

1 **Title:** The muscle damage response in female collegiate athletes following repeated sprint  
2 activity

3 **Brief running head:** EIMD in females following sprint exercise

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26 **Abstract**

27 Exercise induced muscle damage (EIMD) is a well-investigated area, however there is a  
28 paucity of data surrounding the damage response in females. The aim of this study was to  
29 examine the damage responses from a sport-specific bout of repeated sprints in female  
30 athletes. Eleven well-trained females (mean  $\pm$  SD; age  $22 \pm 3$  y, height  $166.6 \pm 5.7$  cm, mass  
31  $62.7 \pm 4.5$  kg) in the luteal phase of the menstrual cycle completed a repeated sprint protocol  
32 designed to induce EIMD ( $15 \times 30$  m sprints). Creatine kinase (CK), countermovement jump  
33 height (CMJ), knee extensor maximum voluntary contraction force (MVIC), muscle soreness  
34 (DOMS), 30 m sprint time and limb girth were recorded pre, post, 24 h, 48 h and 72 h post  
35 exercise. CK was elevated at 24, 48 and 72 h ( $p < 0.05$ ), peaking at 24 h (+418%) and  
36 returning towards baseline at 72 h. CMJ height was reduced immediately post, 24 and 48 h ( $p$   
37  $< 0.05$ ). Sprint performance was also negatively affected immediately post, 24 h, 48 h and 72  
38 h post exercise. Muscle soreness peaked at 48 h ( $p < 0.01$ ) and remained significantly elevated  
39 at 72 h post exercise ( $p < 0.01$ ). Limb girth and MVIC did not alter over time. The current  
40 study provides new information on the EIMD response in trained females following a sport  
41 specific bout of repeated sprints. Importantly, this damage response has the potential to  
42 negatively affect performance for several days post-exercise.

43 **Keywords:** females, muscle function, recovery, exercise-induced muscle damage

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50 **INTRODUCTION**

51 Exercise-induced muscle damage (EIMD) is a popular area of investigation. Numerous signs  
52 and symptoms are associated with EIMD, including elevated muscle soreness, inflammation,  
53 systemic appearance of intramuscular proteins and a concurrent decrement in physical  
54 performance (1). These indices can persist for several days and are precipitated by an initial  
55 mechanical disruption of the contractile apparatus during the damaging exercise and a  
56 secondary inflammatory response (2). The damage response has been well established in  
57 male populations (3-7); however, there is a paucity of literature investigating EIMD in  
58 females.

59

60 Various factors could potentially modulate the EIMD response in females, including oral  
61 contraceptive use, and the potential protective effect of oestrogen (8-13). There is some  
62 evidence suggesting that females are less fatigable than males (14) and the subsequent  
63 recovery following damaging exercise is known to be quicker (15). The faster recovery from  
64 damaging exercise has largely been attributed to the protective effect of oestrogen, but there  
65 has been no attempt to control for the menstrual cycle where large changes in sex hormones  
66 can be seen throughout the course of the menstrual cycle (16). This could potentially  
67 influence both the damage response and recovery process. It therefore makes the expectation  
68 tenable that the damage response in females could be somewhat different to the well-  
69 established response in males. However, it is critical to understand the damage-recovery  
70 response with control over the menstrual cycle to ascertain the implications of damaging  
71 exercise in female athletes.

72

73 In addition to the lack of data on the damage response in female athletes, much of the existing  
74 literature investigating EIMD employs damage protocols that lack specificity to a sporting  
75 context and are often eccentric biased (5, 7, 17-21), and in isolated muscle groups (4, 7, 16-  
76 18). Intermittent sports, such as soccer, rugby and basketball that require periods of high  
77 intensity, repeated sprint activity and changes of velocity (22) and direction (23), elicit  
78 significant muscle damage and prolonged decrements in function. Given the prevalence of  
79 both male and female participation in sports of this nature, further research is warranted with  
80 more sport-specific damage models to better understand the consequences of damaging  
81 repeated sprint activity (5). Establishing these responses in female athletes in particular will  
82 provide new, important information on the damage response in this population that could  
83 influence recovery strategies and exercise prescription. Consequently, the aim of this study  
84 was to examine the magnitude of damage following a sport-specific, repeated sprint protocol  
85 in females. We hypothesised that a repeated sprint exercise protocol would induce muscle  
86 damage in females and negatively affect performance in the subsequent days.

87

## 88 **METHODS**

### 89 **Experimental Approach to the Problem**

90 This investigation employed a repeated sprint protocol with forced deceleration actions,  
91 which has previously been successfully used to induce muscle damage (5). A commonly  
92 used battery of muscle damage indices were measured pre, immediately post and 24, 48, and  
93 72 h post muscle damage; these were lower limb girth, muscle soreness (DOMS), total  
94 creatine kinase (CK) activity, countermovement jump height (CMJ), maximal voluntary  
95 isometric contraction (MVIC) and sprint performance.

96

## 97 **Subjects**

98 Following ethical approval from the University Research Ethics Committee in accordance  
99 with Helsinki declaration, eleven female athletes (mean  $\pm$  SD; age  $22 \pm 3$  years, height  $166.6$   
100  $\pm 5.7$  cm, mass  $62.7 \pm 4.5$  kg) were recruited and informed of the benefits and risks of the  
101 investigation prior to signing an institutionally approved informed consent document to  
102 participate in the study. All participants regularly participated in premier league collegiate or  
103 national league field-based team sports, specifically rugby union (n=2), soccer (n=8) and  
104 netball (n = 1). A menstrual cycle questionnaire was also completed in order to determine  
105 menstrual cycle phase; all testing took place during the early/mid luteal phase. Participants  
106 were free of injury and testing took place out of season. Participants were asked to refrain  
107 from strenuous exercise, alcohol, caffeine, nutritional supplements and any anti-inflammatory  
108 drugs or alternative treatments for the duration of the study.

109

## 110 **Procedures**

111 A 30 m section of an environmentally controlled ( $19^{\circ}$  C and 70% RH) 60-m indoor running  
112 track was marked using cones and two sets of light timing gates (Brower timing systems,  
113 Utah, USA). A further 10 m deceleration zone was also marked at the end of the 30 m  
114 section. Participants first completed a warm up consisting of 400 m self-paced jogging, a  
115 series of dynamic sprint drills including high knees, heel flicks and walking lunges which  
116 were conducted over a measured 10 m section of the aforementioned indoor running track.  
117 This was followed by a series of three practice sprints at the participants perceived 60%, 80%  
118 and 100% of maximum speed. Following the warm up, the participants were given 5 minutes  
119 to prepare themselves for the repeated-sprint protocol, during which time, no static stretching  
120 was performed. Participants then stood 30 cm from the start line to avoid premature

121 triggering of the timing system and completed 15 × 30 m sprints departing every 65 s with  
122 gates set up to record in the reverse order for the next sprint. Participants were told that all  
123 efforts must be maximal and they were instructed to stop within the 10 m deceleration zone.  
124 The rest period was initiated when participants came to a complete halt and the repetition was  
125 completed. Standardized, strong verbal encouragement was provided throughout the protocol.

126

127 *Limb girth.* Lower limb girth was measured at the mid-calf. This was determined at baseline  
128 by the largest girth on the right leg whilst the subject remained standing in anatomical zero.  
129 The location was marked with permanent marker to ensure consistency on subsequent days.  
130 The mean of two measures at each site was used for analysis; the intra-rater CV for this  
131 procedure was < 1.0%.

132

133 *Muscle soreness.* Subjective muscle soreness (DOMS) was measured using a 200 mm visual  
134 analogue scale (VAS) with “no soreness” at one end and “unbearably painful” at the other  
135 and was a reflection of global soreness of the thigh. Soreness was indicated on the VAS after  
136 the participant performed a squat to a knee angle of approximately 90° with the feet shoulder  
137 width apart and then returning to the standing position.

138

139 *Creatine kinase.* Creatine kinase was determined using a capillary blood sample from the  
140 fingertip. A sample of whole fresh blood was analysed immediately using a colorimetric  
141 assay procedure (Reflotron Plus, Roche Diagnostics, UK). The resting normal expected  
142 values for CK when using this equipment are between 50 and 200 IU·L<sup>-1</sup>; the CV for this  
143 instrument was <3%.

144 *Countermovement Jump Height.* Countermovement jump height was assessed using a light  
145 timing system (Optojump, Microgate, Italy). Participants were instructed to squat down and  
146 jump vertically, with their hands on their hips throughout. Participants were advised that all  
147 jumps must be a maximal effort. Three trials with a 60 s rest were performed and the peak  
148 jump height was used for analysis.

149

150 *Maximum Voluntary Contraction.* Maximum isometric voluntary contraction (MVIC) force  
151 of the non-dominant knee extensor musculature was determined using a strain gauge (MIE  
152 Digital Myometer, MIE Medical Research Ltd, Leeds, UK). The knee joint angle was set  
153 before each contraction at 90° using a goniometer to minimise for error derived from  
154 alteration in muscle length (24-26). All participants completed three isometric MVICs of 3 s  
155 duration, separated by 60 s. The peak MVIC from the three contractions was used for  
156 analysis; the CV for this variable was < 5%.

157

158 *30 m Sprint Time.* Participants completed a single maximal effort 30 m sprint where sprint  
159 time was recorded. The sprint was initiated from a line 30 cm behind the start line in order to  
160 prevent false triggering of the timing gates (Brower, Utah, USA).

161

## 162 **Statistical Analyses**

163 Statistical analysis was performed using PASW Statistics 21.0 for Windows (SPSS, Inc.,  
164 Chicago, IL.). Descriptive statistics are reported as means  $\pm$  SD. Fatigue, fastest sprint time,  
165 and mean sprint time were calculated for the repeated sprint protocol: Fatigue =  $[100 \times (\text{total}$   
166  $\text{sprint time} \div \text{ideal sprint time})] - 100$ , in which total sprint time = sum of sprint times from  
167 all sprints and ideal sprint time = the number of sprints  $\times$  fastest sprint time (Fitzsimons et al.,

168 1993). For illustrative purposes, and to account for inter-individual variability, CMJ height  
169 and sprint performance were presented in figure format as a change from baseline. The  
170 absolute scores were analysed using a one-way analysis of variance (ANOVA) with repeated  
171 measures and are presented in Table 1. Mauchly's Test of Sphericity was used to check  
172 homogeneity of variance for all variables; where necessary any violations of the assumption  
173 were corrected using the Greenhouse–Geisser adjustment. Significant effects were followed  
174 up using Tukey *post-hoc* analysis. The alpha level for statistical significance was set at  $p <$   
175  $0.05$  *a priori*.

176

## 177 **RESULTS**

178 The repeated sprint protocol fastest and mean times were  $4.93 \pm 0.23$  and  $5.12 \pm 0.23$  s,  
179 respectively. The mean fatigue score was  $4 \pm 1\%$ . All dependent variables with the exception  
180 of limb girth and MVIC showed significant time effects following the repeated sprints  
181 protocol ( $p < 0.05$ ); illustrating a muscle damage response. DOMS was elevated over time  
182 ( $F = 26.86$ ,  $p < 0.001$ , Figure 1); post-hoc analyses revealed elevations at 24 and 72 h post,  
183 with a peak at 48 h (Table 1). CK was elevated ( $F = 13.34$ ,  $p < 0.05$ ), at every time point  
184 compared to pre-exercise (Table 1, Figure 2). For muscle function measures, there was a  
185 significant main effect for 30 m sprint time ( $F = 8.29$ ,  $p = 0.001$ , Figure 3, panel B) and CMJ  
186 height ( $F = 9.78$ ,  $p < 0.005$ , Figure 3, panel A), but not for MVIC (Table 1). Decrements in  
187 sprint performance were evident across all time points ( $p < 0.05$ ). CMJ height was reduced  
188 immediately post, 24 h and 48 h post exercise ( $p < 0.05$ ), but had returned to near baseline at  
189 72 h.

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191



192 **DISCUSSION**

193 The aim of this study was to ascertain the magnitude of EIMD indices following a repeated  
194 sprint protocol in an athletic female population. Results demonstrated that the repeat sprint  
195 protocol induced muscle damage with increases in DOMS, plasma CK, sprint time and  
196 reductions in CMJ height and 30m sprint time, all of which persisted for several days  
197 following the exercise insult. These data are broadly in agreement with the literature  
198 reporting that EIMD in males is evident soon after strenuous exercise, peaks at 24-48 h post  
199 exercise, and remains elevated for several days (2, 15, 27). Similar results have also been  
200 shown with exercise with a high eccentric component such as downhill running (28) and  
201 plyometric jumps (6). However, this is the first study to specifically document the signs and  
202 symptoms of muscle damage in a female athletic population following a sport-specific EIMD  
203 protocol.

204

205 To date, the majority of research investigating EIMD has used male volunteers and the  
206 differences between the sexes are largely overlooked. There remains some controversy  
207 concerning the presence of sex differences in the response to damaging exercise in humans,  
208 whereas the animal literature clearly shows that females experience less damage than males  
209 (8, 9, 13). The pattern and magnitude of EIMD was somewhat different in our female sample  
210 when compared to previous research in males (5, 29). Firstly, lower peak CK values were  
211 observed in the current study ( $307 \pm 92 \text{ IU}\cdot\text{L}^{-1}$ ) in comparison to previous research using 100  
212 drop jumps (30) and the Loughborough Intermittent Shuttle Test (25), which showed peak  
213 values in excess on  $1000 \text{ IU}\cdot\text{L}^{-1}$ ; and an identical repeated sprint protocol (5) using the same  
214 CK analyser method, but in males volunteers ( $776 \pm 312 \text{ IU}\cdot\text{L}^{-1}$ ). Despite this lower CK  
215 response, soreness levels reported in females in the current study were higher than those  
216 previously reported in males (5) across all time points. However detriments in muscle

217 function post damaging exercise were not as substantial, with no change in MVIC and a  
218 return of CMJ towards basal levels by 48 h. In contrast to previous work that showed  
219 decreases in knee extension force that extended to up to and beyond 48 h, following  
220 damaging exercise (5, 19), there was no change in the current study. However, CMJ was  
221 reduced at 24 h and sprint time was still effected up to 72 h post EIMD. There is little doubt  
222 that training status and the degree to which participants are accustomed to the exercise insult  
223 will affect the damage-recovery profile (2) because of the presence of a repeated bout effect  
224 (4, 31). Although it is beyond the scope of the current work to elucidate the time course  
225 differences in muscle function between studies, we speculate (based on previous work) that  
226 the preferential recruitment (32, 33), and preferential damage of type 2 fibres (22, 34) during  
227 heavy eccentric contractions led to an inability to generate ‘power’ which is an integral  
228 component of dynamic, explosive activity such as CMJ and sprint performance. Collectively  
229 these data suggest the magnitude and pattern of the functional, physiological and perceptual  
230 response to EIMD in female athletes might be different to their male counterparts. However,  
231 further work is required to confirm our observations and to elucidate the possible reasons  
232 underpinning these responses in muscle function.

233

234 There is evidence to suggest that oestrogen may have a protective effect against EIMD by  
235 stabilising membrane properties (35). Oestrogen has been suggested to have the ability to  
236 interact with the phospholipid double layer on the cell membrane thus stabilising the  
237 membrane (36). This interaction has led to a suggestion that the hormone oestrogen might  
238 alleviate muscle damage following a strenuous bout of exercise (15). This potential  
239 attenuation of membrane disruption might account for some of the steroid hormone’s  
240 mitigating effects on creatine kinase and muscle function. Moreover, it has been suggested  
241 that females have a higher CK clearance rate from the blood, which might further explain

242 why CK levels were lower in this current study in comparison to past studies (5, 29).  
243 Although CK release from the muscles is not a direct indicator of muscular damage, it is still  
244 recognised as a surrogate indicator of damage and a loss of sarcolemma integrity (37, 38).

245

246 Another plausible mechanism that could explain the lower degree of damage is the difference  
247 in strength, power, speed, and potentially fatigue resistance, between the sexes (14, 39). Male  
248 soccer players are relatively stronger, quicker and more powerful than females (39), and  
249 during repeated sprint exercise, men experience a greater decline in performance compared to  
250 women (40), which is associated with the initial higher power (41). Males will therefore  
251 typically generate more force during repeated sprint exercise, experience greater fatigue, and  
252 potentially cause greater disturbance to homeostasis and greater EIMD as a result. Further  
253 support for this idea arises from observations that women are more fatigue resistant than men  
254 during isometric (42) and dynamic contractions (43), but not when matched for initial  
255 strength level, at least for sustained sub-maximal contractions (44, 45). Differences in  
256 strength, power, speed and fatigue resistance might explain the lower CK values and faster  
257 return of muscle function observed in females in this study compared to previous literature in  
258 males. Further research is warranted to determine sex difference in the damage response to  
259 exercise, particularly between men and women matched for initial strength level.

260

## 261 **PRACTICAL APPLICATIONS**

262 Our results demonstrate that a bout of sport specific exercise induces muscle damage and  
263 affects functional performance on subsequent days in females. The data provides new  
264 information for athletes, coaches, scientists and practitioners to better understand the  
265 consequences of females engaging in strenuous exercise of this nature. The ability to balance

266 the consequences of training and competition and optimize recovery time in order to be well-  
267 prepared for subsequent training and competition, and to reduce the likelihood of injury is a  
268 constant dichotomous battle when performance schedules are so heavy. Clearly, there is a  
269 requirement for further research to examine the damage responses in this population  
270 following strenuous exercise paradigms and, importantly, if the EIMD response is modulated  
271 differently through phases of the menstrual cycle. Previously, Rampinini et al. (46) proposed  
272 that 48 h is adequate recovery time following a simulated soccer game; based on observations  
273 from the current study, more time is required before full recovery is reached following  
274 repeated sprint activity in female athletes.

#### 275 **Acknowledgements**

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277

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## Figure Legends

**Figure 1.** VAS ratings for perceived muscle soreness before and up to 72 h post muscle damaging repeat sprint exercise. Values presented as mean  $\pm$  SD. \* denotes significantly different from pre-exercise ( $p < 0.05$ )

**Figure 2.** Total CK activity pre and up to 72 h post muscle damaging repeat sprint exercise. Values presented as mean  $\pm$  SD change from baseline. \* denotes significantly different from pre-exercise ( $p < 0.05$ )

**Figure 3.** 30m sprint time (**A**) and CMJ height (**B**) pre and up to 72 h post muscle damaging repeat sprint exercise. Values presented as mean  $\pm$  SD change from baseline. \* denotes significantly different from pre-exercise ( $p < 0.05$ )

Figure 1

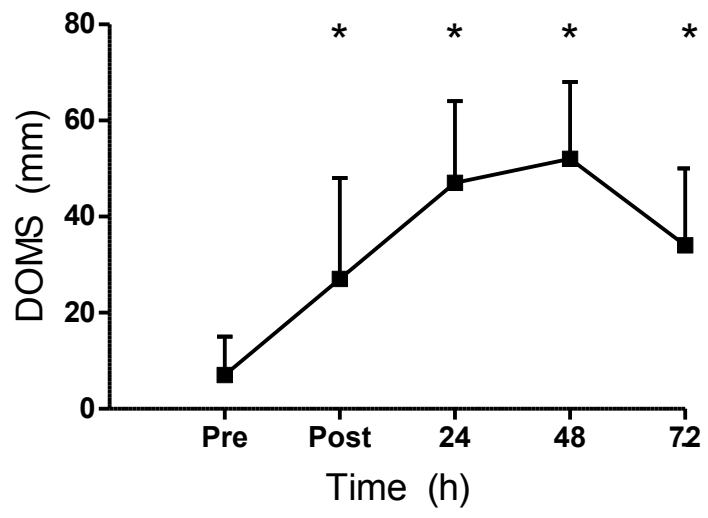


Figure 2

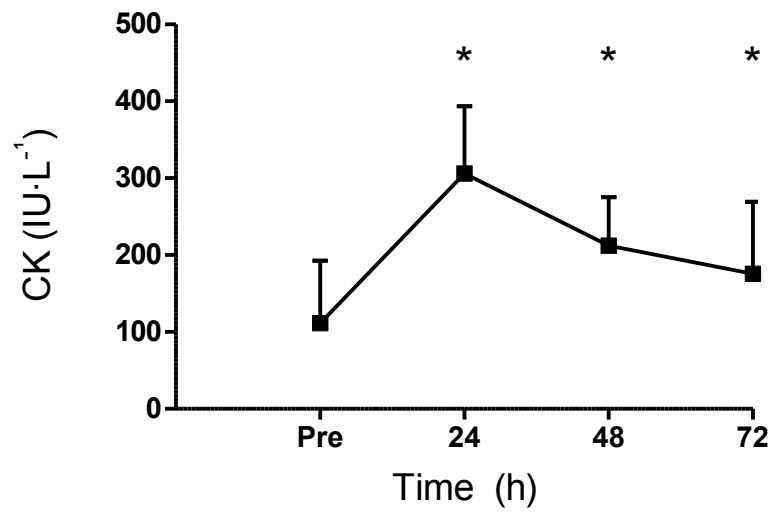
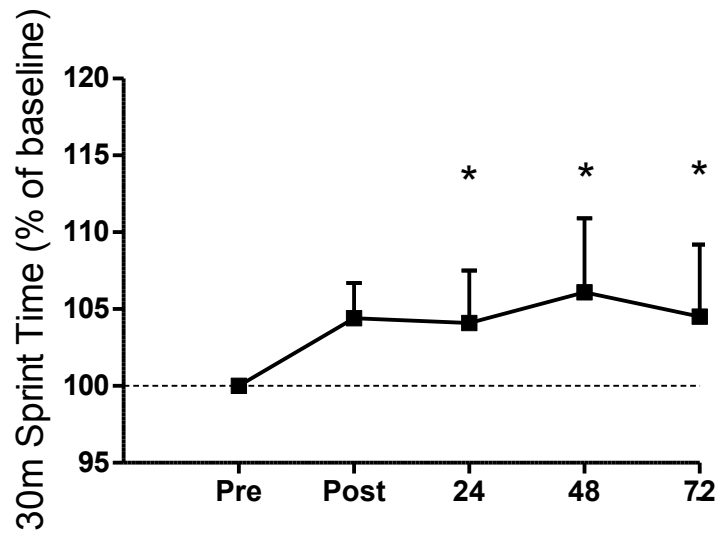
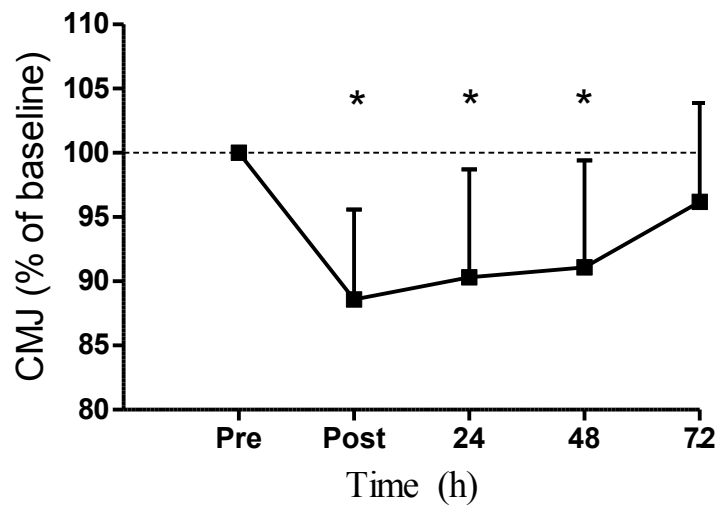


Figure 3

A



B



**Table 1.** Absolute values for dependent variables in response to muscle damaging exercise, mean  $\pm$  SD

Variable	Time post muscle damaging exercise (h)				
	Pre	0	24	48	72
<b>CMJ (cm)</b>	26.4 $\pm$ 3.3	23.4 $\pm$ 4.0*	23.9 $\pm$ 3.9*	24.1 $\pm$ 3.8*	25.3 $\pm$ 3.2
<b>Limb Girth (cm)</b>	57.3 $\pm$ 3.2	57.3 $\pm$ 2.9	56.9 $\pm$ 3.0	56.9 $\pm$ 2.8	57.0 $\pm$ 3.4
<b>MVC (N)</b>	470 $\pm$ 73	426 $\pm$ 91*	440 $\pm$ 78	450 $\pm$ 95	449 $\pm$ 91
<b>Sprint Time (s)</b>	4.95 $\pm$ 0.24	5.16 $\pm$ 0.31*	5.15 $\pm$ 3.30*	5.25 $\pm$ 0.40*	5.17 $\pm$ 0.37*

All values are means  $\pm$  SD (n=11). Significant difference between baseline and post intervention (immediately, 24, 48 and 72 h) (repeated measures ANOVA): \* denotes significantly different from pre-exercise (p<0.05) CK, creatine kinase; CMJ, counter movement jump; MVC, maximal voluntary contraction