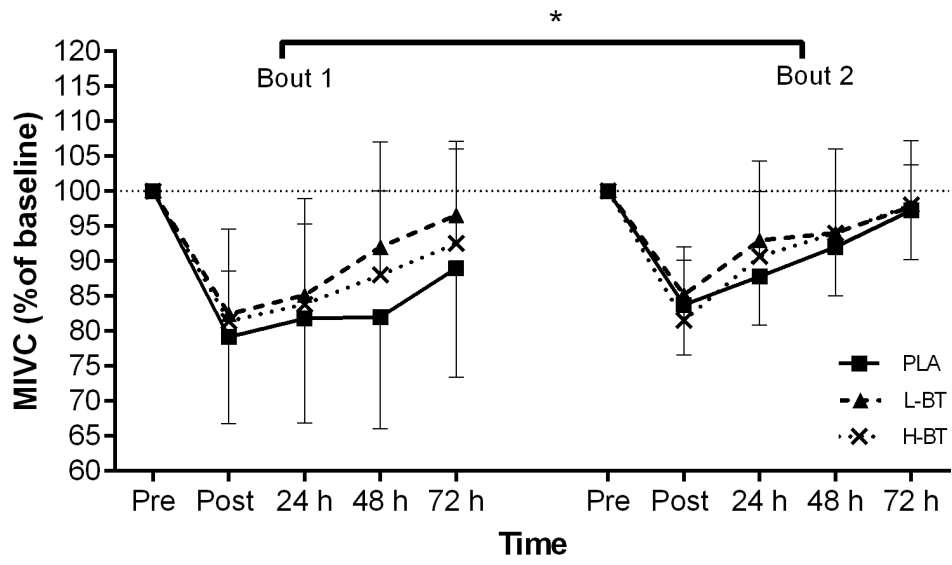
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↓ = CMJ, MIVC, PPT and CK recorded.

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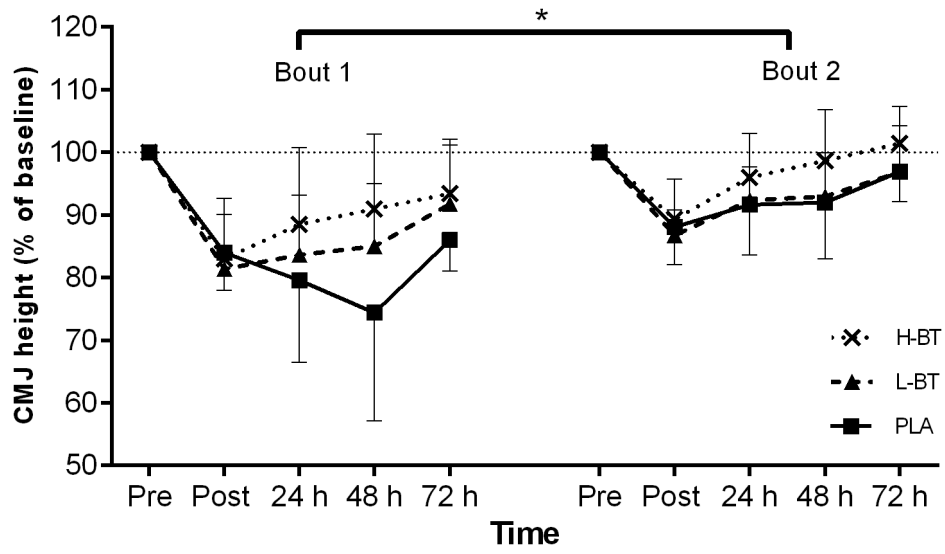
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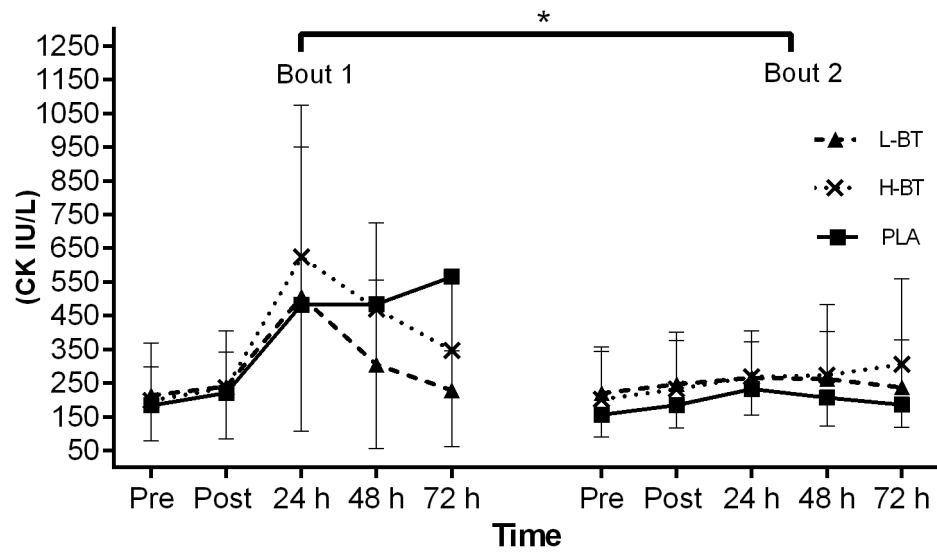
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684 **Table 1:** Macronutrient composition of the higher beetroot juice (H-BT), low beetroot  
 685 juice (LBT) and placebo (PLA) supplements.

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

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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
<b>PPT</b>	<b>HBT</b>	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
	<b>L-BT</b>	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
	<b>PLA</b>	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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