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Title: Lower volume muscle-damaging exercise protects against high volume muscle-damaging exercise and the detrimental effects on endurance performance

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Abstract

Purpose: this study examined if lower volume exercise-induced muscle damage (EIMD) performed two weeks before high volume muscle-damaging exercise protects against its detrimental effect on running performance. **Methods:** 16 male participants were randomly assigned to a lower volume (5 sets of 10 squats, *n*=8) or high volume (10 sets of 10 squats, *n*=8) EIMD group and completed baseline measurements for muscle soreness, knee extensor torque, creatine kinase (CK), a 5-min fixed-intensity running bout and a 3 km running time-trial. Measurements were repeated 24 and 48 h after EIMD, and the time-trial running after 48 h. Two weeks later, both groups repeated the baseline measurements, 10 sets of 10 squats and the same follow-up testing (Bout 2). **Results:** Data analysis revealed increases in muscle soreness and CK and decreases in knee extensor torque 24 – 48 h after the initial bouts of EIMD. Increases in oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}_E$) and rating of perceived exertion (RPE) were observed during fixed-intensity running 24 – 48 h after EIMD Bout 1. Likewise, time increased, speed and $\dot{V}O_2$ decreased during a 3 km running time-trial 48 h after EIMD. Symptoms of EIMD, responses during fixed-intensity and time-trial running were attenuated in the days after the repeated bout of high volume EIMD performed two weeks after the initial bout. **Conclusion:** this study demonstrates that the protective effect of lower volume EIMD on subsequent high volume EIMD is transferable to endurance running. Furthermore, time-trial performance was found to be preserved after a repeated bout of EIMD.

Key words

Exercise-induced muscle damage; repeated bout effect; oxygen uptake; electromyography; endurance running

Abbreviations

CK	creatine kinase
CO_2	carbon dioxide
EIMD	exercise-induced muscle damage
EMG	electromyography
HR	heart rate
[La]	blood lactate
LTP	lactate turnpoint
MVC	maximal voluntary contraction
O_2	oxygen
RBE	repeated bout effect
RPE	rating of perceived exertion
SF	stride frequency
SL	stride length
VAS	visual analogue scale
$\dot{V}_E$	minute ventilation
$\dot{V}O_2$	oxygen uptake
VL	vastus lateralis
VM	vastus medialis
$\dot{V}O_{2peak}$	peak oxygen uptake

Introduction

It is well established that the signs and symptoms of exercise-induced muscle damage (EIMD) are attenuated after a repeated bout of muscle-damaging exercise (McHugh 2003; McHugh et al. 1999). These adaptations are now known to be extended to the observed effects of EIMD on running performance, whereby the negative alterations in the physiological, metabolic, perceptual and kinematic responses during fixed-intensity running are reduced after a repeated bout of EIMD performed two weeks later (Burt et al. 2013).

An interesting observation is that a prior bout of lower volume EIMD protects the muscle against damage in the days after high volume muscle-damaging exercise (Brown et al. 1997; Chen 2003; Clarkson and Tremblay 1988; Nosaka et al. 2001). Howatson et al. (2007) demonstrated that 10 maximal eccentric contractions protected the elbow flexors against 45 maximal eccentric contractions performed two weeks later. Using surface EMG, the authors attributed the ‘repeated bout effect’ (RBE) to a reduction in median freqency. They suggested that an increased number of more resilient type I fibres were activated to protect the muscle against damage during the higher volume eccentric exercise. However, it is not known whether the protective effect of lower volume muscle-damaging exercise performed prior to a high volume of the same exercise preserves running performance. If it does, this would have have a ‘real-world’ application for those endurance athletes engaging in periodized resistance training for the first time. That is to say, where endurance athletes are contemplating concurrent endurance and resistance exercise to improve performance, performing lower volume resistance exercise two weeks before engaging in concurrent training might pre-condition the muscle to withstand high bouts of muscle-damaging exercise and its subsequent effects on endurance performance.

The effects of EIMD on endurance performance are often examined using fixed-intensity sub-maximal workloads (Braun and Dutto 2003; Burt et al. 2013; Chen et al. 2009; Kyröläinen et al. 2000). Whilst such protocols enable physiological responses to be measured during steady-state exercise, they lack ecological validity (Currell and Jeukendrup 2008). Alternatively, time-trials provide a more appropriate measurement of endurance performance that is impaired after EIMD. Indeed, decreases in distance covered (~6%), average power output (~12%) and oxygen cost (~11%) have been observed during cycling and running time-trials performed after EIMD (Burt and Twist 2011; Marcora and Bosio 2007; Twist and Eston 2009). The mechanims posited to explain the decrement in time-trial performance include an altered sense of effort, a reduction in neural drive and an increased production of inflammatory cytokines (Burt and Twist 2011; Marcora and Bosio 2007; Twist and Eston 2009). However, whether this reduction in time-trial performance is still evident after a repeated bout of EIMD remains unknown. Therefore, the aims of this study were (i) to investigate if a lower volume muscle-damaging exercise can protect the muscle against the detrimental effects on the physiological, metabolic, kinematic and perceptual responses during fixed-intensity running after subsequent high volume EIMD, and (ii) to examine the effects of repeated bouts of muscle-damaging exercise on running time-trial performance.

Methods

Participants and experimental design

After institutional ethical approval, 16 healthy male participants (see Table 1), who regularly participated in endurance exercise (2 – 3 sessions per week) and had not completed any lower limb resistance exercise in the previous six months, volunteered to participate in the study. Prior to data collection, participants provided informed consent and completed a medical health questionnaire. Participants were tested on eight separate occasions over a 5-week period. During the initial visit, they completed an incremental exhaustive running test to

determine lactate turnpoint (LTP) and peak oxygen uptake ($\dot{V}O_{2peak}$), followed by habituation procedures relating to measures of perceived muscle soreness, peak knee extensor torque (isometric and isokinetic) and 3 km running time-trial. After 48–72 h, participants completed isometric maximal voluntary contractions (MVCs) of the knee extensors, during which EMG activity of the vastus medialis (VM) and vastus lateralis (VL) was recorded. Upon completion, participants were randomly assigned to either a lower volume (Low-High; n = 8) or high volume (High-High; n = 8) muscle damage group. After a further 48 – 72 h, participants provided baseline measures for perceived muscle soreness, isokinetic peak knee extensor torque, creatine kinase (CK) activity, 5-min fixed-intensity running at LTP, a 3 km running time-trial and either lower or high volume lower limb resistance exercise designed to develop symptoms of EIMD. Baseline procedures were then repeated 24 and 48 h later, with the exception of the time-trial at 24 h. Two weeks later, when symptoms associated with the initial bout of muscle damage had disapated (Hortobagyi et al. 1998), participants repeated the baseline procedures, a repeated bout of high volume muscle-damaging exercise and the same follow-up testing at 24 and 48 h (except for the time-trial at 24 h). Participants were requested to avoid exercise 24 h prior to each visit, maintain their normal diets and avoid using any analgesic agents.

Insert Table 1 here

Procedures

Assessment of lactate turnpoint and peak oxygen uptake

Incremental running to determine individual LTP and $\dot{V}O_{2peak}$ was performed on a motorized treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany). Running speed commenced at 9 $km \cdot h^{-1}$ and was increased by 1.0 $km \cdot h^{-1}$ every 4 min until participants reached volitional exhaustion. Fingertip capillary blood samples were taken at the end of each 4-min

stage and immediately analyzed for blood lactate ([La]) (Lactate Pro analyser, Arkray, Kyoto, Japan). Lactate turnpoint was confirmed as the speed at which a second rise in [La] occurred above baseline values (Jones et al., 2009). Expired air was collected continuously using an online metabolic system (Oxycon Pro, Hoechberg, Germany) that was calibrated prior to each test with a span gas mixture of 16% O_2 and 5% CO_2. Gas exchange variables (including $\dot{V}O_2$) were recorded breath-by-breath and later averaged over 30 s epochs. Heart rate, monitored via telemetry (Polar Electro, Polar Beat, Oy, Finland), and ratings of perceived exertion (RPE; Borg 1998) were recorded in the final 15 s of each stage. $\dot{V}O_{2peak}$ was accepted as the highest $\dot{V}O_2$ averaged over 30 s. Reliability data for $\dot{V}O_{2peak}$ and HR at exhaustion demonstrated a coefficient of variation (CV) of 3.5% and 2.4%, respectively.

Indirect markers of muscle damage

After squatting to a knee angle of 90°, participants indicated the perceived soreness felt in their knee extensors by moving a marker along a visual analogue scale (VAS). The VAS comprised written cues from left (no muscle soreness) to right (muscles too sore to move), which corresponded to a number (0-10 unseen by the participant) on the reverse side. This scale has been used successfully in previous research (Twist & Eston 2009) and has been established as a valid and reliable measurement tool of soreness (Price et al. 1983).

To assess muscle function after the initial and repeated bouts of EIMD, isokinetic peak knee extensor torque was determined from the dominant limb using an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA). After a standardized warm-up comprising 3 min of sub-maximal cycling at 50 W and five sub-maximal and one maximal

familiarization trials, participants performed five maximal efforts at a velocity of 60 deg·s^{-1}. The highest of the five contractions was accepted as the peak isokinetic knee extensor torque. Visual feedback and consistent encouragement were used during each test to promote maximal effort. Institutional reliability data for peak isokinetic knee extensor torque at 60 degs·s^{-1} revealed a CV of 4.9%.

After pre-warming the hand in warm water (~42ºC), a 30 µl sample of fingertip capillary whole blood was collected, pipetted to a test strip, separated for plasma and analyzed for CK using a colorimetric assay procedure (Reflotron, Boehringer Mannheim, Germany). Reliability data for CK revealed a CV of 10.9%.

Fixed-intensity running and stride pattern

Participants performed 5 min of fixed-intensity running at their previously determined LTP on a motorized treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany). Expired air, heart rate (HR), RPE and [La] were recorded as previously described above. Reliability, assessed using CV, determined errors of 3.3%, 5.4%, 2.3%, 3.8%, and 6% for $\dot{V}O_2$, $\dot{V}_E$, HR, RPE, and [La], respectively. The final 10 s of each 5-min running bout was recorded using a high speed video camera (Casio Exilim, Pro Ex-F1) and then downloaded and digitized using motion analysis software (Qunitec Biomechanics 9.03 v 14). Stride length (SL) and stride frequency (SF) during each running bout were determined from the methods previously described by Braun and Dutto (2003) and Chen et al. (2009). In brief, SL for each full stride was calculated from the formula: SL (m) = velocity (m·s^{-1}) x stride time (s), with an average SL used for analysis. Stride frequency was determined by measuring the time between the first and last heel contacts, and then dividing the number of full strides by time. For example, if 12 full strides were completed in 9.4 s, 12.8 strides would be completed in 10 s. Reliability data for SL and SF revealed a CV of 1.2% and 1% respectively.

Electromyography

During each fixed-intensity running bout, surface EMG (Noraxon, Scottsdale, AZ, USA) was recorded from the VM and VL. The location of each muscle site was in accordance with the "Surface EMG for Non-Invasive Assessment of Muscles" (SENIAM) recommendations (Hermens et al. 1999). Briefly, electrodes for the VM were placed at four-fifths of the distance between the anterior superior iliac spine and the joint space in front of the anterior border of the medial ligament. The VL electrode site was located at two-thirds of the distance from the anterior superior iliac spine and the lateral side of the patella. A ground reference electrode was placed over the tibia. Before the placement of electrodes, body hair was removed, the skin lightly abraded and cleansed with an alcohol swab. After the first trial, the position of each electrode was marked with permanent ink to ensure the same placement across the bouts. In agreement with recommendations from SENIAM, dual silver/silver chloride electrodes (10 mm diameter contact area, 20 mm inter-electrode distance and 40 mm x 22 mm adhesive gel surface) were aligned with the orientation of the muscle fibre. Once in place, the electrode-skin impedance was checked before each trial to ensure it was < 10 KΩ. Electrodes were connected, via wires with built-in pre-amplifiers, to a transmitter unit that relayed data to an analogue-to-digital converter through telemetry. The wires and pre-amplifiers were taped to the skin to prevent any movement artefact. The raw EMG signals were sampled at a frequency of 1500 Hz, amplified by 500 and underwent a 12-bit analog-to-digital conversion. The common mode rejection ratio was > 100 dB, the baseline noise < 2 μV and the input impedance > 10 MΩ (Konrad 2005). Data were collected during all of the 5-min fixed-intensity running bouts, with the last 10 contractions analyzed for peak EMG amplitude. To calculate the peak EMG amplitude, the raw data were full-wave rectified using

root mean squared averaging with a 50 ms time window and then filtered with a band-pass of 10 – 500 Hz (Konrad 2005). All peak EMG amplitude data was normalized by dividing the value during each fixed-intensity running bout by the peak EMG amplitude obtained during isometric MVCs of the knee extensors (Ansley et al. 2004). Peak isometric force was measured from the dominant limb at an angle of 80° using the isokinetic dynamometer described above (Byrne et al. 2001). After five sub-maximal and one maximal familiarization trials, participants performed two MVC trials of 3 s duration, with a 60 s rest between each (Byrne et al. 2001).

Three kilometre time-trial protocol

Participants were required to complete 3 km in the quickest time possible on a motorized treadmill (Woodway PPS 55sport-I, Woodway GmbH, Germany). Feedback on distance covered was freely available, however participants were not able to view time, treadmill speed or heart rate. As previously described by Marcora and Bosio (2007), the time-trial commenced with the participants standing on the treadmill whilst the speed was increased to 9 $km \cdot h^{-1}$. Once this speed was attained, participants were able to adjust the speed freely. Time, RPE and a fingertip capillary sample analyzed for [La] were collected upon completion of the time-trial. Expired air and HR were measured throughout the time trial, with mean $\dot{V}O_2$ and HR determined for analysis. Average speed was calculated as distance divided by time.

Exercise-induced muscle damage protocol

The muscle-damaging protocol required participants to perform Smith-machine assisted squats at a resistance corresponding to 80% of body mass. The Low-High group completed an initial bout of resistance exercise comprising 5 sets of 10 squats, followed two weeks later by a high volume bout of 10 sets of 10 squats. The High-High group completed an initial bout of 10 sets of 10 squats, followed two weeks later by repeating the same high volume bout.

Each set was interspersed with a 2-min rest period. Before the exercise, participants performed an unloaded squat during which a goniometer (Cranlea and Co., Birmingham, UK) was used to determine a 90^{o} knee angle. Markers were then placed on either side of the Smith-machine to ensure that the range of motion was maintained during the squatting protocol and between bouts. The bar was positioned on the participant's shoulders, with his back straight, legs fully extended and feet hip-width apart. The eccentric phase of the squat involved lowering the bar until a knee angle of 90^{o} was achieved. Participants then lifted the bar back to the starting position to complete the concentric phase. To stress the eccentric component, participants were instructed to ensure that the downward and upward phases of the squat lasted for 2 and 1 s, respectively. Previous research has shown this protocol to be successful in inducing symptoms of muscle damage to the knee extensors (Davies et al. 2009).

Statistical analysis

Descriptive statistics (mean ± SD) were calculated for all performance variables. To assess the variability of the indirect markers of EIMD and dependent variables during the fixed-intensity running protocol, a series of separate three-way mixed factor (Group [2] x Bout [2] x Time [3]) repeated measures ANOVAs were used. The dependant variables during the time-trial were also analyzed using a mixed factor ANOVA (Group [2] x Bout [2] x Time [2]). Assumptions of sphericity were assessed using Mauchly's test, with any violations adjusted via the Greenhouse-Geisser correction. Where significant interaction effects were observed, independent *t*-tests were used to identify pair-wise differences. The alpha level was set at $P < 0.05$.

Results

Indirect markers of muscle damage

Perceived muscle soreness demonstrated a significant Bout ($F_{(1,14)}$ = 33.9, $P \leq 0.0005$) and Bout x Time ($F_{(2,28)}$ = 24.3, $P \leq 0.0005$) effect, however, no significant Group x Bout x Time interaction was observed. Similarly, there was no Group x Bout x Time interaction for isokinetic peak knee extensor torque, although Bout ($F_{(1,14)}$ = 15.4, P = 0.002) and Bout x Time ($F_{GG(1.2,16.2)}$ = 11.6, P = 0.003) effects were observed. CK activity changed over Bout ($F_{(1,14)}$ = 28.2, $P \leq 0.0005$) and across Bout x Time ($F_{GG(1.2,17.3)}$ = 37.8, $P \leq 0.0005$). A Group x Bout x Time interaction ($F_{GG(1.2,17.3)}$ = 7.9, P = 0.009) was also observed, with *post hoc* analysis revealing that CK activity was significantly greater in the High-High group at 24 and 48 h after Bout 1 (Figure 1).

Insert Figure 1 here

Fixed-intensity running responses to repeated bouts of muscle-damaging exercise

Most of the physiological, metabolic perceptual and kinematic responses during fixed-intensity running revealed significant Bout ($\dot{V}O_2$ ($F_{(1,14)}$ = 30.2, $P \leq 0.0005$); $\dot{V}_E$ ($F_{(1,14)}$ = 9.4, P = 0.008); [La] ($F_{(1,14)}$ = 25.4, $P \leq 0.0005$); RPE ($F_{(1,14)}$ = 20.1, P = 0.001); SL ($F_{(1,14)}$ = 34.2, $P \leq 0.0005$); SF ($F_{(1,14)}$ = 36.5, $P \leq 0.0005$)) and Bout x Time effects ($\dot{V}O_2$ ($F_{(2,28)}$ = 16.1, $P \leq 0.0005$); $\dot{V}_E$ ($F_{(2,28)}$ = 18.2, $P \leq 0.0005$); [La] ($F_{(2,28)}$ = 5.9, P = 0.007); RPE ($F_{(2,28)}$ = 25.6, $P \leq 0.0005$); SL ($F_{(2,28)}$ = 13.7, $P \leq 0.0005$); SF ($F_{(2,28)}$ = 15.2, $P \leq 0.0005$)). Heart rate demonstrated a Bout effect ($F_{(1,14)}$ = 7.1, P = 0.019) only. There was also no significant Group x Bout x Time effect on $\dot{V}O_2$, $\dot{V}_E$, [La], RPE, HR, SL or SF (Table 2).

Insert Table 2 here

Electromyography

Peak EMG amplitude of the VM demonstrated Bout ($F_{(1,14)}$ = 17.5, P = 0.001) and Bout x Time ($F_{(2,28)}$ = 9.8, P = 0.001) effects only, whilst Peak EMG amplitude of the VL was only significant across Bouts ($F_{(1,14)}$ = 27.4, $P \leq 0.0005$; Table 3).

Insert Table 3 here

Time trial responses to repeated bouts of muscle-damaging exercise

All of the dependent variables during the running time-trial, except for end RPE, revealed a significant main effect of Bout (time ($F_{(1,14)}$ = 39.3, $P \leq 0.0005$); average speed ($F_{(1,14)}$ = 29.9, $P \leq 0.0005$); mean $\dot{V}O_2$ ($F_{(1,14)}$ = 15.9, P = 0.001); end [La] ($F_{(1,14)}$ = 33.8, $P \leq 0.0005$); mean HR ($F_{(1,14)}$ = 7.2, P = 0.018)) and an interaction effect of Bout x Time (time ($F_{(1,14)}$ = 29.7, $P \leq$ 0.0005); average speed ($F_{(1,14)}$ = 25.5, $P \leq 0.0005$); mean $\dot{V}O_2$ ($F_{(1,14)}$ = 19.1, P = 0.001); end [La] ($F_{(1,14)}$ = 15.2, P = 0.002); mean HR ($F_{(1,14)}$ = 21.1, $P \leq 0.0005$)). Furthermore, there was no significant interaction of Group x Bout x Time on time, average speed, mean $\dot{V}O_2$, end [La], end RPE or mean HR (Fig. 2).

Insert Figure 2 here

Discussion

The initial bout of squatting exercise, independent of volume, resulted in symptoms of exercise-induced muscle damage. Both groups experienced heightened perceptions of muscle soreness, decreases in peak knee extensor torque and increases in CK activity in the 24 and 48 h after lower or high volume squats. Nonetheless, symptoms of EIMD were attenuated in the days after the second bout of muscle-damaging exercise. Furthermore, this reduction occurred regardless of whether participants performed lower or high volume squatting

exercise in Bout 1, and reaffirms previous observations that lower volume resistance exercise protects the muscle against high volume muscle-damaging exercise (Brown et al. 1997; Chen 2003; Clarkson and Tremblay 1988; Howatson et al. 2007; Nosaka et al. 2001).

As expected, the physiological, metabolic and perceptual responses during fixed-intensity running were altered in the days after Bout 1. Each group displayed increases in $\dot{V}O_2$, $\dot{V}_E$, [La], and RPE during fixed-intensity running at 24 and 48 h after the initial bout of EIMD. We postulate that muscle soreness experienced after the initial squatting bout was responsible for the increase in $\dot{V}O_2$ and $\dot{V}_E$ during fixed-intensity running. In attempts to limit discomfort associated with muscle soreness, participants might have altered their running stride pattern (as shown by changes in SL and SF), leading to the increase in $\dot{V}O_2$ (Braun & Dutto 2003). Furthermore, the activation of group III and IV afferent fibres, which are responsible for muscle soreness, might have provided an additional stimulus to drive $\dot{V}_E$ during fixed-intensity running (Davies et al. 2009). The elevated $\dot{V}_E$ response could also explain the concomitant increase in RPE during fixed-intensity running. Feelings of breathlessness might have provided a central cue to inform RPE, whilst it is also possible that muscle soreness experienced in the knee extensors provided a peripheral cue to alter the sense of effort (Davies et al. 2009; Jameson and Ring 2000; Twist and Eston 2009).

This is the first study to confirm that EMG amplitude is increased during fixed-intensity running after EIMD. Given that knee extensor strength had decreased after the initial lower (~11%) and higher volume (~16%) squatting exercise, a compensatory increase in motor unit recruitment occurred to ensure that participants were able to complete the same running

speed (Kyröläinen et al. 2000). Moreover, the compensatory increase in central motor command could also explain the elevation in $\dot{V}O_2$, [La], and RPE during fixed-intensity running after the initial bout of EIMD. Indeed, since there is a linear relationship between EMG activity and oxygen uptake (Bigland-Ritchie and Woods 1974), it is plausible that the observed increase in $\dot{V}O_2$ was reflective of an increase in EMG amplitude. RPE is described as a 'sensation of innervation' generated by the conscious awareness of the central motor command sent to the exercising muscle (Marcora 2009). Therefore, the increased motor unit recruitment required to produce the same baseline running speed after Bout 1 could also explain the elevation in RPE. Likewise, the observed increase in EMG activity might reflect an increase in the recruitment of non-damaged, more glycolytic, type II fibres, which caused the increase in [La] during fixed-intensity running after Bout 1 (Braun and Dutto 2003).

The detrimental effects of muscle-damaging exercise on fixed-intensity running were less prominent after a repeated bout performed two weeks later (i.e. Bout 2). These findings occurred irrespective of the initial volume of EIMD and endorse those of Burt et al. (2013). It is likely that the initial bout strengthened muscle fibre integrity, which subsequently reduced muscle soreness and maintained muscle function after the repeated bout. This enabled the preservation of stride pattern and motor unit activation during fixed-intensity running, and possibly explains why $\dot{V}O_2$, $\dot{V}_E$, [La], and RPE remained unchanged after Bout 2.

For the first time, this study reports on the effects of repeated bouts of muscle-damaging exercise on running time-trial performance. As anticipated, running time-trial performance was impaired after the initial bout of resistance exercise, where the time to complete 3 km

was increased by 7% and 9% after lower and high volumes of damaging exercise, respectively. This corresponds with previous studies, whereby distance covered during cycling (Burt and Twist 2011; Twist and Eston 2009) and running (Marcora and Bosio 2007) time-trials is decreased by 4 – 6% as a result of EIMD. However, this is the first study to demonstrate that the detrimental effects of EIMD on running time-trial performance are less prevalent after a second bout.

In support of Marcora and Bosio (2007), participants in our study ran at a slower speed despite their RPE being similar to pre-damage during the time-trial after Bout 1. It is speculated that muscular pain incurred as a result of the initial bout of squatting exercise altered the sense of effort during the time-trial, which resulted in participants adopting a slower speed and the subsequent lower $\dot{V}O_2$, $\dot{V}_E$, HR and [La] responses. The absence of any increase in muscle soreness as a result of the RBE would have enabled the same sense of effort during the time-trial after Bout 2, which meant time, speed, $\dot{V}O_2$, HR and [La] remained unaltered.

What is also original from this study is that the responses after a second bout of damaging exercise were not different between the Low-High and High-High groups. This informs that the physiological, metabolic and perceptual responses during fixed-intensity and time-trial running after repeated bouts of higher volume EIMD were attenuated independent of whether this was preceded by lower or high volume muscle-damaging exercise.

We can only speculate about the possible mechanisms of action that explain how initial lower or high volume damaging exercise results in a similar RBE. CK activity after the second bout of high volume squatting was reduced by the initial lower volume bout, meaning it is unlikely that the RBE was exclusively due to the removal and replacement of weak sarcomeres as

proposed by some studies (e.g. Armstrong et al. 1983; Byrnes et al. 1985; McHugh et al. 2003; McHugh et al. 1999). Indeed, if weak sarcomeres were still intact after the lower volume bout of squatting then they would have been damaged during the more strenuous high volume bout (Nosaka et al. 2001). Therefore, the lower CK in Bout 2 suggests that the initial stress in Bout 1, albeit of a lower intensity, provided a stimulus to strengthen the muscle cell membrane against further damage (Clarkson and Tremblay 1988; Howatson et al. 2007). The protective effect of lower volume muscle-damaging exercise against subsequent high volume exercise could also be due to changes in motor unit recruitment during squatting Bout 2 (Chen 2003; Howatson et al. 2007; McHugh 2003; McHugh et al. 1999). Unfortunately EMG activity was measured during running and not the damaging exercise, which means that we are unable to confirm if alterations in motor unit activity occurred.

Despite being the first study to demonstrate that motor unit recruitment during endurance exercise is altered by EIMD, we recognise the limitations of surface EMG. Attempts were made to ensure accurate placement of electrodes on the skin surface, but despite this control, we accept that changes in pH and blood flow might have occurred between visits (Kupa et al. 1995). Moreover, we also acknowledge that the extent of EIMD amongst individuals was variable. Although the group means revealed a significant effect after EIMD, the large standard deviations on most dependent variables suggest that some participants demonstrated a greater susceptibility to EIMD than others. This inter-individual response to EIMD is common and might reside in certain genetic polymorphisms (Hubal et al. 2010).

Conclusions

This investigation reaffirms that EIMD causes alterations to fixed-intensity and time-trial running performance. However, lower volume muscle-damaging exercise (5 sets of 10 squats at 80% body mass) provides protection against a high volume bout (10 sets of 10 squats at 80% body mass) performed two weeks later. Furthermore, in the days after this repeated bout

of EIMD, symptoms of muscle damage and alterations to fixed-intensity and 3 km time-trial running were all reduced. From a practical standpoint, where endurance athletes are contemplating concurrent endurance and resistance exercise to enhance performance, this study shows that performing lower volume resistance exercise pre-conditions the muscle to withstand high bouts of muscle-damaging exercise and its detrimental effects on fixed-intensity and time-trial running.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical standards

The authors declare that the experiments carried out complied with current UK laws.

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Table 1.

	Low-High (n = 8)	**High-High (n = 8)**
Age (y)	26 ± 5	27 ± 4
Stature (m)	1.79 ± 0.05	1.77 ± 0.1
Body Mass (kg)	79.5 ± 7.8	76.7 ± 9.8
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.8 ± 3.5	54.2 ± 4.1
HR_{peak} ($b \cdot min^{-1}$)	191 ± 10	191 ± 9
LTP_{speed} ($km \cdot h^{-1}$)	12.4 ± 1.1	12.6 ± 0.9

Table 2.

Variable	Bout	Low-High Baseline	Low-High 24	Low-High 48 h	High-High Baseline	High-High 24 h	High-High 48 h
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1}) ‡ #	1	46.9 ± 2.3	48.7 ± 3.1	48.9 ± 3.0	47.3 ± 2.8	49.5 ± 2.7	49.9 ± 3.1
	2	46.7 ± 2.4	47.1 ± 2.5	46.9 ± 2.7	47.5 ± 2.8	47.4 ± 3.1	47.4 ± 2.8
$\dot{V}_E$ (l·min^{-1}) ‡ #	1	101.3 ± 11.7	110.8 ± 12.4	109.3 ± 12.4	101.3 ± 21.9	112.4 ± 26.5	114.3 ± 26.3
	2	102.6 ± 11.8	104.4 ± 10.9	103.3 ± 11.9	100.3 ± 16.3	102.9 ± 15.6	103.1 ± 18.4
[La] (mmol.l^{-1}) ‡ #	1	4.8 ± 1.0	5.5 ± 1.2	5.1 ± 1.2	5.7 ± 1.5	6.1 ± 1.4	6.6 ± 1.8
	2	4.6 ± 1.1	4.8 ± 1.1	4.6 ± 1.0	5.6 ± 1.6	5.7 ± 1.6	5.7 ± 1.4
RPE ‡ #	1	12.6 ± 0.9	14.1 ± 1.1	13.8 ± 1.0	13.0 ± 1.5	14.8 ± 1.6	14.3 ± 1.6
	2	12.6 ± 0.7	12.9 ± 1.1	12.6 ± 0.7	13.3 ± 1.2	13.5 ± 1.3	13.5 ± 1.3
HR (b·min^{-1}) ‡	1	163.4 ± 11.6	166.4 ± 11.5	165.8 ± 11.2	172.5 ± 10.2	172.9 ± 11.2	173.5 ± 9.5
	2	163.3 ± 11.4	164.5 ± 10.1	163.9 ± 10.0	170.8 ± 11.0	171.0 ± 11.6	171.3 ± 11.1
SL (m^{-1}) ‡ #	1	2.39 ± 0.17	2.33 ± 0.19	2.33 ± 0.18	2.43 ± 0.20	2.35 ± 0.19	2.33 ± 0.18
	2	2.41 ± 0.18	2.40 ± 0.18	2.40 ± 0.18	2.45 ± 0.21	2.44 ± 0.20	2.43 ± 0.20
SF ‡ #	1	14.4 ± 1.1	14.8 ± 1.2	14.8 ± 1.2	14.5 ± 0.8	14.9 ± 0.7	15.1 ± 0.7
	2	14.3 ± 1.1	14.3 ± 1.1	14.4 ± 1.1	14.4 ± 0.8	14.4 ± 0.8	14.5 ± 0.8

Table 3.

Variable	Bout	Low-High Baseline	Low-High 24 h	Low-High 48 h	High-High Baseline	High-High 24 h	High-High 48 h

EMG Peak VM (%) ‡ #	1	66.8 ± 22.3	72.5 ± 24.4	71.0 ± 22.3	49.3 ± 14.7	62.6 ± 15.0	59.8 ± 19.4
	2	66.2 ± 21.9	63.3 ± 21.5	65.5 ± 23.0	50.2 ± 20.7	48.4 ± 18.0	48.1 ± 17.8
EMG Peak VL (%) ‡	1	68.8 ± 17.3	72.4 ± 14.7	71.4 ± 11.6	52.0 ± 13.0	57.0 ± 17.0	56.7 ± 14.4
	2	61.8 ± 16.1	62.0 ± 14.7	61.5 ± 13.8	51.8 ± 15.5	51.2 ± 7.7	48.6 ± 11.1

Table legends

Table 1. Participant characteristics

Table 2. Mean (± SD) physiological, metabolic, perceptual and kinematic responses during fixed-intensity running after repeated bouts of muscle-damaging exercise. ‡ significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$).

Table 3. Mean (± SD) peak EMG amplitude responses during fixed-intensity running after repeated bouts of muscle-damaging exercise. ‡ significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$).

Figure legends

Figure 1. Changes in **a** perceived muscle sorness, **b** knee extensor torque and **c** CK after repeated bouts of muscle-damaging exercise. Values are shown as means ± SD. ‡ denotes a significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # denotes a significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$). * denotes a Group x Bout x Time interaction; High-High group > Low-High Group at 24 – 48 h after Bout 1 ($P < 0.05$).

Figure 2. Changes in **a** time, **b** average speed, **c** mean $\dot{V}O_2$, **d** mean heart rate, **e** end blood lactate and **f** end RPE during time-trial running after repeated bouts of EIMD. Values are shown as means ± SD. ‡ significant effect for Bout; Bout 1 > Bout 2 ($P < 0.05$). # significant Bout x Time interaction; values 24 – 48 h after Bout 1 > values 24 – 48 h after Bout 2 ($P < 0.05$).