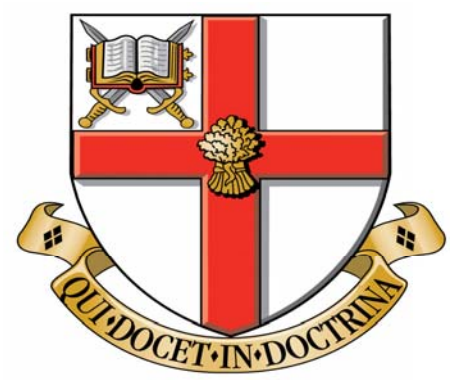

THE EFFECTS OF REPEATED BOUTS OF MUSCLE DAMAGING EXERCISE ON THE PHYSIOLOGICAL AND PERCEPTUAL RESPONSES DURING SINGLE-LEG CYCLING: EVIDENCE FOR CROSS-TRANSFER DURING ENDURANCE EXERCISE

by

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No portion of the work referred to in this Research Project has been submitted in support of an application for another degree or qualification of this, or any other University or institute of learning.

The project was supervised by a member of academic staff, but is essentially the work of the author.

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Abstract

Unaccustomed eccentric exercise commonly induces the immediate and prolonged symptoms of exercise-induced muscle damage (EIMD). Whilst an initial bout impairs sub maximal endurance performance, a repeated bout of the same eccentric exercise attenuates symptoms of EIMD. This is known as the repeated bout effect (RBE). Furthermore, it is claimed that training the unilateral limb, increases the strength of the contralateral homologous limb. This is manifested through neural adaptations and is known as cross-transfer. Accordingly, this study investigated if an initial bout of eccentric exercise in the unilateral leg would provide protection from the detrimental effects of EIMD on endurance performance after a second bout of eccentric exercise in the contralateral leg. After institutional ethical approval, 12 healthy, recreational participants were randomly assigned to two groups (ipsilateral, n = 6 both bouts performed in the same leg; contralateral, n = 6 one bout performed in each leg) who performed 10 x 10 eccentric contractions. Strength, perceived soreness and sub maximal cycling exercise was measured at baseline, 24 h and 48 h. Two weeks later, when symptoms of EIMD had dissipated, all procedures were repeated. Results revealed that perceived muscle soreness and isometric strength ($P < 0.05$) were significantly altered following the eccentric exercise at 24-48 h after an initial bout of EIMD. However, after a repeated bout, the symptoms of EIMD were attenuated in both the ipsilateral and contralateral groups. Furthermore, this had no significant effect on sub maximal endurance cycling. EMG data provided evidence for RBE after decreases in MF and increases in peak amplitude. This data further supports the evidence for a RBE, and supports observations that protective adaptations transfer to the untrained limb. EMG data supports this neural adaptation.

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List of Abbreviations

ANOVA – Analyses of variance

BF – Breathing frequency

Bla – Blood lactate

CNS – Central nervous system

DOMS – Delayed onset muscle soreness

EIMD – Exercise-induced muscle damage

EMG – Electromyography

FFT – Fast Fourier transformations

h – Hours

HR – Heart rate

MF – Median frequency

MVC – Maximal voluntary contraction

P_{max} – Maximum power output

PO – Power output

RBE – Repeated bout effect

RMS – Root mean squared

RPE – Ratings of perceived exertion

rpm – Revolutions per minute

s - Seconds

SD – Standard deviation

VAS – Visual analogue scale

$\dot{V}_E$ – Ventilation

VL – Vastus lateralis

VM – Vastus medialis

$\dot{V}O_2$ – Oxygen uptake

$\dot{V}O_{2\,peak}$ – Peak oxygen uptake

W – Watts

Introduction

It is well documented that concurrent resistance and endurance training improves running (Paavolainen, Häkkinen, Hämäläinen, Nummela & Rusko, 1999; Millet, Jaouen, Borrani & Candau, 2002; Jung, 2003) and cycling (Levin, McGuigan & Laursen, 2009; Sunde, Støren, Bjerkaas, Larsen, Hoff & Helgerud, 2010; Rønnestad & Mujika, 2013) economy and performance, as well as promoting health benefits such as elevating energy expenditure and fat utilization (Paschalis *et al.,* 2011). However, if the resistance exercise is unaccustomed, an initial bout can lead to immediate and prolonged symptoms of exercise-induced muscle damage (EIMD). This can lead to alterations in the physiological, metabolic, perceptual and kinematic responses to exercise and impairs performance in the days after (Davies, Rowlands & Eston, 2009; Twist & Eston, 2009; Burt & Twist, 2011). EIMD leads to ultrastructural changes in the muscle, including disruption of the sarcomere, damage to t-tubules and “z-band streaming” (Morgan & Allen, 1999; Byrne, Twist & Eston, 2004; Falvo & Bloomer, 2006). Accompanying these changes are typical symptoms, including swelling, delayed onset muscle soreness (DOMS), loss of strength, increased muscle stiffness and elevated concentrations of blood myofibre proteins (MacIntyre, Reid & McKenzie, 1995; Cleak & Eston, 1999; Morgan & Allen, 1999; Clarkson & Hubal, 2002; Byrne *et al*., 2004). EIMD alters the physiological and perceptual responses during endurance exercise (Davies *et al.,* 2009; Twist & Eston, 2009; Burt & Twist, 2011). Previous literature postulates an increased in oxygen uptake ($\dot{V}O_2$) is required, in order to perform the same task in a damaged state, due to the greater reliance on type I muscle fibres, after preferential damage to type II fibres (Burt & Twist, 2011). This recruitment of type I fibres would maintain normal metabolic function, and therefore, no alterations in blood lactate (Bla) during sub maximal exercise, below the lactate threshold (LT) (Scott, Rozenek, Russo,

Crussemeyer & Lacourse, 2003). Contrary to this, several studies have identified that Bla does increase after EIMD and is more pronounced after exercise above the LT, due to the recruitment of non-damaged type II fibres to try and maintain pre-damaged force levels (Gleeson, Blannin, Zhu, Brooks & Cave, 1995; Braun & Dutto, 2003). Increases in perception of pain are commonly observed during sub maximal exercise following EIMD, and is simultaneously associated with increases in ratings of perceived exertion (RPE) and ventilation ($\dot{V}_E$) (Scott *et al.,* 2003; Davies *et al.,* 2009). This is modulated by afferent fibres located in and around the blood vessels of the exercising muscle, which are responsible for increases in $\dot{V}_E$ (Davies *et al.,* 2009). Muscle function is similarly impaired following EIMD, regarding power output (PO) and consequently cadence. Previous research has suggested that PO decreases due to an increased perception of pain, which may limit the attempt to work within intolerable limits (Twist & Eston, 2009; Burt & Twist, 2011), whilst others argue that the reduction in PO is due to alterations in force generating capacity (Hotta *et al.,* 2006).

After recovery from an initial bout of muscle damaging exercise, the same exercise performed ~2 weeks later results in attenuated signs and symptoms of EIMD (Connolly, Reed & McHugh, 2002; Nosaka, 2008; Burt, Lamb, Nicholas & Twist, 2012). This protective effect is referred to as the repeated bout effect (RBE; McHugh, 2003), the mechanism of which is thought to be neurally, cellular and/or mechanically orientated (McHugh, 2003). While no unified theory exists, the extent of muscle damage is reduced in a repeated bout due to an improved efficiency in motor unit recruitment (Golden & Dudley, 1992; Connolly *et al.,* 2002), greater distribution of workload amongst muscle fibres (Nosaka & Clarkson, 1995) and/or changes in the number of stress susceptible fibres (Chen, Nosaka & Sacco, 2007). Adaptation after the initial bout might also lead to intermediate filament remodelling that attenuates the magnitude of

damage after the repeated bout (Connolly *et al.,* 2002; Thiebaud, 2012).

Typically, the RBE alters the response to a second bout of muscle-damaging exercise, either by attenuating the magnitude of EIMD markers or by enhancing recovery (McHugh, 2003; Nosaka, 2008); whilst soreness is still elevated compared to baseline, it does not occur to as great an extent as the first bout of damaging exercise (Connolly *et al.,* 2002). Similar findings have also been shown for isometric strength and creatine kinase (Kamandulis, Skurvydas, Brazaitis, Škikas & Duchateau, 2010). Whilst an initial bout of muscle-damaging exercise alters the physiological and perceptual responses to sub maximal endurance exercise (Elmer, McDaniel & Martin, 2010; Burt & Twist, 2011), these changes are not observed after the same damaging exercise performed two weeks later (Burt *et al*., 2012). Such observations indicate that the RBE extends to protect the negative impact of EIMD on endurance performance, and is of an applied interest since individuals participate in multiple training sessions.

Cross-transfer is a physiological phenomenon associated with increases in the contralateral (untrained) limb, after training in the ipsilateral limb (Connolly *et al.,* 2002; Howatson & van Someren, 2007). Previous meta-analysis of randomized control trials highlighted that whilst strength gains of 35% were observed in the trained limb of pooled data, the contralateral limb demonstrated a 7.8% ($P < 0.05$) increase in strength (Munn, Herbet & Gandevia*,* 2004). This has implications in rehabilitation scenarios where individuals are unable to train a limb due to injury, i.e. training of the uninjured limb might confer some protection and adaptation of the injured limb. Previous research has shown cross-transfer of the RBE (Howatson & van Someren, 2007; Starbuck & Eston, 2011). For example, in a group of young men, reductions in muscle soreness and maximal voluntary contraction (MVC) were lower in magnitude in the ipsilateral limb when the same damaging exercise was repeated two weeks later. Interestingly, a

similar group performed the same protocol but damaged the contralateral arm in the second bout of exercise and also reported a significant reduction in muscle soreness and MVC compared to after the initial bout, albeit of a lower magnitude. Two viable hypotheses exist to explain the mechanism of cross-transfer (Carroll, Herbert, Munn, Lee & Gandevia, 2006; Lee & Carroll, 2007). Since the nervous system is involved in strength training adaptations, unilateral tasks increase cortical activity to both the unilateral and contralateral limb, which prompts the notion of the neural drive "spilling-over" which is accessible to the untrained limb (Lee & Carroll, 2007; Adamson, MacQuaide, Helgerud, Hoff & Kemi, 2008; Starbuck & Eston, 2011). Electromyography (EMG) shows an increase in slow twitch motor unit recruitment during a repeated bout of eccentric exercise (Starbuck and Eston, 2011), resulting in a greater distribution of workload and less mechanical stress on the muscle (McHugh, 2003). This suggests that neural adaptations are likely to be centrally mediated by spinal and cortical motor pathways (Lee & Carroll, 2007; Starbuck & Eston, 2011). The second hypothesis is associated with the central nervous system (CNS), and suggests that motor engrams which improve performance of the trained limb, are accessible to the contralateral limb (Starbuck & Eston, 2011). This is examined as motor learning, and as such individuals learn how to create an internal representation of specific patterns of muscle recruitment and neural activity (Carroll *et al.,* 2006; Lee & Carroll, 2007). Moreover, it is suggested that strength is increased by functional changes such as, learning to inhibit the antagonist muscle or improve coordination of synergists (Lee & Carroll, 2007).

Evidence for cross-transfer is limited, and despite several studies addressing the RBE during performance, no study to date has examined both cross-transfer and the RBE during sub maximal cycling performance. Knowledge of cross-transfer during cycling exercise can be adopted as a tool in clinical and rehabilitation settings, whereby a bout of eccentric exercise in the uninjured limb may provide protection to the injured limb.

This might reduce the symptoms of detraining including atrophy and strength reductions, and provide protection for when rehabilitation exercise begins, promoting a more beneficial recovery. Therefore, the purpose of this study was to investigate if an initial bout of eccentric exercise in one leg would provide protection from the detrimental effects of EIMD on endurance performance after a second bout of eccentric exercise in the opposite leg. It was hypothesised that a repeated bout of muscle-damaging exercise would attenuate the symptoms associated with EIMD in both the ipsilateral and contralateral legs, with greater reductions in the ipsilateral leg; and subsequently a reduction in the detrimental effects on sub maximal single leg cycling exercise.

Methods

2.1 Participants

After institutional ethical approval was granted by the Faculty of Applied Sciences Research Ethics Committee, a sample of twelve healthy, recreational male and female participants were recruited for the study. Participants all engaged in regular physical activity (2-3 endurance exercise sessions per week), but had not undertaken any form of lower limb resistance exercise in the previous six months. Participant characteristics are shown in table 1. The sample size was calculated using the sample size calculator G*Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007) and an effect size of 0.47 determined from previous data on isokinetic peak torque (Burt *et al.,* 2012), alongside a power of 80% and a significance level of 0.05. All participants provided written informed consent before taking part and were asked to refrain from any strenuous exercise during the period of the study, maintain a normal balanced diet, refrain from alcohol and caffeine 24 h prior to testing and avoid using any analgesic agents.

Table 1: Participants characteristics. Values are shown as mean ± SD.

	Ipsilateral Group (n = 6)	**Contralateral Group (n = 6)**
Age (y)	20 ± 1.7	22.5 ± 1.5
Stature (m)	1.8 ± 0.05	1.75 ± 0.1
Mass (Kg)	74.8 ± 4.7	70.6 ± 10.1
$\dot{V}O_{2\,peak}$ **Dominant limb (ml·kg^{-1}·min^{-1})**	31.2 ± 8.4	29.4 ± 6.4
$\dot{V}O_{2\,peak}$ **Non-Dominant limb (ml·kg^{-1}·min^{-1})**	35.5 ± 10.3	27.8 ± 8.1
P_{max} Dominant limb (W)	177.5 ± 3.7	157.5 ± 17.5
P_{max} Non-Dominant limb (W)	177.5 ± 25.8	163.3 ± 25.8

2.2 Design

The study was a repeated measures, experimental design involving seven laboratory visits over a five week period (see Figure 1). Participants initially performed exhaustive incremental cycling trials on the dominant and non-dominant leg to establish peak oxygen uptake ($\dot{V}O_{2\,peak}$) and maximum power output (P_{max}) of each limb. This was followed by habituation to the procedures used to measure perceived muscle soreness, isometric strength and single-leg cycling exercise. At 24-72 h later participants performed baseline measurements of perceived muscle soreness, isometric strength and two bouts of single-leg cycling at 75% P_{max}. Cycling, strength and soreness data was collected from the dominant or non-dominant limb, which was randomly selected beforehand. Despite the random allocation, previous research suggests that leg dominance does not effect the magnitude of EIMD (Hody, Rogister, Leprince, Laglaine & Croisier, 2013). During the same visit, participants experienced a bout of eccentric exercise on the selected limb designed to induce muscle damage. Measurements were then repeated 24 h and 48 h after the eccentric exercise. Two weeks later, participants were randomly allocated to an ipsilateral (same leg) or contralateral (opposite leg) group. The participants in the ipsilateral group repeated the baseline measurements, the eccentric exercise and measurements at 24 and 48 h all on the same leg. Conversley, the contralateral group performed the baseline measurements, the eccentric exercise and measurements at 24 and 48 h, but this time on the opposite leg.

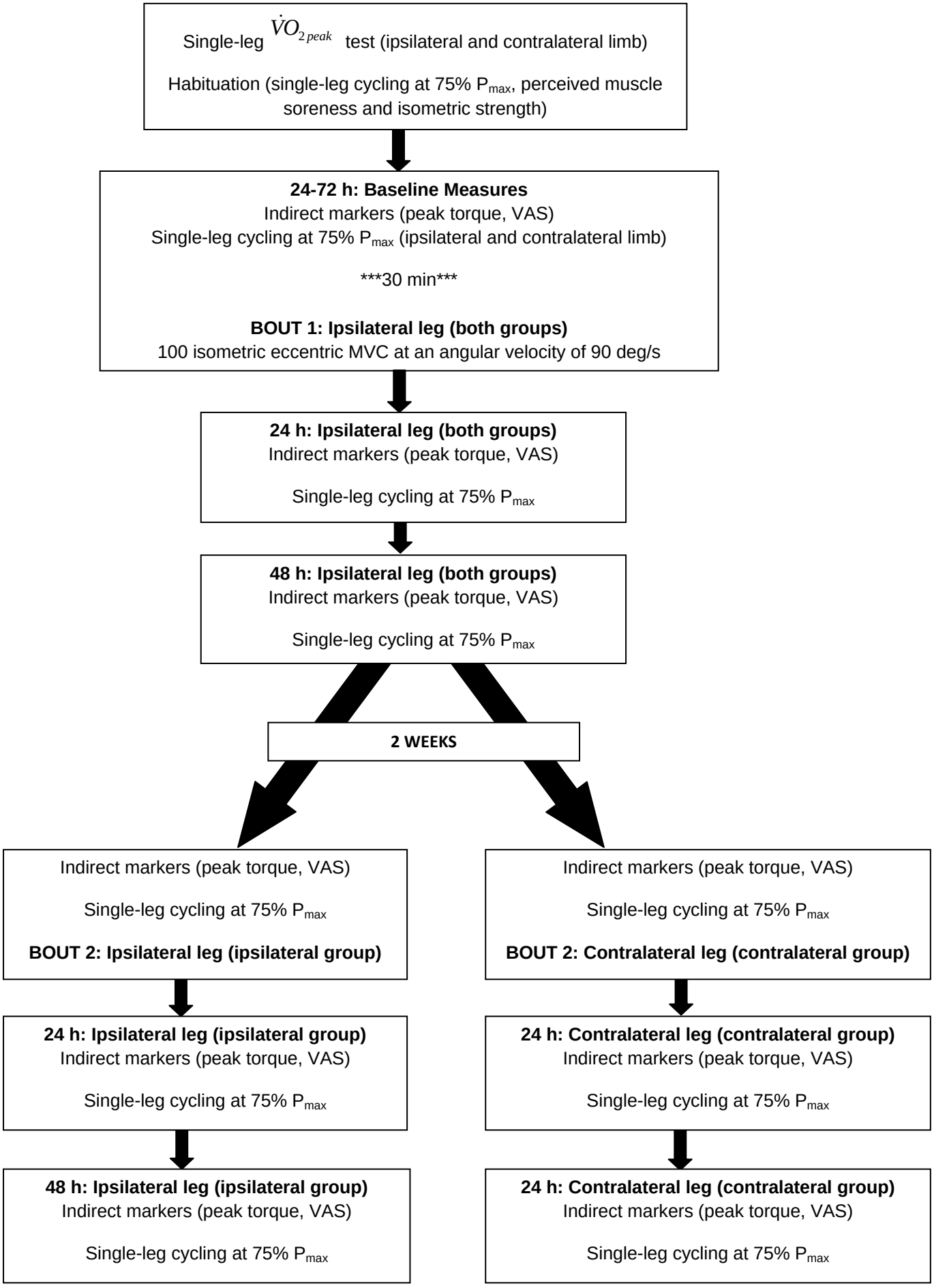

Figure 1: Schematic of experimental design

2.3 Procedures

2.3.1 Assessment of $\dot{V}O_{2\,peak}$ and P_{max}

Participants were asked to perform a ramp protocol cycle test to exhaustion on the dominant and non-dominant leg using an electronically braked cycle ergometer (Lode Excalibur Sport, Lode Medical Technology, Groningen, The Netherlands). After a 5-minute warm up at 25 W, the test commenced at an initial workload of 25 W and increased by 15 W every 30 s until volitional exhaustion. Participants were instructed to maintain a pedal cadence between 60 and 80 rpm. The test was terminated when the participant could no longer maintain the required cadence. Expired air was collected continuously throughout the test using an online metabolic system (Oxycon Pro, Hoechberg, Germany), which was calibrated according to the manufacturer's instructions prior to each test. $\dot{V}O_{2\,peak}$ was taken as the highest $\dot{V}O_2$ recorded over 30 s and P_{max} was defined as the minimal power output that elicited a $\dot{V}O_2$ reading within 2 $ml \cdot kg^{-1} \cdot min^{-1}$ of the previous reading, despite an increase in workload (Laursen, Shing, Peake, Coombes & Jenkins, 2005). Heart rates (HR), monitored via telemetry (Polar Electro, Polar Beat, Oy, Finland), was recorded in the final 15 s of each exercise bout. After a 15-minute recovery period, the procedures were repeated on the opposite leg.

2.3.2 Perceived muscle soreness

Participants were asked to indicate perceived muscle soreness in the knee extensors using a 0-10 visual analogue scale (VAS). The scale consists of written cues from left (no soreness) to right (muscles too sore to move), which corresponded to a number on the reverse side, unseen by the participant. To indicate lower body muscle soreness, participants were asked to place their hands on their hips and heels to the floor, and perform a unilateral squat to approximately 90º and move the pointer to indicate their rating of soreness on the scale. The corresponding number was accepted as the value

of perceived muscle soreness. This technique has been used successfully in previous studies (Marcora & Bosio, 2007; Twist & Eston, 2009; Burt & Twist, 2011).

2.3.3 Peak isometric torque

Peak isometric torque of the knee extensors was measured at a knee angle of 80 deg using an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA). Testing was preceded by a standardized warm-up of 3-minutes cycling at 50 W (Monark, 874E, Monark, Varberg, Sweden) followed by stretching of the knee extensor muscle group. After five sub-maximal and one maximal warm-up trial, participants performed five maximal voluntary contractions with 10 s beteen each trial. The highest value ($N \cdot m$) recorded was used for analysis.

2.3.4 Single-leg cycling protocol

The single-leg cycling protocol was performed using an electronically braked cycle ergometer (Lode Excalibur Sport, Lode Medical Technology, Groningen, The Netherlands) and required the participant to cycle for 5-minutes at a workload corresponding to 75% Pmax. Participants were instructed to maintain a pedal cadence between 60 and 80 rpm. When cycling with only one leg, the rider must typically “pull up” once the pedal reaches bottom dead centre. This requires recruitment of the less powerful and more fatigable hip flexor muscle group, which can be uncomfortable and limits the maximal exercise intensity that can be attained. Therefore, during single-leg cycling, a counterweight system (10 kg) was attached to the contralateral pedal on the cycle ergometer; this system assisted with the upward phase of the pedalling action, thus preserving normal double-leg cycling biomechanics (Abbiss *et al*., 2011). Expired air was measured continuously during each trial. HR, RPE, pedal force and cadence was recorded every minute, and Bla concentration at the end of each trial.

Measurements of $\dot{V}O_2$, $\dot{V}_E$ and breathing frequency (BF) were taken for analysis. EMG activity (see below) was recorded throughout the 5-minute period.

2.3.5 Electromyography (EMG) activity

During the single-leg cycling protocol and eccentric exercise EMG signals were recorded from bipolar surface electrodes placed over the vastus medialis (VM) and vastus lateralis (VL). The tibialis anterior was used as the reference point. This was in order to investigate any potential alterations in motor unit recruitment a) during the eccentric exercise bouts, b) as a result of muscle-damaging exercise during cycling. After palpation to locate the midline of the muscle's belly, the participant's skin was prepared using alcohol based cleaning wipes. Electrodes (Noraxon USA, Inc., Scottsdale, AZ) were then placed on the midline of the belly, whilst the muscle was contracted. The raw signal was recored via telemetry to a laptop computer. Electrode placement and preparation was based on previous work by De Luca (1997). A band pass filter from 10 to 500 Hz and a sampling rate of 1,500 Hz was applied to the data using MyoResearch XP 1.07.41 software (Noraxon USA, Scottsdale, AZ). For the amplitude of EMG activity the raw EMG data was rectified using root mean squared (RMS) averaging with a 10 ms time constant. The peak values were obtained and recorded for each bout of eccentric exercise. To compute the median frequency (MF), the raw EMG data was passed through a Hamming window and processed using Fast Fourier transformations (FFT) to provide a power density spectrum.

2.3.6 Muscle-damaging exercise

Each participant performed a bout of 100 isokinetic eccentric MVCs at an angular velocity of 90 deg/s on an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA) (Byrne, Eston & Edwards, 2001). The eccentric actions were

performed as 10 sets of 10 repetitions with 10 s rest between repetitions and 60 s between sets. EMG was recorded throughout the procedure as described above.

2.4 Statistical Analysis

The Shapiro-Wilk test was firstly used to verify the normal distribution of the data ($P > 0.05$). Independent samples *t*-tests were performed on all baseline measures between the ipsilateral and contralateral groups to ensure no significant differences existed and that the random allocation of participants, formed groups with similar baseline measures. Changes in perceived muscle soreness and isometric strength were analysed using separate three-way (Group [2] x Bout [2] x Time [3]) analyses of variance (ANOVA). Separate three-way (Group [2] x Bout [2] x Time [3]) ANOVA with repeated measures on time were calculated to assess changes to each performance variable in the sub-maximal protocol. *Post-hoc* Tukey tests were used to follow up any significant results. All statistical comparisons were performed using SPSS software (version 20, IBM SPSS, Chicago, Illinois). Where appropriate, values are reported as mean ± standard deviation (SD), and in all cases, the alpha level was initially set at $P < 0.05$.

Results

Independent samples *t*-tests on baseline values indicated that the majority of the data was normally distributed ($P > 0.05$), therefore parametric tests were adopted for statistical analysis.

3.1 Indirect markers of muscle damage

3.1.1 Perceived muscle soreness

There was a significant main effect for time [$F_{(2, 20)} = 17.273$, $P < 0.001$] which indicated that there was an increase in soreness from baseline for both groups. The main effect for bout approached statistical significance [$F_{(1, 10)} = 3.398$, $P = 0.095$], revealing that the extent of soreness after the second bout of eccentric exercise was lower compared to the first bout. There was no significant interaction of bout x group [$F_{(1, 10)} = 1.553$, $P = 0.241$], bout x time [$F_{(2, 20)} = 1.846$, $P = 0.184$] or bout x time x group [$F_{(2, 20)} = 0.189$, $P = 0.830$] (Figure 2).

3.1.2 Isometric strength

There was a significant main effect for bout [$F_{(1, 10)} = 71.799$, $P < 0.001$] indicating that the decrements after the second bout of eccentric exercise were attenuated when compared to the first bout. Changes in isometric strength were also significantly lower over time [$F_{(2, 20)} = 126.205$, $P < 0.001$]. Interestingly, there was a greater percentage increase for strength during bout 2 for the contralateral group, compared to the ipsilateral group. There was also a significant bout x time interaction for isometric strength [$F_{(2, 20)} = 103.334$, $P < 0.001$]. However, there was no significant bout x group [$F_{(1, 10)} = 1.271$, $P = 0.286$] or bout x time x group interaction [$F_{(2, 20)} = 1.904$, $P = 0.175$].

When percentage strength changes were compared to baseline values there was no

significant main effect for bout [$F_{(1, 10)} = 2.340$, $P = 0.157$], or bout x group [$F_{(1, 10)} = 0.303$, $P = 0.594$], or time [$F_{(2, 20)} = 1.528$, $P = 0.241$], or bout x time [$F_{(2, 20)} = 1.692$, $P = 0.210$] or bout x time x group [$F_{(2, 20)} = 0.494$, $P = 0.617$] (Figure 3).

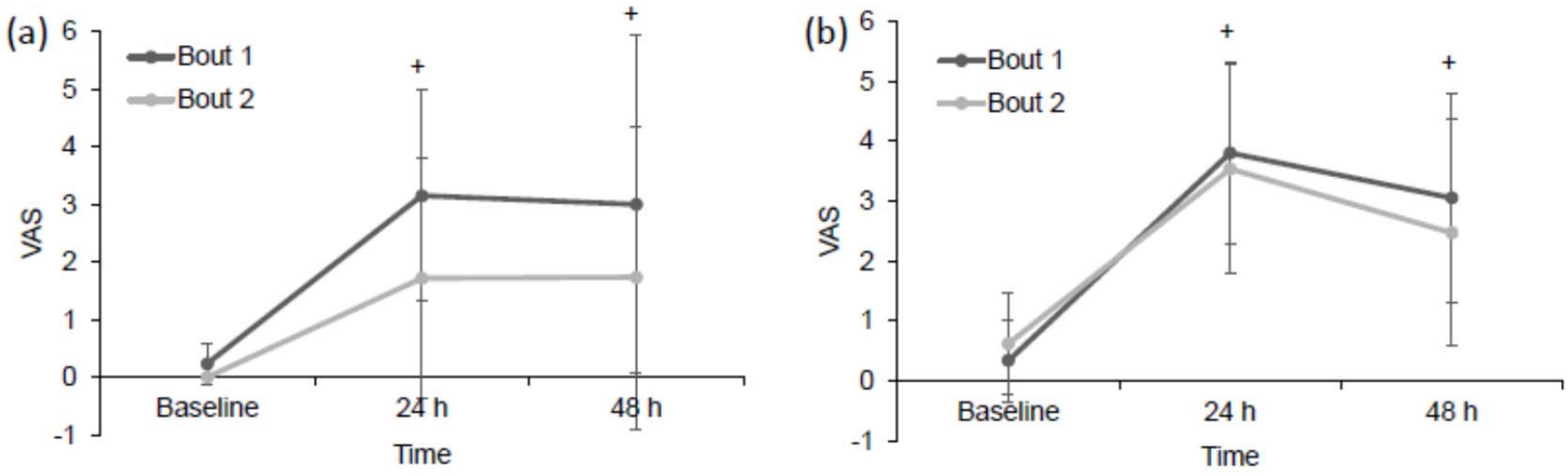

Figure 2: Mean ± SD for perceived muscle soreness after both bouts of eccentric exercise for (a) ipsilateral group, and (b) contralateral group. Soreness increased over time in both bouts after the muscle damaging protocol ($P < 0.05$). $^{+}$ Denotes significant difference to baseline.

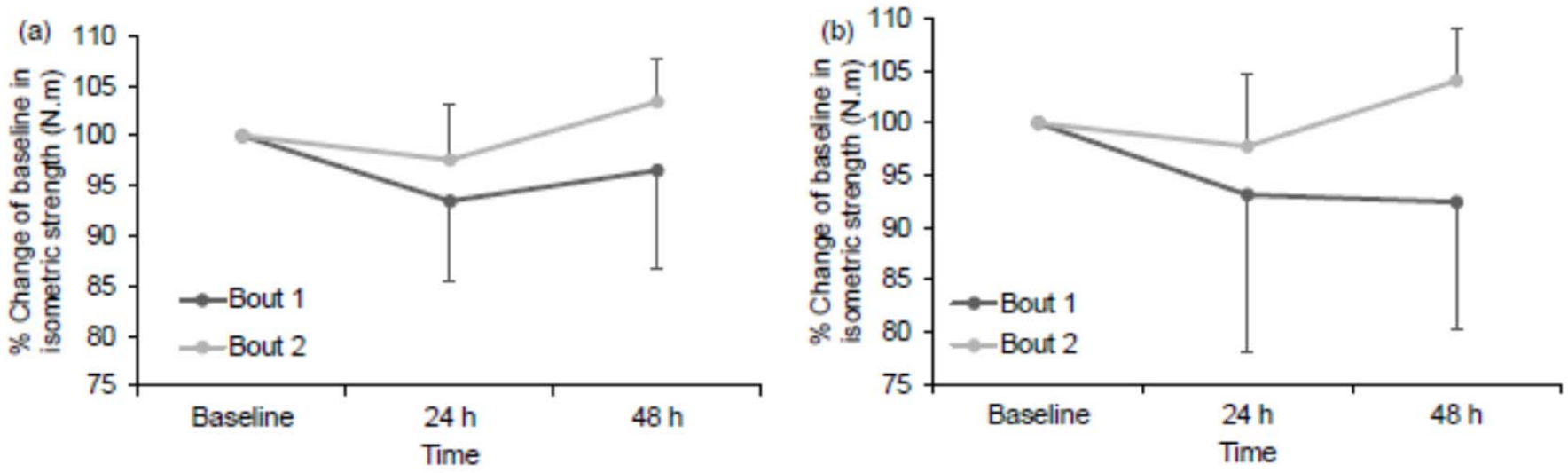

Figure 3: Mean ± SD for percentage change from baseline for isometric strength following both bouts of eccentric exercise for (a) ipsilateral group, and (b) contralateral group.

3.1.3 EMG

The EMG analysis for MF and peak amplitude during the last set of each bout of eccentric exercise is displayed in Table 2. There was no bout effect for either MF in VM or VL, [$F_{(1, 10)} = 0.042$, $P = 0.842$] or [$F_{(1, 10)} = 1.283$, $P = 0.284$], respectively. Similarly, there was no bout x group effect for MF in VM or VL [$F_{(1, 10)} = 0.044$, $P = 0.838$] or [$F_{(1, 10)} = 0.008$, $P = 0.930$], respectively. The main effect for bout approached statistically significance [$F_{(1, 10)} = 3.465$, $P = 0.092$] which revealed that the peak amplitude VM during the second bout of eccentric exercise was lower compared to the first bout of eccentric exercise. There was no significant effect of peak amplitude VM for bout x group [$F_{(1, 10)} = 0.728$, $P = 0.413$]. Similarly for peak amplitude VL there was no significant main effect for either bout [$F_{(1, 10)} = 0.058$, $P = 0.814$] or bout x group [$F_{(1, 10)} = 0.009$, $P = 0.928$].

Table 2: EMG during the last set of eccentric exercise for bout 1 and bout 2. Values are shown as mean ± SD

	Median frequency (Hz)		**Peak amplitude (mV)**	
	VM	**VL**	**VM**	**VL**
Bout 1				
Ipsilateral	63.4 ± 2.4	68.7 ± 5.0	654.5 ± 93.7	534.6 ± 83.7
Contralateral	65.1 ± 4.9	69.6 ± 5.6	745.0 ± 134.0	716.3 ± 125.3
Total	64.3 ± 1.2	69.1 ± 0.6	699.8 ± 64.0	625.4 ± 128.5
Bout 2				
Ipsilateral	64.8 ± 2.1	64.4 ± 3.6	710.8 ± 109.4	545.3 ± 69.1
Contralateral	65.0 ± 6.8	63.7 ± 6.0	930.5 ± 194.3	742.9 ± 146.8
Total	64.9 ± 0.2	64.0 ± 0.5	820.7 ± 155.3	644.1 ± 139.7

3.2 Sub-maximal single leg cycling responses to repeated bouts of eccentric exercise

There was a significant bout effect for HR [$F_{(1, 10)}$ = 4.992, P = 0.049] indicating that HR was significantly greater in bout 2 compared to bout 1. However there was no bout x time x group interaction [$F_{(2, 20)}$ = 2.424, P = 0.114]. RPE approached statistical significance for time [$F_{(2, 20)}$ = 2.938, P = 0.076] and bout x time x group [$F_{(2, 20)}$ = 3.012, P = 0.980]. There was no bout x time x group interaction observed for $\dot{V}O_2$ [$F_{(2, 20)}$ = 1.860, P = 0.182] or $\dot{V}_E$ [$F_{(2, 20)}$ = 1.473, P = 0.253]. However, there was a bout x time interaction for $\dot{V}_E$ [$F_{(2, 20)}$ = 3.640, P = 0.045], indicating that the increases that occurred at 24 h and 48 h in bout 1, did not follow a similar pattern during bout 2. There was no bout x time x group interaction for BF [$F_{(2, 20)}$ = 0.070, P = 0.933] or peak force [$F_{(2, 20)}$ = 0.593, P = 0.562]. The main bout effect for peak force approached statistical significance [$F_{(1, 10)}$ = 4.060, P = 0.072], likewise for bout x time interaction [$F_{(2, 20)}$ = 4.778, P = 0.054]. There was no bout x time x group interaction observed for cadence [$F_{(2, 20)}$ = 1.103, P = 0.351] or [Bla] [$F_{(2, 20)}$ = 1.049, P = 0.480]. All physiological data for sub-maximal single leg cycling responses to repeated bouts of muscle damage are shown in Table 3.

3.2.1 EMG during sub maximal cycling protocol

The EMG data recorded during the sub maximal cycling protocol demonstrated no bout x time x group interaction (Table 4). There was a significant bout effect for MF VL [$F_{(1, 10)}$ = 11.002, P = 0.008].The main effect for bout approached statistical significance for peak amplitude VM [$F_{(1, 10)}$ = 4.347, P = 0.064]. All raw data obtained for both groups is shown in Table 4.

Table 3: Physiological responses to sub maximal single leg cycling protocol during the final minute, following repeated bout of muscle damage. Values are shown as mean ± SD.

Variable	Group	Bout	Baseline	24 h	48 h
HR (b·min^{-1})	**Ipsilateral**	**Bout 1**	142 ± 29	145 ± 28	142 ± 27
		Bout 2	146 ± 27	152 ± 24	152 ± 22 *
	Contralateral	**Bout 1**	133 ± 12	136 ± 9	136 ± 13
		Bout 2	144 ± 21	142 ± 22	140 ± 18
RPE	**Ipsilateral**	**Bout 1**	14.2 ± 2.8	13.8 ± 3.4	13.5 ± 2.6
		Bout 2	13.67 ± 2.1	13.67 ± 2.1	13.5 ± 2.4
	Contralateral	**Bout 1**	13.8 ± 1.7	15.5 ± 1.5	15.5 ± 1.1
		Bout 2	15.7 ± 2.1	15.7 ± 2.1	15.5 ± 1.9
$\dot{V}O_2$ **(ml·min^{-1})**	**Ipsilateral**	**Bout 1**	1824.2 ± 359.7	1642.0 ± 418.9	1741.8 ± 401.7
		Bout 2	1682.8 ± 374.7	1684.8 ± 168.2	1816 ± 308.1
	Contralateral	**Bout 1**	1627.0 ± 388.2	1497.7 ± 580.5	1657.3 ± 312.2
		Bout 2	1598.8 ± 551.3	1348.8 ± 558.6	1591.3 ± 640.3
$\dot{V}_E$ **(l·min^{-1})**	**Ipsilateral**	**Bout 1**	59.7 ± 20.0	61.2 ± 25.5	60.5 ± 21.3
		Bout 2	56.0 ± 9.5	58.5 ± 13.0	57.3 ± 11.6
	Contralateral	**Bout 1**	54.8 ± 11.5	60.2 ± 10.0	57.7 ± 8.6
		Bout 2	63.7 ± 19.8	59.3 ± 14.3	62.8 ± 19.7
BF (min^{-1})	**Ipsilateral**	**Bout 1**	33.5 ± 10.1	36.7 ± 14.5	34.3 ± 12.6

		Bout 2	34.0 ± 7.7	32.0 ± 8.4	31.5 ± 7.4
	Contralateral	**Bout 1**	34.0 ± 5.1	36.7 ± 5.7	34.7 ± 4.0
		Bout 2	38.2 ± 8.8	36.2 ± 7.0	31.2 ± 8.6
Peak force (N·m)	**Ipsilateral**	**Bout 1**	95.8 ± 12.4	98.1 ± 22.5	89.3 ± 21.1
		Bout 2	96.5 ± 21.8	91.9 ± 25.6	92.5 ± 18.3
	Contralateral	**Bout 1**	67.4 ± 10.3	61.3 ± 15.6	80.5 ± 19.5
		Bout 2	92.8 ± 24.9	82.6 ± 19.7	92.6 ± 29.3
Cadence (rpm)	**Ipsilateral**	**Bout 1**	75.7 ± 7.8	77.1 ± 2.7	77.1 ± 2.5
		Bout 2	78.6 ± 1.7	78.4 ± 1.9	79.1 ± 1.4
	Contralateral	**Bout 1**	76.7 ± 3.1	74.5 ± 4.3	74.7 ± 4.5
		Bout 2	73.9 ± 4.5	73.9 ± 4.9	74.5 ± 3.8
[Bla] (mmol·l^{-1})	**Ipsilateral**	**Bout 1**	5.6 ± 2.6	5.4 ± 3.0	4.71 ± 1.5
		Bout 2	5.1 ± 1.5	5.3 ± 2.1	5.1 ± 1.4
	Contralateral	**Bout 2**	6.9 ± 2.4	5.7 ± 2.6	6.2 ± 1.4
		Bout 1	5.4 ± 1.8	5.4 ± 1.4	4.6 ± 1.1

* Denotes a significant bout effect.

Table 4: EMG during the last minute sub maximal cycling protocol, at baseline, 24 h and 48 h. Values are shown as mean ± SD.

		Group	Bout	Baseline	24 h	48 h	Bout x Time x Group Interaction
Median frequency (Hz)	**VM**	**Ipsilateral**	**Bout 1**	57.6 ± 10.6	56.4 ± 13.4	58.7 ± 12.9	$F_{(2, 20)} = 1.398$, $P = 0.270$
			Bout 2	55.4 ± 11.5	56.4 ± 9.3	54.0 ± 10.9	
	VM	**Contralateral**	**Bout 1**	53.2 ± 9.9	52.1 ± 9.6	51.9 ± 9.4	
			Bout 2	53.1 ± 9.1	54.3 ± 8.1	55.2 ± 10.6	
	VL	**Ipsilateral**	**Bout 1**	59.0 ± 18.6	52.6 ± 11.0	50.9 ± 7.3	
			Bout 2	49.6 ± 10.8	50.7 ± 10.3	49.3 ± 8.9	$F_{(2, 20)} = 0.929$, $P = 0.411$
	VL	**Contralateral**	**Bout 1**	58.1 ± 5.7	58.7 ± 6.3	59.6 ± 6.6	
			Bout 2	52.3 ± 9.2	53.4 ± 7.2	50.15 ± 8.2	
Peak amplitude (mV)	**VM**	**Ipsilateral**	**Bout 1**	543.7 ± 245.7	423.0 ± 247.9	483.7 ± 220.8	
			Bout 2	567.8 ± 267.2	592.8 ± 237.1	558.8 ± 250.0	
	VM	**Contralateral**	**Bout 1**	506.7 ± 251.2	591.0 ± 371.2	534.7 ± 464.3	$F_{(2, 20)} = 2.594$, $P = 0.100$
			Bout 2	53.1 ± 9.1	774.3 ± 674.7	755.2 ± 654.3	
	VL	**Ipsilateral**	**Bout 1**	340.7 ± 86.1	425.8 ± 319.9	390.8 ± 175.8	
			Bout 2	454.7 ± 161.2	488.2 ± 165.6	443.5 ± 211.1	

VL	**Contralateral**	**Bout 1**	418.3 ± 216.9	461.7 ± 278.1	407.3 ± 251.8	$F_{(2, 20)} = 0.567$, $P = 0.576$
		Bout 2	535.5 ± 330.7	488.2 ± 371.7	527.7 ± 399.4	

Discussion

In conjunction with previous research adopting eccentric exercise to induce muscle damage (Byrne *et al.,* 2001; Paulsen *et al.,* 2005), this study demonstrated that an initial bout of eccentric exercise increased perceived muscle soreness, and decreased isometric strength. There was a significant increase in muscle soreness over 48 h for both groups, with results approaching statistical significance suggesting that perceived muscle soreness was generally lower following the second bout of eccentric exercise. However, there were no comparable differences between groups. This study observed a ~4-8% decrease in isometric strength during the 48 h after bout 1, for both the ipsilateral and contralateral groups. Following a second bout of eccentric exercise, the magnitude of force loss observed in the ipsilateral and contralateral groups, was ~3%. The relatively small reductions in force-generating capacity (< 20%), suggests mild EIMD (Paulsen, Mikkelsen, Raastad & Peake, 2012). These results provide further evidence that an initial bout of eccentric exercise attenuates the symptoms association with EIMD, when the same bout of exercise is repeated (McHugh, 2003). However, in accordance with previous research (Howatson & van Someren, 2007; Starbuck & Eston, 2011), there was no notable difference between groups to provide evidence of a cross-transfer effect. Interestingly, the contralateral group exhibited a ~4% increase in isometric strength during the second bout, despite no significant differences between baseline measures. Previous research has documented different motor control strategies employed by dominant and non-dominant limbs, may impact the cross-transfer effect (Ferreira, Pereira, Hackney & Machado, 2012; Pereira, Freire, Cavalcanti, Luz & Neto, 2012). Research by Farthing (2009) suggests that greater cortical adaptations are shared between limbs, when the dominant limb is used for exercise in the first instance. In the present study, limb dominance was randomly

assigned, as recent work by Hody et al. (2013) suggest that limb dominance does not affect the magnitude of EIMD; however, it is plausible that the damage protocol did not sufficiently damage participants enough to elicit a marked difference between the ipsilateral and contralateral groups (Hody *et al.,* 2013). It is possible that limb dominance explains the large variability in cross-transfer, due to preferential skill transfer (Farthing, 2009); similarly, insufficient habituation may have also been a limiting factor – although all participants were familiarised with the protocol beforehand, the 2 week dissemination period between bouts, might have been long enough to induce a learning effect during the second bout. This might explain why the contralateral group improved their isometric strength by ~4% compared to baseline.

Furthermore, EMG was used to provide further understanding of the potential neural adaptations during the two bouts of eccentric exercise. Although not statistically so, there was an ~11% increased peak amplitude for the total VM during the second bout for both groups. This suggests an increase in motor unit recruitment, potentially to reduce the stress placed on individual motor units, and attenuate the effects of EIMD during the second bout of eccentric exercise (McHugh, 2003); thus supporting evidence for a RBE in the ipsilateral group. The pattern of changes for perceived soreness, isometric strength and peak amplitude observed in the contralateral group suggest a possible cross-transfer (Howatson & van Someren, 2007). Although not statistically significant, this pattern of response may be hindered by the large variability between participants, due to their different training backgrounds. Despite being calculated to achieve a significance, the small sample size may also have influenced these results.

In addition, this study investigated the effects of repeated bout of eccentric exercise on sub maximal cycling – the majority of physiological and perceptual responses, were unaltered following the eccentric exercise; however, there was a significant increase in

HR during bout 2 compared to bout 1. This is surprising as there were no further findings to suggest an increase in effort; moreover, results show a protective effect over the muscle, so lower heart rates would have been expected. This could be explained by inter-day variability, yet this is surprising. Furthermore, RPE was elevated following the initial bout of muscle-damaging exercise within both groups: after the second bout of eccentric exercise, perceived exertion was attenuated in the ipsilateral group, whilst there was a statistical difference compared to the contralateral limb. These findings are consistent with previous research (Elmer *et al.,* 2011; Burt *et al.,* 2012) which attribute the increase in perceived effort to the increased motor unit recruitment.

The EMG data recorded during the sub maximal cycling, demonstrated (although not significant) an increase for peak amplitude VM during the second bout of exercise, supporting this statement. This response in motor unit recruitment may have provided a cue to maintain or even better the baseline peak force achieved prior to the muscle-damaging exercise (Elmer *et al.,* 2011; Burt *et al.,* 2012); this would seem plausible considering there were no significantly different alterations in cadence, yet peak force approached statistical significance between bouts. As expected, $\dot{V}_E$ increased at 24 h following the eccentric exercise which is consistent with previous findings (Davies *et al.,* 2009; Burt *et al.,* 2012). This is modulated by group III and IV afferent fibres around the muscle, and nociceptive muscle afferents which respond to the increased muscle soreness (Haouzi, Chenuel & Huszczuk, 2004). Following the second bout of eccentric exercise, $\dot{V}_E$ decreased in the ipsilateral group, suggesting a quicker recovery second time around, namely being the RBE, which is similar to previous research (Burt *et al.,* 2012). Interestingly, $\dot{V}_E$ increased in the contralateral limb during the second bout, which may have been influenced by limb dominance and not a cross-transfer effect (Farthing, 2009).

Previous research has documented a reduced MF following a repeated bout of muscle-damaging exercise (Howatson, van Someren & Hortobagyi, 2007; Starbuck & Eston, 2011), highlighting an increased reliance on type I muscle fibres, after preferential damage to type II fibres (Fridén, Sjostrom & Ekblom, 1983; Warren, Hermann, Ingalls, Masselli & Armstrong, 2000). By using surface EMG, this study identified a significant reduction in MF VL across both groups, following the second bout of eccentric exercise. This could have protected the muscle from further damage and enabled normal motor unit recruitment (Chen, 2003; Burt *et al.,* 2012), hence why there was no statistical reduction between bouts, but actually improvements in the contralateral group. This furthers signifies a RBE in the ipsilateral group. Several theories have been hypothesised regarding the mechanism of cross-transfer (Connolly *et al.,* 2002); however, it is plausible from the EMG results, that cross-transfer is attributable from neural mechanisms. This theory is based on the knowledge that type II fibres are damaged in the initial bout of exercise (Fridén *et al.,* 1983; Connolly *et al.,* 2002), and is further supported by the EMG data obtained within this study. EMG demonstrated an increase in motor unit recruitment within both groups, however, the large variability may have accounted for it not being statistically so.

Whilst the study was powered on previous isokinetic peak torque data (Burt *et al.,* 2012), the author recognises that the results are based on a relatively small sample size. Similar studies have also used a relatively small sample size (Starbuck & Eston, 2011), however, the variability between participants as a result of their different training background, might be the reason why not all results approached statistical significance, as expected, despite the notable patterns of response.

It is evident that a greater protection is provided through the RBE, however, the patterns of response suggest that some protection is provided through cross-transfer, providing a contralateral protection. Therefore, this tool could be adopted in a

rehabilitation setting to reduce the symptoms of detraining, and to reduce the extent of EIMD following resistance training, but it appears not to effect the physiological responses during sub maximal cycling.

Conclusion

This was the first study to the author's knowledge to examine both the RBE and cross-transfer effect of the lower limbs, during sub maximal exercise. The adaptation that occurred in the ipsilateral limb, attenuated the magnitude of EIMD symptoms, however, this study did not display any alteration to sub maximal cycling in the days after a repeated bout of unaccustomed eccentric exercise. However, it did attenuate the detrimental effects of EIMD on perceived soreness and isometric strength, reaffirming the likely explanation of the RBE. Surface EMG supports the neural of cross-transfer, after both groups demonstrated increase in peak amplitude; as expected this adaptation was greater in the ipsilateral group, providing a greater protective effect as consequence of the RBE. The primary findings from this study, reemphasise that populations engaging in resistance exercise for the first time using a single limb, need to be aware of the negative effects this has on muscle function in the days following; thereafter, symptoms of EIMD will be attenuated following a repeated bout of resistance exercise. Furthermore, the secondary finding from this study identified that through neural mechanisms, there are changes to the contralateral limb which provide some protection to repeated bouts of eccentric exercise. However, this did not appear to statistically effect endurance exercise.

This area of research warrants further investigation, and future work should consider different damage protocols to induce a greater magnitude of damage. Future work should also wish to consider recruiting participants from a similar athletic population.

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Appendices

Appendix 1: Ethical approval
Appendix 2: Participant information sheet
Appendix 3: Participant informed consent
Appendix 4: Health questionnaire
Appendix 5: Exercise-induced muscle damage: symptoms and advice
Appendix 6: Data collection sheets
Appendix 7: SPSS raw data, EMG data and SPSS output file (CD)

Appendix 1

Faculty of Applied Sciences
Research Ethics Committee
frec@chester.ac.uk

Laura Wade

22nd April 2013

Dear Laura,

Study title: **The effects of repeated bouts of muscle damaging exercise on the physiological and perceptual responses during single-leg cycling: evidence for cross-transfer during endurance exercise.**
FREC reference: **773/13/LW/SES**
Version number: **2**

Thank you for sending your application to the Faculty of Applied Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	March 2013
Appendix 1 – List of References	1	March 2013
Appendix 2 – C.V. for Lead Researcher	1	March 2013
Appendix 3 – Participant Information Sheet	1	March 2013
Appendix 4 – Participant Consent Form	1	March 2013
Appendix 5 – Advertisement Material	1	March 2013
Appendix 6 – Risk Assessment Form	1	March 2013
Appendix 7 – Pre-health Questionnaire	1	March 2013
Appendix 8 – Details of Protocols	1	March 2013
Appendix 9 – Symptoms of Muscle Damage and Advice	1	March 2013
Response to FREC request for further information and clarification		April 2013
Application Form	2	April 2013

Appendix 1 – List of References	1	April 2013
Appendix 2 – C. V. for Lead Researcher	1	April 2013
Appendix 3 – Participant Information Sheet	2	April 2013
Appendix 4 – Participant Consent Form	2	April 2013
Appendix 5 – Advertisement Material	2	April 2013
Appendix 6 – Risk Assessment Form	2	April 2013
Appendix 7 – Pre-health Questionnaire	2	April 2013
Appendix 8 – Details of Protocols	1	April 2013
Appendix 9 – Symptoms if Muscle Damage and Advice	2	April 2013
Appendix 10 – Schematic of Experimental Design	1	April 2013

With the Committee's best wishes for the success of this project.

Yours sincerely,

Prof. Cynthia Burek
Acting Chair, Faculty Research Ethics Committee

Enclosures: Standard conditions of approval.

Cc. Supervisor/FREC Representative

University of Chester

Appendix 2

Participant Information Sheet

The effects of repeated bouts of muscle damaging exercise on the physiological and perceptual responses during single-leg cycling: evidence for cross-transfer during endurance exercise.

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask me if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?
The purpose of this study is to investigate if an initial bout of exercise in one leg will provide protection from the detrimental effects of exercise-induced muscle damage on endurance performance after a second bout of exercise in the opposite leg.

Why have I been chosen?
You have been chosen because you fit the criteria required (aged 18-30), engage in regular physical activity (2-3 endurance exercise sessions per week) and have not engaged in any form of lower limb resistance exercise in the past 6 months.

Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to change your mind or withdraw at any time and without giving a reason. This will not affect the standard of treatment or care you receive.

What will happen to me if I take part?
You will be required to visit the exercise laboratory on seven different occasions over the course of five weeks. The first visit will consist of two incremental single-leg cycling tests (using the dominant and then the non-dominant leg), which will require you to cycle until you reach a point of exhaustion. This will then be followed by a habituation session to the procedures which will be used to measure perceived muscle soreness, strength and single-leg cycling at 75% of your peak power. At 24-72 hours later you will be asked to provide, feelings of muscle soreness and measurements of muscle strength, before completing two bouts of single leg cycling. During the same visit you will also undergo a bout of lower limb exercise designed to cause muscle damage. The creatine kinase, feelings of muscle soreness, strength and single leg cycling will then be repeated 24 and 48 hours later. Two weeks later, you will be asked to return to the laboratory and will be randomly allocated to the “same leg” or the “opposite leg” group. You will then be asked to repeat the baseline measures, lower limb exercise and 24 and 48 hour measurements on the leg, dependent to the group you are in. You will be asked to refrain from any strenuous exercise 24 h prior to each visit, maintain your normal diet, and avoid using any analgesic agents.

What are the possible disadvantages and risks of taking part?
You will experience a short bout of muscle soreness as a consequence of the resistance exercise. This will be most evident approximately 48 hours following the muscle-damaging exercise, after which symptoms will ease and will have disappeared by approximately one week later. These symptoms are common in all exercising populations, particularly following a bout of unaccustomed exercise and have no lasting effect.

What are the possible benefits of taking part?
By taking part in this study, you should be protected from symptoms of muscle damage for approximately six months following other subsequent bouts of muscle-damaging exercise.

What if something goes wrong?
If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Applied and Health Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055..

Will my taking part in the study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research will have access to such information.

What will happen to the results of the research study?
The results will be written up into a dissertation for my final project of my MSc. Individuals who participate will not be identified in any subsequent report or publication.

Who is organising and funding the research?
The research is conducted as part of a MSc in Sports Science (Physiology) within the Department of Sport and Exercise Sciences at the University of Chester. The study is organised with supervision from the department, by Laura Wade, an MSc student.

Who may I contact for further information?
If you would like more information about the research before you decide whether or not you would be willing to take part, please contact:

Name: Laura Wade
University E-mail: @chester.ac.uk

Thank you for your interest in this research.

University of Chester

Appendix 3

Participant Informed Consent

The effects of repeated bouts of muscle damaging exercise on the physiological and perceptual responses during single-leg cycling: evidence for cross-transfer during endurance exercise.

Name of Researcher: Laura Wade (University of Chester)

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my care or legal rights being affected. ☐
3. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Researcher	Date	Signature

(1 for researcher; 1 for participant)

University of Chester

Participant Informed Consent

The effects of repeated bouts of muscle damaging exercise on the physiological and perceptual responses during single-leg cycling: evidence for cross-transfer during endurance exercise.

Name of Researcher: Laura Wade (University of Chester)

Please initial box

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3. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Researcher	Date	Signature

(1 for researcher; 1 for participant)

University of Chester

Appendix 4

PRE-TEST HEALTH QUESTIONNAIRE
(PLEASE NOTE THAT THIS INFORMATION WILL BE CONFIDENTIAL)

Name:…… Age:……

Resting blood pressure (mmHg):...../..... Resting heart rate ($b \cdot min^{-1}$):.....

Project Title: **The effects of repeated bouts of muscle damaging exercise on the physiological and perceptual responses during single-leg cycling: evidence for cross-transfer during endurance exercise.**

Inclusion criteria: Participants must be aged between 18-30 and must all be engaging in regular physical activity (2-3 endurance exercise sessions per week).

Exclusion criteria: Participants will be excluded from the study if they have engaged in lower limb resistance exercise in the previous 6 months or if they are pregnant.

Please answer these questions truthfully and completely. The purpose of this questionnaire is to ensure that you are fit and healthy enough to participate in this research project.

	Yes	**No**
1. Have you in the past suffered from a serious illness or accident. If Yes, please provide details	☐	☐

……
……

	Yes	**No**
2. Have you consulted your doctor the last 6 months If Yes, please provide details	☐	☐

……
……

3. Do you suffer, or have you suffered from:

	Yes	**No**
Asthma	☐	☐
Diabetes	☐	☐
Bronchitis	☐	☐
Epilepsy	☐	☐
High blood pressure	☐	☐

	Yes	No
4. Is there any history of heart disease in your family	☐	☐

	Yes	No
5. Are you suffering from any infectious skin diseases, sores, blood wounds, or infections i.e., Hepatitis B, HIV, etc.?	☐	☐

If Yes, please provide brief details

……
……

	Yes	No
6. Are you currently taking any medication	☐	☐

If Yes, please provide details

……
……

	Yes	No
7. As far as you are aware, are you pregnant?	☐	☐

	Yes	No
8. Are you suffering from a disease that inhibits the sweating process	☐	☐

	Yes	No
9. Is there anything to your knowledge that may prevent you from participating in the testing that has been outlined to you?	☐	☐

If Yes, please provide details

……
……

Your Recent Condition

	Yes	No
• Have you eaten in the last 2 hours?	☐	☐

If Yes, please provide details

……
……

	Yes	No
• Have you consumed alcohol in the last 24hr	☐	☐

	Yes	No
• Have you had any kind of illness or infection in the last 2 weeks	☐	☐

	Yes	No
• Have you exercised in the last 2 days?	☐	☐

If Yes, please describe below
……
……

	Yes	No
• Have you taken part in any lower body resistance training in the past 6 months?	☐	☐

If Yes, please describe below
……
……
……

Persons will not be permitted to take part in any experimental testing if they:

- have a known history of medical disorders (i.e. hypertension, heart or lung disease)
- have a fever, suffer from fainting or dizzy spells
- are currently unable to train because of a joint or muscle injury
- have had any thermoregulatory disorder
- have gastrointestinal disorder
- have a history of infectious diseases (i.e. HIV or Hepatitis B)
- have, if pertinent to the study, a known history of rectal bleeding, anal fissures, haemorrhoids or any other similar rectal disorder.

My responses to the above questions are true to the best of my knowledge and I am assured that they will be held in the strictest confidence.

Name: (Participant)…… Date:…….

Signed (Participant): ……

Name: (Researcher)…… Date:…….

Signed (Researcher): ……..

Appendix 5

Exercise-Induced Muscle Damage: Symptoms and Advice

Symptoms

Due to the muscle damaging exercise it is normal to feel the following symptoms peak at around 48 hours:

- Swelling
- Soreness
- Stiffness
- Aching
- Decreased range of movement around the joint

Advice

These symptoms are normal and expected from muscle damaging exercise. It is important that you **do not** take anti-inflammatory drugs such as aspirin or ibuprofen, or ice the affected area as this will impact the data collected. These symptoms should gradually ease and be disappear in 1-2 weeks.

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Appendix 6

Athletes Profile

Group (Ipsilateral/Contralateral?):.....

Name…… Age …….. D.O.B. ……..

Stature (m)……..... Mass (Kg)……

BioDex Measurements

Description	Knee Measurement
Biodex Width	
Biodex Height	
Seat Height	
Seat Depth	
Back Support Depth	
Leg Length	

Assessment of VAS, CK & Muscle Strength

	Baseline	24 h	48 h	Baseline	24 h	48 h
VAS						
KNEE EXTENSOR Peak Torque 80 deg·s^{-1}(N-M)						

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Assessment of $\dot{V}O_{2peak}$ and P_{max}

Name:……..

Pressure (mmHg):…….. Room Temp (^{0}C):……. Humidity (%):…….

Ramp test to exhaustion
Dominant Leg

Stage	Heart Rate ($b \cdot min^{-1}$)
25 W	
40 W	
55 W	
70 W	
85 W	
100 W	
115 W	
130 W	
145 W	
160 W	
175 W	
190 W	
205 W	
220 W	
235 W	
250 W	
265 W	
280 W	
295 W	
310 W	

Post exercise blood Lactate ($mmol \cdot l^{-1}$)……..

Time to exhaustion (min)……

VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)……

HR_{max} ($b \cdot min^{-1}$)……

Pmax (W)……
75% Pmax (W)……

Non - Dominant Leg

Stage	Heart Rate ($b \cdot min^{-1}$)
25 W	
40 W	
55 W	
70 W	
85 W	
100 W	
115 W	
130 W	
145 W	
160 W	
175 W	
190 W	
205 W	
220 W	
235 W	
250 W	
265 W	
280 W	
295 W	
310 W	

Post exercise blood Lactate ($mmol \cdot l^{-1}$)……..

Time to exhaustion (min)……

VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)……

HR_{max} ($b \cdot min^{-1}$)……

Pmax (W)……

75% Pmax (W)……

Single Leg Cycling Protocol

Group (Ipsilateral/Contralateral?):.....

Baseline

Name:……..
Pressure (mmHg):…….. Room Temp (^{0}C):……. Humidity (%):…….

75% Pmax:.....
Leg:.....

Time (Min)	HR (b·min^{-1})	RPE	Cadence	Average VO_2	VE (l.min^{-1})	BF
1						
2						
3						
4						
5						

Immediately Post-Exercise Lactate (mmol·l^{-1}): _____

	Visit 1
Average Cadence	
Peak Force (N-m)	
Average Force (N-m)	

24 h

Pressure (mmHg):…….. Room Temp (^{0}C):……. Humidity (%):…….

75% Pmax:.....
Leg:.....

Time (Min)	HR ($b \cdot min^{-1}$)	RPE	Cadence	Average VO_2	VE ($l.min^{-1}$)	BF
1						
2						
3						
4						
5						

Immediately Post-Exercise Lactate ($mmol \cdot l^{-1}$): _____

	Visit 1
Average Cadence	
Peak Force (N-m)	
Average Force (N-m)	

48 h

Pressure (mmHg):…….. Room Temp (^{0}C):……. Humidity (%):…….

75% Pmax:.....
Leg:.....

Time (Min)	HR ($b \cdot min^{-1}$)	RPE	Cadence	Average VO_2	VE ($l.min^{-1}$)	BF
1						
2						
3						
4						
5						

Immediately Post-Exercise Lactate ($mmol \cdot l^{-1}$): _____

	Visit 1
Average Cadence	
Peak Force (N-m)	
Average Force (N-m)	

Single Leg Cycling Protocol

Group (Ipsilateral/Contralateral?):.....

Baseline

Name:……..
Pressure (mmHg):…….. Room Temp (^{0}C):……. Humidity (%):…….

75% Pmax:.....
Leg:.....

Time (Min)	HR ($b \cdot min^{-1}$)	RPE	Cadence	Average VO_2	VE ($l.min^{-1}$)	BF
1						
2						
3						
4						
5						

Immediately Post-Exercise Lactate ($mmol \cdot l^{-1}$): _____

	Visit 1
Average Cadence	
Peak Force (N-m)	
Average Force (N-m)	

24 h

Pressure (mmHg):…….. Room Temp (^{0}C):……. Humidity (%):…….

75% Pmax:.....
Leg:.....

Time (Min)	HR ($b \cdot min^{-1}$)	RPE	Cadence	Average VO_2	VE ($l.min^{-1}$)	BF
1						
2						
3						
4						
5						

Immediately Post-Exercise Lactate ($mmol \cdot l^{-1}$): _____

	Visit 1
Average Cadence	
Peak Force (N-m)	
Average Force (N-m)	

48 h

Pressure (mmHg):…….. Room Temp (^{0}C):……. Humidity (%):…….

75% Pmax:.....
Leg:.....

Time (Min)	HR ($b \cdot min^{-1}$)	RPE	Cadence	Average VO_2	VE ($l.min^{-1}$)	BF
1						
2						
3						
4						
5						

Immediately Post-Exercise Lactate ($mmol \cdot l^{-1}$): _____

	Visit 1
Average Cadence	
Peak Force (N-m)	
Average Force (N-m)	

Appendix 7