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

Brief running head: EIMD in females following sprint exercise

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Abstract

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

Keywords: females, muscle function, recovery, exercise-induced muscle damage
INTRODUCTION

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

Various factors could potentially modulate the EIMD response in females, including oral contraceptive use, and the potential protective effect of oestrogen (8-13). There is some evidence suggesting that females are less fatigable than males (14) and the subsequent recovery following damaging exercise is known to be quicker (15). The faster recovery from damaging exercise has largely been attributed to the protective effect of oestrogen, but there has been no attempt to control for the menstrual cycle where large changes in sex hormones can be seen throughout the course of the menstrual cycle (16). This could potentially influence both the damage response and recovery process. It therefore makes the expectation tenable that the damage response in females could be somewhat different to the well-established response in males. However, it is critical to understand the damage-recovery response with control over the menstrual cycle to ascertain the implications of damaging exercise in female athletes.
In addition to the lack of data on the damage response in female athletes, much of the existing literature investigating EIMD employs damage protocols that lack specificity to a sporting context and are often eccentric biased (5, 7, 17-21), and in isolated muscle groups (4, 7, 16-18). Intermittent sports, such as soccer, rugby and basketball that require periods of high intensity, repeated sprint activity and changes of velocity (22) and direction (23), elicit significant muscle damage and prolonged decrements in function. Given the prevalence of both male and female participation in sports of this nature, further research is warranted with more sport-specific damage models to better understand the consequences of damaging repeated sprint activity (5). Establishing these responses in female athletes in particular will provide new, important information on the damage response in this population that could influence recovery strategies and exercise prescription. Consequently, the aim of this study was to examine the magnitude of damage following a sport-specific, repeated sprint protocol in females. We hypothesised that a repeated sprint exercise protocol would induce muscle damage in females and negatively affect performance in the subsequent days.

METHODS

Experimental Approach to the Problem

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

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

Procedures

A 30 m section of an environmentally controlled (19° C and 70% RH) 60-m indoor running track was marked using cones and two sets of light timing gates (Brower timing systems, Utah, USA). A further 10 m deceleration zone was also marked at the end of the 30 m section. Participants first completed a warm up consisting of 400 m self-paced jogging, a series of dynamic sprint drills including high knees, heel flicks and walking lunges which were conducted over a measured 10 m section of the aforementioned indoor running track. This was followed by a series of three practice sprints at the participants perceived 60%, 80% and 100% of maximum speed. Following the warm up, the participants were given 5 minutes to prepare themselves for the repeated-sprint protocol, during which time, no static stretching was performed. Participants then stood 30 cm from the start line to avoid premature
triggering of the timing system and completed 15 × 30 m sprints departing every 65 s with gates set up to record in the reverse order for the next sprint. Participants were told that all efforts must be maximal and they were instructed to stop within the 10 m deceleration zone. The rest period was initiated when participants came to a complete halt and the repetition was completed. Standardized, strong verbal encouragement was provided throughout the protocol.

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

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

Creatine kinase. Creatine kinase was determined using a capillary blood sample from the fingertip. A sample of whole fresh blood was analysed immediately using a colorimetric assay procedure (Reflotron Plus, Roche Diagnostics, UK). The resting normal expected values for CK when using this equipment are between 50 and 200 IU·L⁻¹; the CV for this instrument was <3%.
Countermovement Jump Height. Countermovement jump height was assessed using a light timing system (Optojump, Microgate, Italy). Participants were instructed to squat down and jump vertically, with their hands on their hips throughout. Participants were advised that all jumps must be a maximal effort. Three trials with a 60 s rest were performed and the peak jump height was used for analysis.

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

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

Statistical Analyses

Statistical analysis was performed using PASW Statistics 21.0 for Windows (SPSS, Inc., Chicago, IL.). Descriptive statistics are reported as means ± SD. Fatigue, fastest sprint time, and mean sprint time were calculated for the repeated sprint protocol: Fatigue = [100 × (total sprint time ÷ ideal sprint time)] – 100, in which total sprint time = sum of sprint times from all sprints and ideal sprint time = the number of sprints × fastest sprint time (Fitzsimons et al.,
1993). For illustrative purposes, and to account for inter-individual variability, CMJ height and sprint performance were presented in figure format as a change from baseline. The absolute scores were analysed using a one-way analysis of variance (ANOVA) with repeated measures and are presented in Table 1. Mauchly’s Test of Sphericity was used to check homogeneity of variance for all variables; where necessary any violations of the assumption were corrected using the Greenhouse–Geisser adjustment. Significant effects were followed up using Tukey post-hoc analysis. The alpha level for statistical significance was set at $p < 0.05$ a priori.

**RESULTS**

The repeated sprint protocol fastest and mean times were 4.93 ± 0.23 and 5.12 ± 0.23 s, respectively. The mean fatigue score was 4 ± 1%. All dependent variables with the exception of limb girth and MVIC showed significant time effects following the repeated sprints protocol ($p < 0.05$); illustrating a muscle damage response. DOMS was elevated over time ($F = 26.86, p < 0.001$, Figure 1); post-hoc analyses revealed elevations at 24 and 72 h post, with a peak at 48 h (Table 1). CK was elevated ($F = 13.34, = p < 0.05$), at every time point compared to pre-exercise (Table 1, Figure 2). For muscle function measures, there was a significant main effect for 30 m sprint time ($F = 8.29, p = 0.001$, Figure 3, panel B) and CMJ height ($F = 9.78, p < 0.005$, Figure 3, panel A), but not for MVIC (Table 1). Decrements in sprint performance were evident across all time points ($p < 0.05$). CMJ height was reduced immediately post, 24 h and 48 h post exercise ($p < 0.05$), but had returned to near baseline at 72 h.
The aim of this study was to ascertain the magnitude of EIMD indices following a repeated sprint protocol in an athletic female population. Results demonstrated that the repeat sprint protocol induced muscle damage with increases in DOMS, plasma CK, sprint time and reductions in CMJ height and 30m sprint time, all of which persisted for several days following the exercise insult. These data are broadly in agreement with the literature reporting that EIMD in males is evident soon after strenuous exercise, peaks at 24-48 h post exercise, and remains elevated for several days (2, 15, 27). Similar results have also been shown with exercise with a high eccentric component such as downhill running (28) and plyometric jumps (6). However, this is the first study to specifically document the signs and symptoms of muscle damage in a female athletic population following a sport-specific EIMD protocol.

To date, the majority of research investigating EIMD has used male volunteers and the differences between the sexes are largely overlooked. There remains some controversy concerning the presence of sex differences in the response to damaging exercise in humans, whereas the animal literature clearly shows that females experience less damage than males (8, 9, 13). The pattern and magnitude of EIMD was somewhat different in our female sample when compared to previous research in males (5, 29). Firstly, lower peak CK values were observed in the current study (307 ± 92 IU·L\(^{-1}\)) in comparison to previous research using 100 drop jumps (30) and the Loughborough Intermittent Shuttle Test (25), which showed peak values in excess on 1000 IU·L\(^{-1}\); and an identical repeated sprint protocol (5) using the same CK analyser method, but in males volunteers (776 ± 312 IU·L\(^{-1}\)). Despite this lower CK response, soreness levels reported in females in the current study were higher than those previously reported in males (5) across all time points. However detriments in muscle
function post damaging exercise were not as substantial, with no change in MVIC and a return of CMJ towards basal levels by 48 h. In contrast to previous work that showed decreases in knee extension force that extended to up to and beyond 48 h, following damaging exercise (5, 19), there was no change in the current study. However, CMJ was reduced at 24 h and sprint time was still effected up to 72 h post EIMD. There is little doubt that training status and the degree to which participants are accustomed to the exercise insult will affect the damage-recovery profile (2) because of the presence of a repeated bout effect (4, 31). Although it is beyond the scope of the current work to elucidate the time course differences in muscle function between studies, we speculate (based on previous work) that the preferential recruitment (32, 33), and preferential damage of type 2 fibres (22, 34) during heavy eccentric contractions led to an inability to generate ‘power’ which is an integral component of dynamic, explosive activity such as CMJ and sprint performance. Collectively these data suggest the magnitude and pattern of the functional, physiological and perceptual response to EIMD in female athletes might be different to their male counterparts. However, further work is required to confirm our observations and to elucidate the possible reasons underpinning these responses in muscle function.

There is evidence to suggest that oestrogen may have a protective effect against EIMD by stabilising membrane properties (35). Oestrogen has been suggested to have the ability to interact with the phospholipid double layer on the cell membrane thus stabilising the membrane (36). This interaction has led to a suggestion that the hormone oestrogen might alleviate muscle damage following a strenuous bout of exercise (15). This potential attenuation of membrane disruption might account for some of the steroid hormone’s mitigating effects on creatine kinase and muscle function. Moreover, it has been suggested that females have a higher CK clearance rate from the blood, which might further explain
why CK levels were lower in this current study in comparison to past studies (5, 29).

Although CK release from the muscles is not a direct indicator of muscular damage, it is still recognised as a surrogate indicator of damage and a loss of sarcolemma integrity (37, 38).

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

PRACTICAL APPLICATIONS

Our results demonstrate that a bout of sport specific exercise induces muscle damage and affects functional performance on subsequent days in females. The data provides new information for athletes, coaches, scientists and practitioners to better understand the consequences of females engaging in strenuous exercise of this nature. The ability to balance
the consequences of training and competition and optimize recovery time in order to be well-prepared for subsequent training and competition, and to reduce the likelihood of injury is a constant dichotomous battle when performance schedules are so heavy. Clearly, there is a requirement for further research to examine the damage responses in this population following strenuous exercise paradigms and, importantly, if the EIMD response is modulated differently through phases of the menstrual cycle. Previously, Rampinini et al. (46) proposed that 48 h is adequate recovery time following a simulated soccer game; based on observations from the current study, more time is required before full recovery is reached following repeated sprint activity in female athletes.

Acknowledgements

The authors would like to thank the participants in the current study for their participation.
REFERENCES


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 ± 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 ± 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 ± SD change from baseline. * denotes significantly different from pre-exercise (p<0.05)
Figure 1

A line graph showing DOMS (mm) over time (h). The graph displays the following data points:

- Pre
- Post
- 24
- 48
- 72

The graph indicates a gradual increase in DOMS from Pre to Post, with a peak at 72 hours, followed by a decrease. Significance markers (*) are present at the 24, 48, and 72-hour marks.
Figure 2

![Graph showing CK levels over time](image)

CK (IU·L⁻¹)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Pre</th>
<th>24</th>
<th>48</th>
<th>72</th>
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<td>CK</td>
<td>100</td>
<td>300</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 3

A

30m Sprint Time (% of baseline)

Time (h)

B

CMJ (% of baseline)

Time (h)
### Table 1. Absolute values for dependent variables in response to muscle damaging exercise, mean ± SD

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

All values are means ± 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