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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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5 Sciences, Northumbria University, Newcastle Upon Tyne, UK

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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 ± 3 y, height 166.6 ± 5.7 cm, mass
31 62.7 ± 4.5 kg) in the luteal phase of the menstrual cycle completed a repeated sprint protocol
32 designed to induce EIMD (15×30 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 ± 3 years, height 166.6
100 ± 5.7 cm, mass 62.7 ± 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° 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⁻¹; 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 ± 0.23 and 5.12 ± 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.

190

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

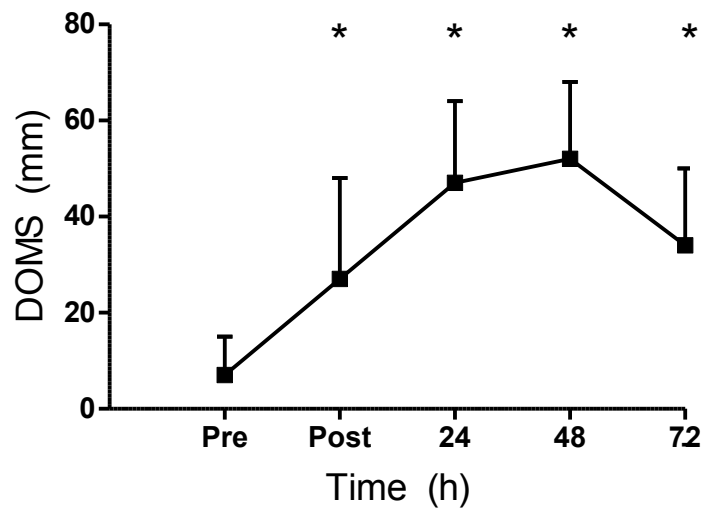


Figure 2

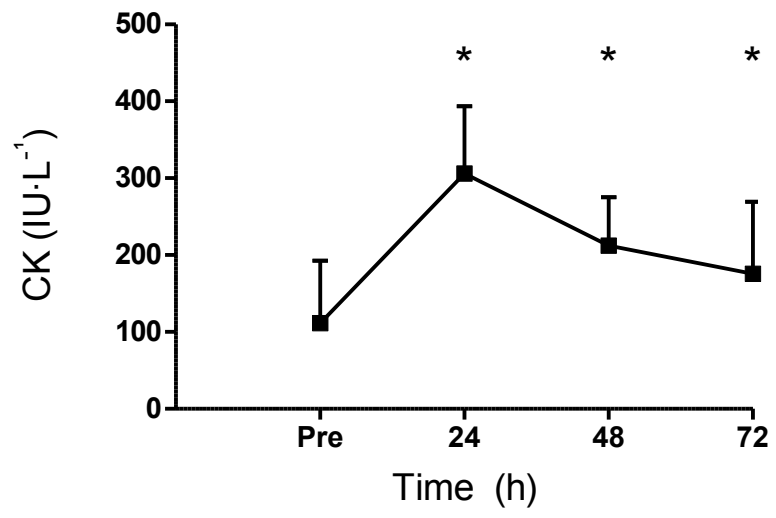
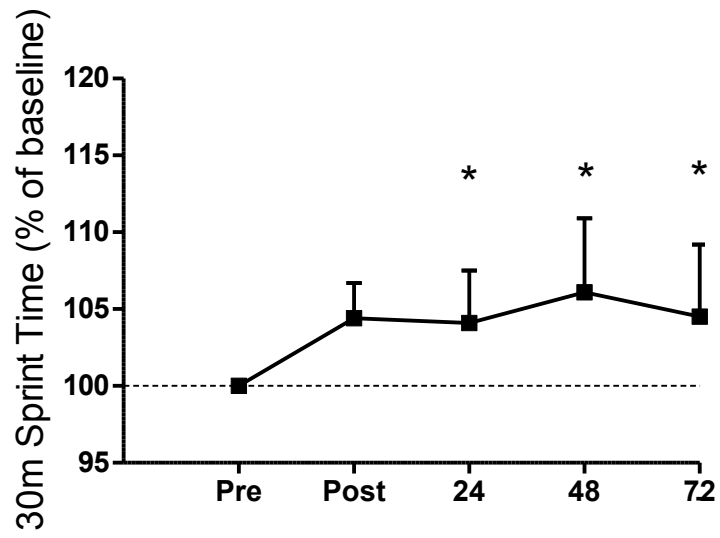


Figure 3

A



B

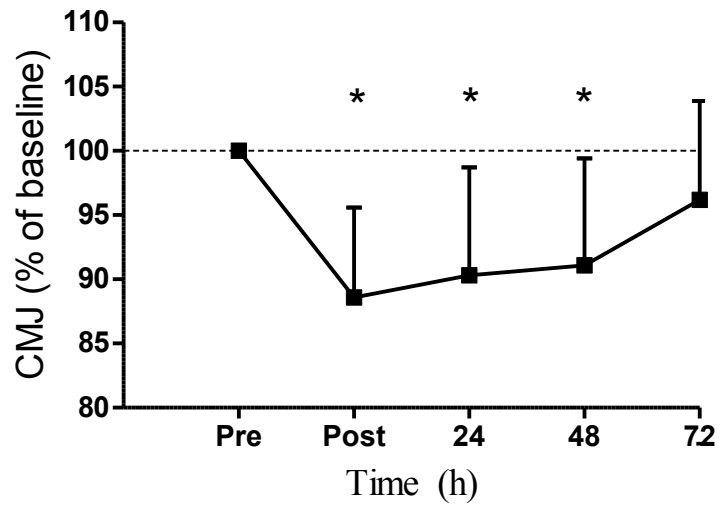


Table 1. Absolute values for dependent variables in response to muscle damaging exercise, mean \pm SD

Variable	Time post muscle damaging exercise (h)				
	Pre	0	24	48	72
CMJ (cm)	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
Limb Girth (cm)	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
MVC (N)	470 \pm 73	426 \pm 91*	440 \pm 78	450 \pm 95	449 \pm 91
Sprint Time (s)	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