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THE REPEATED BOUT EFFECT CAN OCCUR WITHOUT MECHANICAL AND NEUROMUSCULAR CHANGES AFTER A BOUT OF ECCENTRIC EXERCISE

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Abstract

Changes in muscle fascicle mechanics have been postulated to underpin the repeated bout effect (RBE) observed following exercise-induced muscle damage (EIMD). However, in the medial gastrocnemius (MG), mixed evidence exists on whether fascicle stretch amplitude influences the level of EIMD, thus questioning whether changes in fascicle mechanics underpin the RBE. An alternative hypothesis is that neural adaptations contribute to the RBE in this muscle. The aim of this study was to investigate neuromechanical adaptations during and after repeated bouts of a highly controlled muscle lengthening exercise that aimed to maximise EIMD in MG. Twenty subjects performed two bouts of 500 active lengthening contractions (70% of maximal activation) of the triceps surae, separated by 7 days. Ultrasound constructed fascicle length-torque (L-T) curves of MG, surface electromyography (EMG), maximum torque production and muscle soreness were assessed before, 2 hours and 2 days after each exercise bout. The drop in maximum torque (4%) and the increase in muscle soreness (24%) following the repeated bout were significantly less than following the initial bout (8% and 59% respectively), indicating a RBE. However, no shift in the L-T curve, nor changes in EMG parameters were present. Furthermore, muscle properties during the exercise were not related to the EIMD or RBE. Our results show that there are no global changes in gastrocnemius mechanical behaviour or neural activation that could explain the observed RBE in this muscle. We suggest that adaptations in the non-contractile elements of the muscle are likely to explain the RBE in the triceps surae.

(250/250 words)

Introduction

Exercise-induced muscle damage (EIMD) often occurs after the performance of an unaccustomed bout of exercise that contains eccentric (lengthening) contractions. The effects of EIMD typically include muscle soreness that peaks 24–48 hr post-exercise and a sustained decrease in the force producing capacity of the muscle [1]. Although the in vivo mechanisms

underpinning the mechanical adaptations following EIMD are still debated, mechanical factors such as stretch amplitude [2–4], intensity of contraction [5], and the number of contractions [6], have all been shown to alter the degree of EIMD and particularly force loss after an initial bout of exercise. However, the response also seems to be muscle dependent, with lower limb muscles less susceptible to damage under similar loading conditions than upper limb muscles [7,8]. Regardless of the stimulus that induces EIMD, it seems that neuro-muscular adaptations after damage afford protection from the effects of muscle damage in subsequent bouts of similar exercise; known as the repeated bout effect (RBE) [9,10]. There are several suggested mechanisms for the RBE, ranging from sarcomerogenesis (addition of serial sarcomeres in fibres) [1,11], changes in connective tissue composition [12,13], and changes in neural control [10,14,15]. While it is uncertain whether one or a combination of these mechanisms are responsible for the RBE, it is clear is that the RBE is a multifactorial phenomenon which is likely to be highly muscle and exercise specific.

Recent investigations into muscle damage in the human gastrocnemius muscle have shown that this muscle is relatively resilient to muscle damage, most likely due to the fact that the muscle is protected from excessive stretch by its long and compliant Achilles tendon [16,17]. However, there is recent conflicting evidence as to what factors lead to force loss due to EIMD in this muscle group. While the amplitude of fascicle stretch is most likely related to stretch amplitude in isolated muscle fibres [2], for the most part the gastrocnemius primarily acts on the ascending limb of its length-tension relationship [16], where it is likely to be less susceptible to fibre damage [2]. In vivo studies on the human medial gastrocnemius (MG) have shown mixed evidence as to whether the stretch amplitude of the fascicles (measured using ultrasound imaging) has an effect on overall EIMD. Hoffman et al. [16], employed a backward-downhill walking protocol (1 hr) to induce EIMD and found no relationship between the final length to which the muscle fascicles were stretched and the presence or amount of muscle damage incurred. By contrast, Guilhem et al. [4] suggested that the amount of strain elicited by 10 sets of 30 maximal isokinetic eccentric contractions of plantar flexors, had a strong linear influence on the amount of drop in maximum voluntary torque found immediately after the exercise. There is a clear difference in the protocols used to induce EIMD which might account for the different results. In addition, while the Guilhem et al. study suggested that larger amplitude stretches would elicit larger force drops, they did not relate torque drops to optimum fascicle lengths and their MVC torque results immediately

post-exercise may be influenced by muscle fatigue [5]. Thus, the relationship between fascicle strain and EIMD during a bout of exercise requires further investigation.

Although the gastrocnemius, and indeed soleus, muscles appear less susceptible to EIMD than upper body muscles, these muscles have been shown to adapt after an initial bout of exercise and hence confer the RBE. For instance, the triceps surae muscle group as a whole shows reduced force loss and attenuated muscle soreness following subsequent bouts of eccentric exercise [17]. The previous study by Hoffman et al.[17], did not demonstrate a shift in the optimal length of the gastrocnemius muscle in the week following the initial damaging bout with no apparent difference in the fascicle strains experienced during the exercise. In addition there was no change in the operating length of the fascicles during the repeated bout of exercise. This suggests that adaptations other than whole scale sarcomerogenesis might be at play in protecting the muscle from damage in subsequent bouts. However, the strains experienced during the exercise in this study (backward walking) were relatively low (~10% optimum length), and involved variable muscle activation, different joint kinematics and diverse fascicle stretch in every stride. While some studies suggest that in vastus lateralis a lesser fascicle strain and earlier tendon elongation during the exercise are associated with the RBE[18], this may not be true for the gastrocnemius, due to its tendons capacity to buffer fibre elongation [17]. Overall, the muscle properties during the exercise bout that are related with EIMD and RBE are still not well understood.

At the neural level, reports of decreased frequency parameters without a significant change in signal amplitude in the elbow flexor surface electromyogram (EMG) have been interpreted as a sign that reduced activity of fast-twitch motor units during the second eccentric bout might contribute to the RBE [14,19]. However, these findings have not been found in lower limb muscles like the hamstrings [20]. It is possible that adaptations in the sarcolemma, resulting in a slowing of action potential conduction velocity, may explain the altered spectral frequency characteristics in the elbow flexors rather than specific damage to its fast-twitch fibres. To our knowledge, there are no studies that have investigated the neural adaptations related to the RBE in the human gastrocnemius muscle.

To better understand the mechanics of EIMD and the RBE in the gastrocnemius muscle, we employed here a highly controlled eccentric contraction protocol over a relatively large range of joint motion to examine the response of the neuro-muscular system to the

repeated bout. Further, because mechanical adaptations that underpin the RBE may occur during the exercise bout[18] (e.g. changes in fascicle strain or operating length range), we will explore muscle properties during the damaging protocol. Therefore, the aim of this study was to quantify any perceptual, mechanical and neural changes that occur as a result of two separated bouts of highly controlled eccentric exercise in the MG muscle. A second aim was to explore any mechanical and neural changes that occur during the stretch phase of the exercise task in repeated bouts. We combined ultrasound imaging and supramaximal tibial nerve stimulation to create fascicle length–torque curves after each exercise bout. Further, our ultrasound measurements and electromyographic recordings were combined to explore the controlled isokinetic exercise bouts. We hypothesised that MG would be protected from damage for the repeated bout of exercise, and that this would be driven by changes in either mechanical or neural adaptations or behaviour of the muscle during the damaging task.

Materials and methods

Subjects

Based on being able to detect a drop of 10% in the maximum voluntary contraction (MVC) torque [21], which is equivalent to a medium effect size (d=0.5), a sample size of 15 subjects was required (α=0.05 and β=0.20). Twenty healthy participants (11 male, age 25 ± 3 yrs, mass 71.8 ± 6 kg, height 174 ± 5 cm; 9 female, age 25 ± 4 yrs, mass 58.4 ± 6 kg, height 164 ± 7 cm) volunteered to participate in the study and provided written, informed consent. Participants were excluded if they had any pre-existing lower limb injuries, had surgery on the lower limb within the past five years and had undertaken eccentric training exercises specific to the calf muscle group (e.g. heel drop exercises) within 6 months prior to the first testing session. The protocol was approved by the local university ethics committee and conducted according to the Declaration of Helsinki.

Experimental protocol

Fascicle length-torque (L-T) data from the MG and indirect markers of muscle damage from the triceps surae were collected before (PRE), 2 hours (2H) after, and 2 days (2D) after each bout (two bouts in total) of eccentric exercise. These exercise bouts were separated by seven days, and each bout consisted of 500 voluntary, isolated and controlled,

lengthening contractions of the triceps surae muscles performed on an isokinetic dynamometer (Humac Norm, CSMi Computer Sports Medicine, Stoughton, USA). With the participant attached to the dynamometer and lying in the prone position, the exercise cycle consisted of an initial isometric plantar flexion contraction to reach a target torque level, followed by a series of rapid eccentric contractions driven by the dynamometer at an ankle angular velocity of 50°/s (Figure 1). The target torque level was determined prior to the exercise as the maximum torque obtained during a supramaximal electrical stimulation of the triceps surae at the most plantar flexed joint position, which was approximately 70% of a maximum voluntary plantar flexion contraction at the same joint angle. Participants received approximately 30 s of rest between every cycle of 25 contractions to minimise the effects of fatigue. Verbal encouragement and visual feedback of the torque they were generating was given consistently across subjects to ensure they maintained the required torque output across the set.

Muscle mechanical properties

We used an adapted version of the peripheral nerve stimulation protocol outlined in the papers by Hoffman and colleagues [16,21] to examine the L-T relationship of MG. Subjects lay prone with their right knee extended to ~175° and their right foot tightly attached to a footplate connected to the same dynamometer as described above. At the beginning of each L–T data collection session, MG fascicle length was measured during a single passive rotation at constant velocity through the full range of ankle motion. This provided a reference length relative to passive torque to ensure consistency in the length measurements across tracked ultrasound trials. A constant-current stimulus (500 μs width; DS7AH, Digitimer, Welwyn Garden City, UK) was applied cutaneously at the popliteal fossa to depolarise the tibial nerve and evoke a maximal twitch of the relaxed triceps surae muscle. The amount of current that elicited the largest twitch was increased by 20% and was subsequently used for all stimulations. The maximal peak-to-peak M-wave amplitude (Mmax) for a supramaximal pulse was also recorded from MG. Supramaximal stimuli using single and triple (50 Hz) pulses were used to evoke resting torque twitches at several different ankle joint angles across the range of motion; typically 7 to 11 joint angles in 3° increments from 15° of plantar flexion to each subject's individual maximum dorsiflexion angle. The order in which measurements were taken at each angle was pseudorandomized until each joint angle had been measured twice. A single current pulse was used 3-5 s prior to the triplet to minimize

any thixotropic effect [22]. Plantar flexion torque and ankle joint position were measured by the dynamometer and collected at a sampling rate of 1 kHz using a Micro 1401-3 and Spike 2 software (Cambridge Electronic Design, Cambridge, UK). At the same time, ultrasound images of MG fascicles were captured using B-mode ultrasonography (6 MHz, ~110 fps, 42 x 51 mm field of view) using a 96-element multi-frequency transducer (LV7.5/60/96; Telemed, Vilnius, Lithuania) attached to a PC-based ultrasound system (Echoblaster 128, UAB; Telemed). The flat shaped transducer was strapped to the medial aspect of MG using an elastic bandage so that it could not move relative to the leg. The location of the transducer relative to the skin was marked with an indelible marker for consistent placement of the transducer for subsequent testing sessions.

Indirect markers of muscle damage

In the same position as described above and with an ankle angle of 15° dorsiflexion, subjects performed 3 x 3-s isometric MVCs separated by a minimum of 1 min rest. During each isometric contraction, isometric plantar flexor torque and surface EMG of the lateral gastrocnemius (LG), MG and soleus (SOL) were recorded. EMG was recorded using a bipolar configuration of two electrodes (8 mm recording diameter, Ag/AgCl, Covidien, Mansfield, USA) placed over the bellies of each muscle with an inter-electrode spacing of 2 cm (centre to centre) according to reported guidelines (Hermens et al., 1999). EMG signals were amplified 1000 times and band-passed filtered between 10–500 Hz (Neurolog System, Digitimer Ltd., Hertfordshire, UK) prior to being sampled at 2 kHz using the same instrumentation and software as described earlier.

Self-reported muscle soreness was assessed using a 10-point analogue scale (0 represents no soreness and 10 represents the worst soreness ever felt) using three different modalities [23]: 1) having the experimenter independently palpate the relaxed muscle bellies of MG, LG, and SOL to a depth of the width of their thumb; 2) the experimenter using an algometer (Wagner instruments, Riverside, USA) to apply 2.5 kg of pressure in the middle of the belly of the same muscles and 3) subjects walking for 15–20 m and rating the soreness they felt in their calf while walking.

Muscle mechanical and neural behaviour during the exercise bout

Using the ultrasonography and EMG methods described above, fascicle length changes of MG and RMS of the triceps surae were measured over 10 contractions at the beginning and end of each exercise bout. Initial and final fascicle strains were then calculated for each bout. Triceps surae EMG root mean square (RMS) activity and plantar flexion torque were also averaged over the same 10 contractions to evaluate any changes in muscle activation.

Data analysis

Length-tension curve construction

Custom software [24] was used offline to track MG muscle fascicle length changes from ultrasound images by applying a Lucas–Kanade optical flow algorithm with affine transformation [25]. As previously described, [16,21] active and passive fascicle L-T curves were constructed by plotting fascicle length against the peak torque produced from the triplet stimulation. The passive L-T relationship was first determined from passive ankle rotations as previously mentioned. The relationship between passive fascicle length and passive ankle torque was fitted with a third order polynomial and slack length estimated from the point on this curve that intersected through 0 Nm. An exponential fit was applied to all values above slack length, with its coefficient representing triceps surae passive stiffness [26]. Active torque was calculated as the difference between the torque produced by the stimulation (i.e., the peak of the triplet twitch) and the passive torque associated with the fascicle length during the contraction; as per Hoffman et al [21]. An active L-T curve was then fitted through the active L-T data using a physiologically appropriate model [21,26]. From the fitted L-T curves the maximum torque (T_{max}), the muscle fascicle length at which T_{max} occurred (optimal fascicle length, L_0) and the passive curve stiffness (k) were determined. The active and the passive curve parameters for all 20 subjects were then averaged and the mean active and passive curves were created for each measurement time point both pre- and post-exercise (Figure 2).

Isometric MVC torque and EMG activity

From the three MVCs performed, the attempt that yielded the highest maximum torque was used for subsequent EMG and torque analyses. Maximum voluntary isometric

torque was calculated by averaging the torque recorded during the middle 1 s of each MVC where the torque signal was considered stable. The EMG RMS amplitude (50 ms window) was calculated over the same 1-s period for each of the three muscles. During the same stable 1 s section of the MVC, median power frequency (MPF) was estimated over 500 ms non-overlapping epochs for each muscle. The power spectrum for MPF estimation was computed using the Welch method by applying a 512 points Hanning window with 15% overlap between successive sub-epochs [14]

Fascicle strain, torque and EMG activity during the exercise bouts

For each exercise bout, muscle fascicle length was calculated for each of the initial and final ten contractions (as previously described) and normalised to L_0 of the pre-exercise L-T curve. The torque (normalised to the triplet twitch elicited for the M-wave calculation) and EMG RMS (normalised to Mmax) data were divided in dorsiflexion-plantar flexion cycles and an average curve was created from the ten contractions, for each subject. The stretch phase (from ankle plantar flexion to dorsiflexion) was considered for statistical analysis.

Statistical analysis

Dependent variables (T_{max}, L_0, *k*, isometric MVC torque, EMG RMS, muscle MPF and soreness scores) were analysed using a two-way repeated measures analysis of variance (ANOVA) with bout (1^{st} and 2^{nd}) and time (PRE, 2H, and 2D) as factors. Greenhouse-Geisser corrections were applied to those significance tests that failed to meet the assumption of sphericity. A two-way repeated measures ANOVA, with contraction time (initial and final contractions of each bout) and bout as factors was used to compare stretch parameters, torque and EMG activation within and between the exercise bouts. A priori planned comparisons (Bonferroni contrasts) were incorporated into the ANOVA design to compare the effects of time and bout over the variables in analysis. All values presented are mean ± SD and statistical significance was set at $P \leq 0.05$.

Results

Markers of muscle damage

L-T relationship

A significant drop in T_{max} occurred following the initial bout of lengthening contractions (Figure 3). This was identified through a significant main effect of time ($F_{2, 38}$= 19.7, P< 0.01) but not of bout ($F_{1, 19}$ = 0.369, P = 0.55). No interaction (time x bout) was found ($F_{2, 38}$ = 1.22, P = 0.31). When compared to PRE, T_{max} was significantly reduced at 2H (P <0.01) before recovering to pre-exercise levels at 2D for the initial bout (Figure 3). For the repeated bout (Figure 3), there was no significant change in T_{max} at 2H (P = 0.21) or 2D (P = 0.33) compared with PRE (Figure 3).

There was no significant main effect of time ($F_{2,38}$ = 1.49, P = 0.24) or bout ($F_{1,19}$ = 1.38, P = 0.25) and no interaction (time x bout) ($F_{2,38}$ = 0.358, P = 0.7) of the optimal fascicle length to generate T_{max}. Further, there was no significant effect of time ($F_{2,38}$ = 0.184, P = 0.83), bout ($F_{1,19}$ = 0.466, P = 0.5) or interaction ($F_{2,38}$ = 0.52, P = 0.6) in '*k*' (stiffness) of the passive curves (Figure 3). Thus, L-T curves presented no significant changes in muscle mechanical behaviour (i.e. no rightward shift) when comparing between bouts (Figure 4).

Muscle soreness

The eccentric exercise protocol performed by subjects successfully elicited muscle soreness as indicated by increased muscle soreness scores following the initial bout (Figure 5). A significant interaction (time x bout) was found for all three soreness scores (palpation, $F_{2,38}$ = 8.4; algometer, $F_{2,38}$ = 7.11; walking, $F_{2,38}$ = 9.43; all P < 0.01) with main effects for time (palpation, $F_{2,38}$ = 36.38; algometer, $F_{2,38}$ = 47.22; walking, $F_{2,38}$ = 32.91; all P < 0.01) and bout (palpation, $F_{1,19}$ = 8.73; algometer, $F_{1,19}$ = 12; walking, $F_{1,19}$ = 9.43; all P < 0.01). Multiple comparisons revealed a significant increase in soreness for all scores at 2H (palpation, algometer and walking, P < 0.01); and 2D after the initial bout (palpation, algometer and walking P < 0.01).

Following the repeated bout, a significant increase in soreness was evident for all scores at 2H (palpation, P = 0.02; algometer, P < 0.01; walking, P = 0.01). At 2D, a significant increase in soreness compared to PRE repeated bout was found only for palpation (P < 0.01) and algometer (P < 0.01) but not for walking (P = 0.14). At 2D after the repeated

bout, the increase in soreness for all scores was lower (palpation, $P < 0.01$; algometer $P < 0.01$; walking, $P < 0.01$) in comparison to the same scores at 2D after the initial bout.

Isometric MVC torque and EMG parameters

There was a significant main effect of time ($F_{2,34} = 7.38$, $P < 0.01$) but not of bout ($F_{1,17} = 0.45$, $P = 0.51$) on isometric MVC torque production (Table 1). No interaction (time x bout) was found ($F_{2,34} = 0.26$, $P = 0.78$). Pre-exercise MVC torque was not different between bouts. At 2H, multiple comparisons revealed that the reduction in isometric MVC torque was significant after the initial bout ($P = 0.01$) but not after the repeated bout ($P = 0.09$). MVC peak torque decreased 15% from PRE to 2H for the initial bout, and 11% from PRE to 2H for the repeated bout. At 2D, MVC torque was not significantly different to PRE values for both bouts (Table 1).

EMG RMS during the isometric MVC task was similar between bouts for all muscles (Table 1), with no significant main effect of time (MG, $F_{2,34} = 1.55$, $P = 0.23$; LG, $F_{2,34} = 0.35$, $P = 0.7$; SOL, $F_{2,34} = 0.54$, $P = 0.59$), bout (MG, $F_{1,17} = 0.24$, $P = 0.62$; LG, $F_{1,17} = 0.18$, $P = 0.68$; SOL, $F_{1,17} = 0.01$, $P = 0.9$) or interaction (time x bout) (MG, $F_{2,34} = 2.21$, $P = 0.12$; LG, $F_{2,34} = 1.11$, $P = 0.34$; SOL, $F_{2,34} = 0.04$, $P = 0.96$). MPF recorded during the isometric MVC task showed a significant main effect of time in MG ($F_{2,32} = 4.24$, $P = 0.02$) and of bout in SOL ($F_{1,17} = 7.28$, $P = 0.02$). After the first exercise bout, multiple comparisons revealed a significant reduction in MPF from PRE to 2H for LG and MG: from 100 to 84 Hz in MG ($P = 0.04$) and from 98.4 to 84.1 Hz in LG ($P = 0.04$). No significant changes in MPF were found after the second bout in the muscles evaluated (MG, LG, SOL all $P > 0.99$).

Muscle mechanical and neural behaviour during the exercise bout

Overall, the results showed no major neuromechanical changes during the exercise bout for either bout one or two. Fascicle strain, EMG RMS and plantar flexor torque production during the initial and repeated bouts are presented in Figure 6. There was no significant main effect of contraction time ($F_{1,15} = 0.15$, $P = 0.7$), bout ($F_{1,15} = 0.6$, $P =$

0.45), or interaction (contraction time x bout) ($F_{1, 15}$ = 0.25, P = 0.63) on peak fascicle stretch during the eccentric contractions. Mean fascicle stretch was 11.2 ± 6.4 mm for the initial bout and 10.9 ± 5.1 mm for the repeated bout. This equated to a stretch of 17.9% and 17.2% relative to $L_{0,}$ respectively for the two bouts. The normalised EMG of the MG and LG revealed no significant main effect of contraction time (MG, $F_{1, 15}$ = 0.03, P = 0.87; LG, $F_{1, 15}$ = 0.86, P = 0.37), bout (MG, $F_{1, 15}$ = 1.17, P = 0.3; LG, $F_{1, 15}$ = 0.33, P = 0.57) or interaction (contraction time x bout) (MG, $F_{1, 15}$ = 0.23, P = 0.64; LG, $F_{1, 15}$ = 0.03, P = 0.86) during the stretch phase of the isokinetic exercise bouts. A significant main effect of contraction time ($F_{1,14}$ = 8.92, P < 0.01) but not bout ($F_{1,14}$ = 0.01, P = 0.95) or interaction effect (contraction time x bout) ($F_{1,14}$ = 1.4, P =0.26) was found for SOL EMG. This finding revealed increased SOL activity during the stretch phase in the final contractions of the initial bout (P = 0.04). Analysis of the torque during the stretch phase of the exercise bouts showed significant main effect of contraction time ($F_{1, 15}$ = 5.97, P = 0.03) but not of bout ($F_{1, 15}$ = 3.58, P = 0.08) and no interaction effect ($F_{1, 15}$ = 3.12, P =0.1).

Discussion

The present study employed a highly controlled eccentric exercise task, designed to produce EIMD in the triceps surae, to explore mechanical and neural responses that may underpin the RBE. We found a reduced loss of evoked torque and reduced muscle soreness following the repeated bout of the same eccentric exercise, indicating the presence of a RBE for this particular exercise protocol. A smaller reduction in the isometric MVC torque after the second bout also supports this conclusion. However, contrary to our hypothesis, we found no significant change in the optimum MG fascicle length to produce T_{max} after the initial bout and no obvious change in fascicle strain or excitation of the triceps surae across the two exercise sessions. We also found no relationship between the amount of fascicle strain experienced by individuals during the initial bout contractions and the subsequent force decrement from the first bout. These results suggest that MG was afforded protection during the second bout of exercise without macro level changes in mechanical or neural behaviour. We therefore suggest that either the damage was not sufficient to elicit measurable changes, or that micro-level adaptations might be sufficient to protect the gastrocnemius muscle from damage.

Fascicle mechanics post initial exercise bout

Despite markers of EIMD, our results showed no rightward shift in the L-T curve either 2H or 2D post the initial bout. This is in contrast with results derived from studies that exercise the elbow flexor that show significant changes in angle-torque curves after a single bout of exercise [27,28]. However, care must be taken when comparing angle-torque curves with fascicle length-torque curves, as joint angle data do not take into account the actual length of the muscle fibres. Despite this difference in methodology, we suggest that the lack of a rightward shift in this study may be due to the fact that MG fascicle stretch during the eccentric contractions is buffered by the overall elastic properties of the muscle-tendon unit. Focusing on the change in L_0, which is the parameter that may identify muscle fibre adaptations after damage, the CI of the difference between PRE and 2H post IB is -1.86 to 1.19 mm (see table in supplementary material). Considering that mean fascicle length during PRE IB was 62.38 mm, the CI suggest that the true difference between PRE and 2H post IB is unlikely to be more than 5% in each direction of L_0. Since significant shifts in L_0 have been reported with changes of at least 10% in L_0 [16], a maximum potential difference of 5% may be considered trivial.

Here, during the exercise bout, stretch of the fascicles was approximately 17% of their optimum length and the fascicles were operating primarily on the ascending limb and plateau of their L-T curve. Although most elbow flexor studies did not quantify fascicle strain during the exercise bout, it has been suggested that damaging protocols in these muscles involved greater length changes (~30 – 50 % of the optimum length) on the descending limb of the angle-torque curve [27]. The potentially higher tendon compliance of MG [29] allows its fascicles to operate mainly at shorter lengths and moderate force through the physiological range of motion of the ankle joint. This is likely the reason for the observed small decrease in voluntary isometric torque and electrically evoked twitch seen here, compared with the much larger decreases in torque (even up to 50%) reported for the elbow flexors [5,27,28].

L-T adaptation during the RBE

A reduction in T_{max} was present after the first but not the second bout of exercise. There was also no change in the optimal fascicle length after, or between, bouts. In an attempt to cause a significant level of damage, our exercise protocol consisted of a high number of repetitions over a large range of ankle motion, with muscles activated to approximately 70% of their voluntary maximum. However, even with this tightly controlled and focused protocol, we had relatively small amounts of soreness and torque decrement comparable to those reported after 30 mins of backward downhill walking [17]. While our study used a more focused exercise protocol, which elicited greater amplitude of stretch compared to backward walking (17% in current study compared to 10% in the study by Hoffman et al [16]), there was little difference in the damage produced. Because the elastic component (e.g. Achilles tendon) stretches in response to the increased force production during the eccentric contraction, this buffers the stretch of the muscle fascicles, potentially limiting the amount of induced damage. It is possible that a greater number of contractions would induce more cumulative damage and larger effects (e.g. 1 hr of backward walking[16] elicits greater damage than 30 mins of backward walking[17]). However, it is clear that the MG is well designed to minimise strain of its force generating structures as a result of its high tendon compliance. In this situation, changes that are more subtle and difficult to measure (e.g. changes to the extracellular matrix or subtle remodelling of sarcomere arrangement) may better explain the mechanism for the RBE in this muscle.

Contrary to our results, some studies speculate that the RBE is associated with a rightward shift in the angle–torque relationship after repeated bouts of exercise [1,5]. It is proposed that following muscle damaging exercise, there is a longitudinal addition of sarcomeres (sarcomerogenesis) that limits the amount of strain experienced by each individual sarcomere for subsequent bouts of the same eccentric exercise task [1,30]. Although assumptions of sarcomere changes made from angle-torque curves in vivo are problematic due to potential disassociation between changes in sarcomere length and joint angle [21], this discrepancy may suggest that changes in muscle mechanical behaviour during the RBE are muscle specific. Chen and colleagues [5] reported that, for the elbow flexors, maximal eccentric contractions elicited a significant shift in the optimum angle to produce torque in subsequent bouts of exercise. However, this shift was short lived after submaximal eccentric contractions, with the elbow flexors still eliciting a RBE. The results taken together suggest that a sustained rightward shift in the length-tension relationship is not a prerequisite for

conferral of the protective effect. Thus, a significant addition of sarcomeres in series is unlikely to be the primary mechanism of the RBE, at least in the gastrocnemius muscle.

Although our method of constructing fascicle L-T curves is more accurate than the angle-torque method for the detection of mechanical adaptations at the muscle level, it is still limited in the evaluation of architectural changes at the fibre level. Thus, while in this study sarcomerogenesis was not detected at the fascicle level, as determined by ultrasound, this adaptation may still occur at a level not detectable by our measurement techniques. An alternative interpretation of our results is that our small reductions in voluntary isometric torque and T_{max} reflect the damage of a small number of muscle fibres. Therefore, any sarcomerogenesis that may have occurred within those damaged fibres may be masked by the fibres that were not damaged and remain perfectly suited for the task, with their contractile properties dominating the overall (muscle level) length-tension relationship. It has been demonstrated that different regions of the same muscle show different levels of adaptation in response to eccentric exercise, according to starting muscle length and neural activation [3]. This may be related to the architecture of the individual fibres and muscles [3], which may explain the results seen here in comparison to other studies [5,30].

Neuromuscular adaptations during the RBE

Our results showed no adaptation in EMG RMS that can explain the RBE observed here. Similar results have been reported by McHugh and colleagues [20] who indicated that the RBE was not due to neural adaptation, as measured by surface EMG in the hamstring muscles. Like other studies [20] there was no significant change in EMG RMS, suggesting that the level of drive to the triceps surae muscle was similar across the two bouts. Some studies have reported attenuation of high-frequency content of the EMG signal at the time of the second bout [14,19], where they suggest selective recruitment of slow-twitch fibres or decruitment of fast-twitch fibres due to the damage. While our results show changes in MPF after the first bout, it is not possible to say this was due to changes in motor unit recruitment or perhaps more likely, a slowing of action potential conduction velocity as they travel beneath the recording electrodes on the muscle. Thus, the use of surface EMG may be insufficient for the detection of neural adaptations such as changes in motor unit recruitment order and/or motor unit synchronization, that may still play a role in the RBE.

Adaptations during the bouts of exercise

Overall, the results of this study show no major change in the neuromechanical measures made here during the stretch phase of the exercise bouts (Figure 6). There was no difference between the bouts of exercise in triceps surae neural drive, no change in fascicle stretch magnitude, and no change in fascicle operating length range. Thus, there seems to be no obvious fascicle level changes in mechanical strain during the exercise that may underpin a mechanical protective adaptation. Conversely, a reduction in vastus lateralis [18] and biceps brachii [31] fascicle lengthening has been reported after repeated bouts of submaximal cycling and maximal eccentric elbow flexor contractions respectively, which may be related to lower stretch damage in the later bouts. Shorter/stiffer tendons may involve less utilization of tendon elastic properties, eliciting more adaptations at fascicle level in comparison to the gastrocnemius where elastic tissue compliance is higher. Overall, these results suggest that the adaptations in fascicle stretch during the eccentric exercise are specific to the muscle that is exercised.

Possible mechanisms of the RBE in the gastrocnemius muscle

Assuming that sarcomerogenesis and/or alterations in neural activation are not the main cause of the RBE in gastrocnemius, we suggest that adaptations in the non-contractile elements of the muscle fibres are a possible mechanism. Disruption of the cytoskeleton, specifically the protein desmin, is one of the earliest occurring events in eccentric contraction-induced damage [32]. Therefore, it would seem plausible that an adaptation in the non-contractile elements of the muscle fibres may be a first line of defence in protection against repeated damage [10]. As proposed by Mackey and colleagues [12], muscles exposed to one bout of damaging stimulus present an overexpression of anabolic matrix growth factors and collagen, favouring the strengthening of the extracellular matrix for repeated bouts, particularly in the zones (fibres) where muscle damage is the greatest [13]. Thus, a diminished degradation/increased protection of the damaged muscle and its connective tissue when

subjected to the second bout of exercise may explain the mechanical adaptations present during the RBE in our study, suggesting a close interaction between the matrix cells and the contracting skeletal muscle cell [33]. Other adaptions might include increased recruitment of immune cells [34], which may facilitate an accelerated repair to the damaged fibres, thus contributing to the RBE.

In this study, the gastrocnemius muscle presented levels of soreness that were decreased after the repeated bout, in absence of mechanical alterations (no change in the L-T relationship) or large contractile damage (evidenced by the small drop in T_{max}). Even though it has been documented that damage to contractile machinery may be important for muscle soreness [35], our results indicate that fascicle damage can be independent of muscle soreness. It has been demonstrated that in lower limb muscles, an initial bout of eccentric exercise mediates the hyperexcitability of the spinal nociceptive pathways that elicit a decreased central sensitization during the repeated bout [36]. This central sensitization seems to be independent from altered contractile properties, since it can be elicited by contralateral exercise [37], and may be related with a lower perception of soreness in the RBE. Moreover, it seems that connective tissue damage and inflammation are more responsible for the soreness than muscle fibre damage and inflammation [38]. Overall, this indicates that the RBE, in terms of soreness levels, can be conferred in absence of significant muscle damage.

The results of this study should be interpreted considering certain limitations. It is important to highlight that the triceps surae has been shown to be less prone to changes after EIMD because of its structure and regular function. For instance, soleus muscle has a larger proportion of type I fibres[39], which may help to buffer or to compensate gastrocnemius function and contractile behaviour. Further, as previously stated, the ultrasound imaging and EMG recordings implemented here may not be sensitive enough to depict adaptations at sarcomere and motor unit level. Future research should consider the use of more advanced techniques such as in vivo imaging of human sarcomeres[40] and or high-density EMG[41], which may be better suited to investigate potential eccentric exercise induced changes.

Conclusions and Perspective

In conclusion, the results of this study show that in the gastrocnemius muscle, the RBE occur without mechanical adaptations during or after repeated bouts of controlled

eccentric exercise. Moreover, it seems that the neuromuscular adaptations measured here do not contribute to the RBE, at least for gastrocnemius. We suggest that adaptations in non-contractile elements of the muscle may better explain the protection of the muscle during the repeated bout. This response is possibly specific for the gastrocnemius muscle due to its physiological and architectonic features. The results from this study suggest that the adaptations of the gastrocnemius muscle to eccentric exercise that prevent future damage (repeated bout effect) are relatively subtle. Diverse studies using elbow flexors [5,19,27,28] have shown much higher levels of muscle damage after similar or lower amounts of muscle work in comparison to this study, which likely influences the adaptations that occur within the muscle. It is likely that the repeated bout effect is multifactorial and that different mechanisms dominate depending on the muscle type and mechanical stimulus. Overall, our results suggest that the gastrocnemius muscle damage is buffered from significant stretch through its range of motion by its elastic tendinous tissue, and that connective tissues may be a primary target for the adaptations that protect this muscle from future damage. This increased resilience to muscle damage may be an advantage from a physiological point of view, since it can be speculated that the muscle is more prepared for submaximal eccentric loading during daily life activities [8].

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Table 1. Changes in EMG activation and plantarflexor torque during isometric contractions at preexercise (PRE), two hours (2H) postexercise, and two days (2D) after two bouts of 500 isokinetic contractions.

	IB			RB		
	PRE	2H	2D	PRE	2H	2D
MG (RMS)	0.23 ± 0.14	0.29 ± 0.17	0.21 ± 0.09	0.26 ± 0.14	0.24 ± 0.14	0.24 ± 0.16
LG (RMS)	0.21 ± 0.08	0.20 ± 0.09	0.18 ± 0.07	0.20 ± 0.08	0.21 ± 0.13	0.21 ± 0.13
SOL (RMS)	0.12 ± 0.04	0.11 ± 0.05	0.11 ± 0.05	0.12 ± 0.06	0.13 ± 0.05	0.10 ± 0.05
Torque (Nm)	126 ± 40	107 ± 26*	122 ± 34	127 ± 33	113 ± 29	123 ± 31

MG, medial gastrocnemius; LG, Lateral Gastrocnemius; SOL, soleus; IB, initial bout; RB, repeated bout. Values are presented as mean ± SD. *Significant difference compared with the preexercise measurement of the same bout ($P < 0.05$)

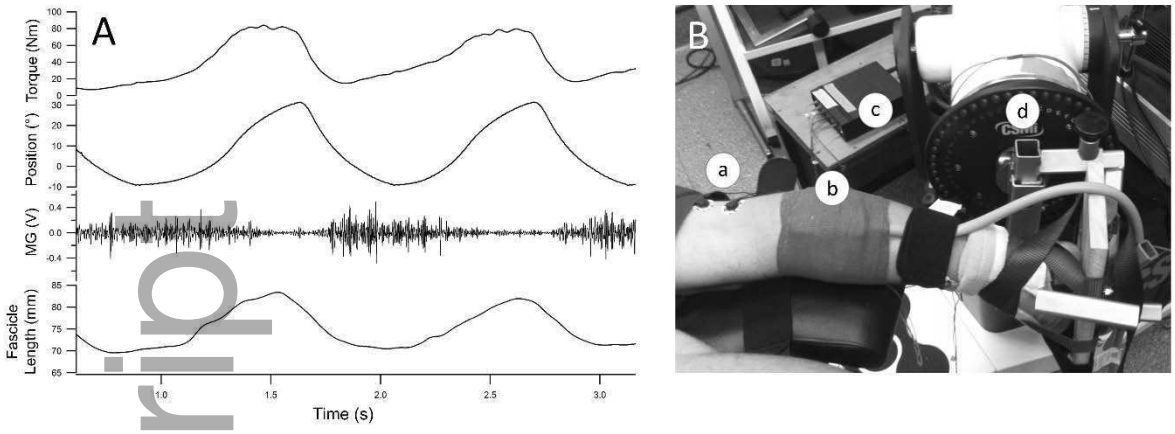

Figure 1. Experimental design to explore mechanical and neural changes that may occur during an eccentric plantar flexor exercise task and to create fascicle length–torque curves.Panel A, example of raw data (top to bottom: plantar flexor torque, ankle joint position, medial gastrocnemius (MG) muscle activity and MG fascicle length) recorded during the exercise bouts. Panel B, the experimental setup showing a) electrical stimulation electrodes, b) ultrasound probe fixed beneath an elastic bandage, c) EMG amplifier, d) isokinetic dynamometer. The surface electromyography electrodes recording MG activity are situated over the belly of MG and are beneath the elastic bandage.

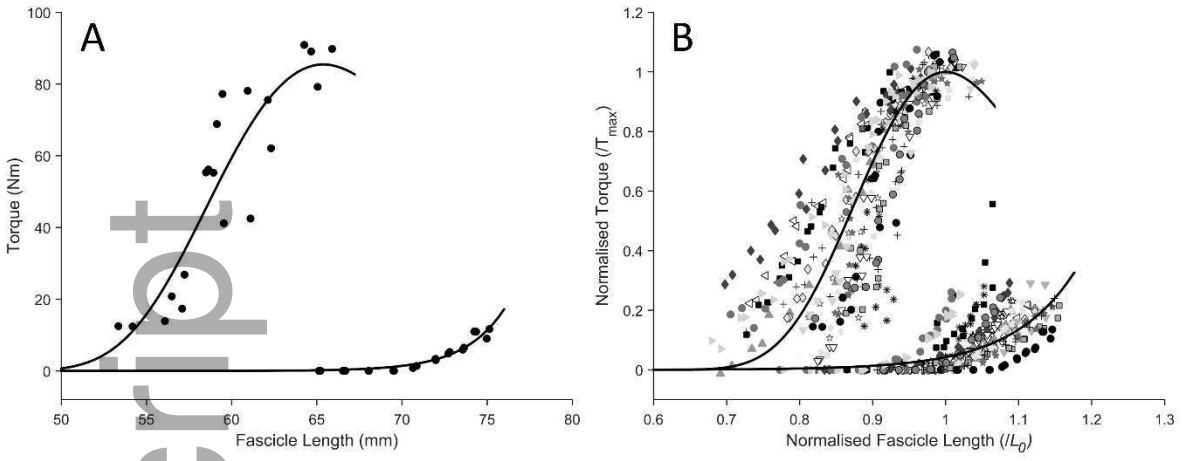

Figure 2. A) Data points and the fitted active and passive fascicle length–torque curves from a representative subject prior to the inital bout. B) each unique set of symbols represents data from each of the 20 individuals, while the fitted lines represent the group mean active and passive fascicle length-torque curves. Fascicle length is normalized to the calculated optimum length (L_0), and torque is normalized to the calculated maximum stimulated twitch torque produced by three consecutive stimuli at 50 Hz (T_{max}).

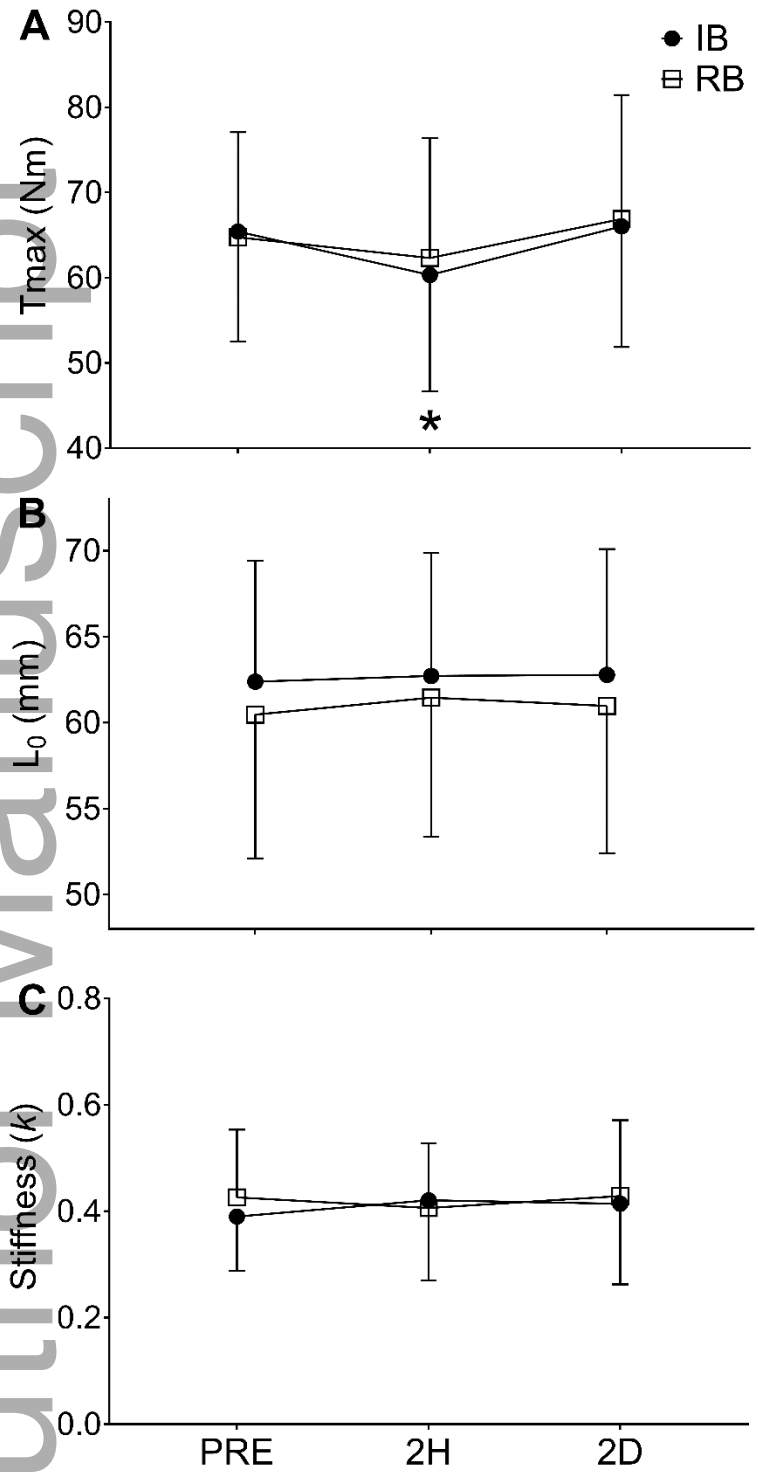

Figure 3. Active and passive length–torque curve parameters (A, maximum twitch torque B, optimal length and C, passive stiffness) before (PRE), 2 hours (2H), and 2 days (2D) after the initial (IB) and repeated (RB) bouts of eccentric exercise. Values are presented as mean and error bars indicate the SD. * Significant difference compared with the PRE measurement of the same bout ($P \leq 0.05$).

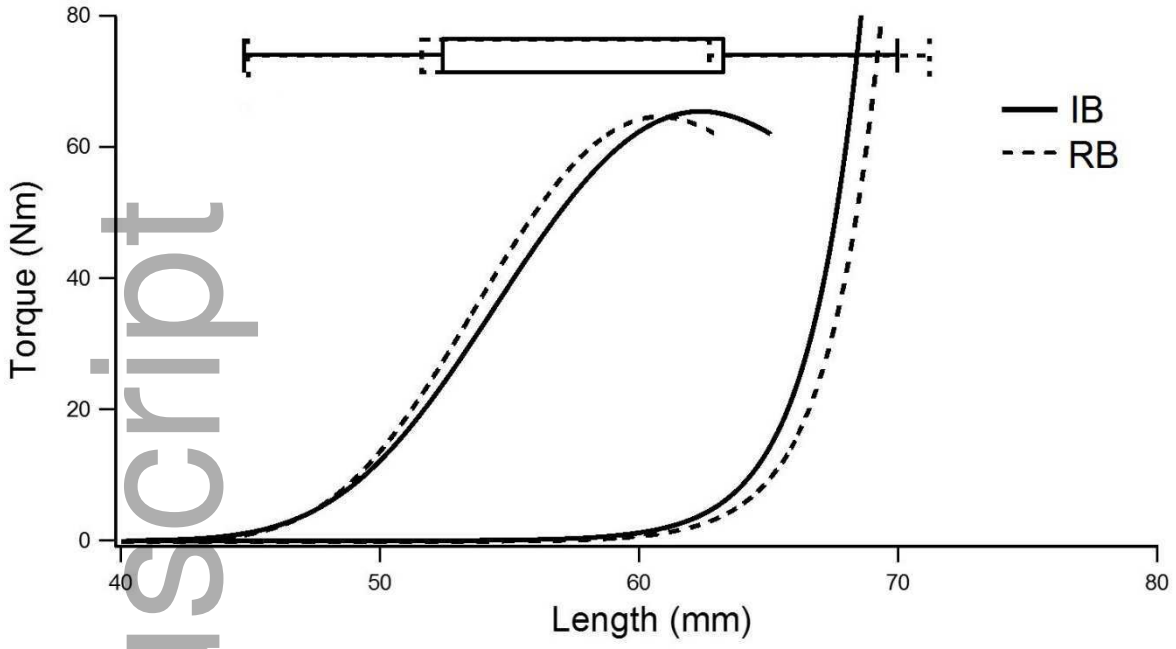

Figure 4. Group mean active and passive pre-exercise fascicle length–torque (L-T) curves for the initial (IB) and the repeated bout (RB). The horizontal rectangles above indicates the mean fascicle stretch amplitude during the exercise bout (i.e., the fascicle stretches from the ascending limb to the plateau region). The horizontal error bars indicate the SD of the group mean initial and final fascicle stretch lengths.

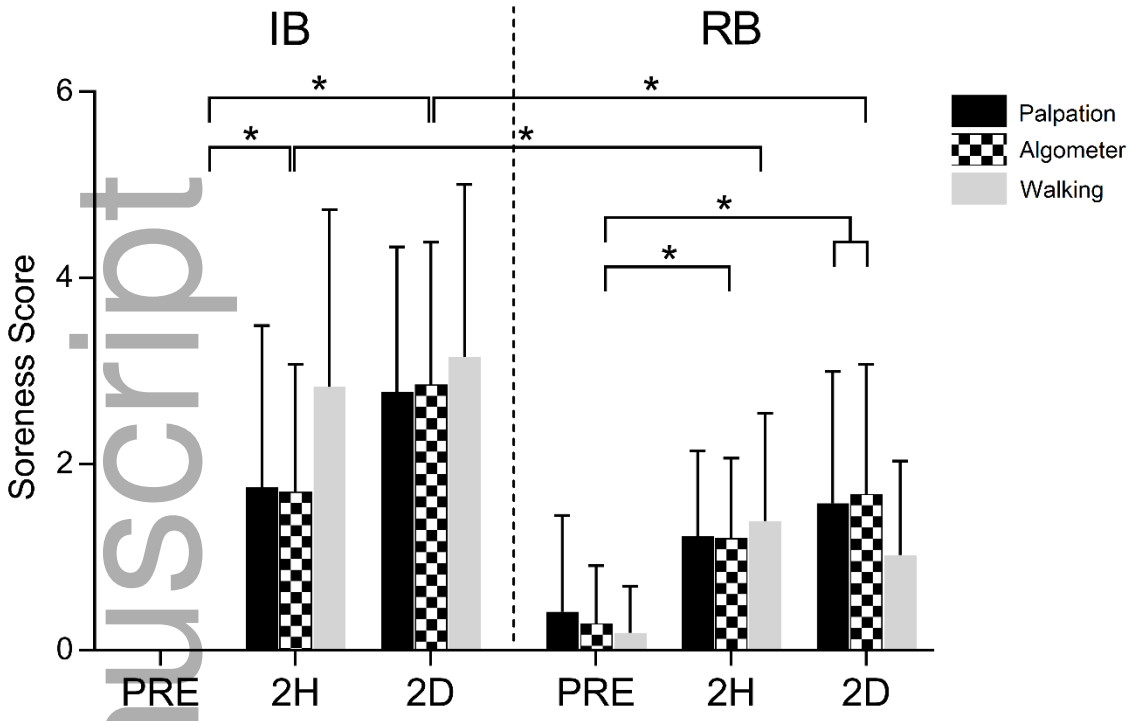

Figure 5. Mean ± SD soreness scores (0–10) from palpation, algometer, and walking in the combined muscle bellies of the triceps surae. Measurements were made pre-exercise (PRE), 2 hours post-exercise (2H), and 2 days post-exercise (2D) for the initial (IB) and repeated bouts (RB). *Represents significant differences at $P \leq 0.05$. Note that there are no values for PRE-IB as all subjects rated their level of soreness as 0 of 10.

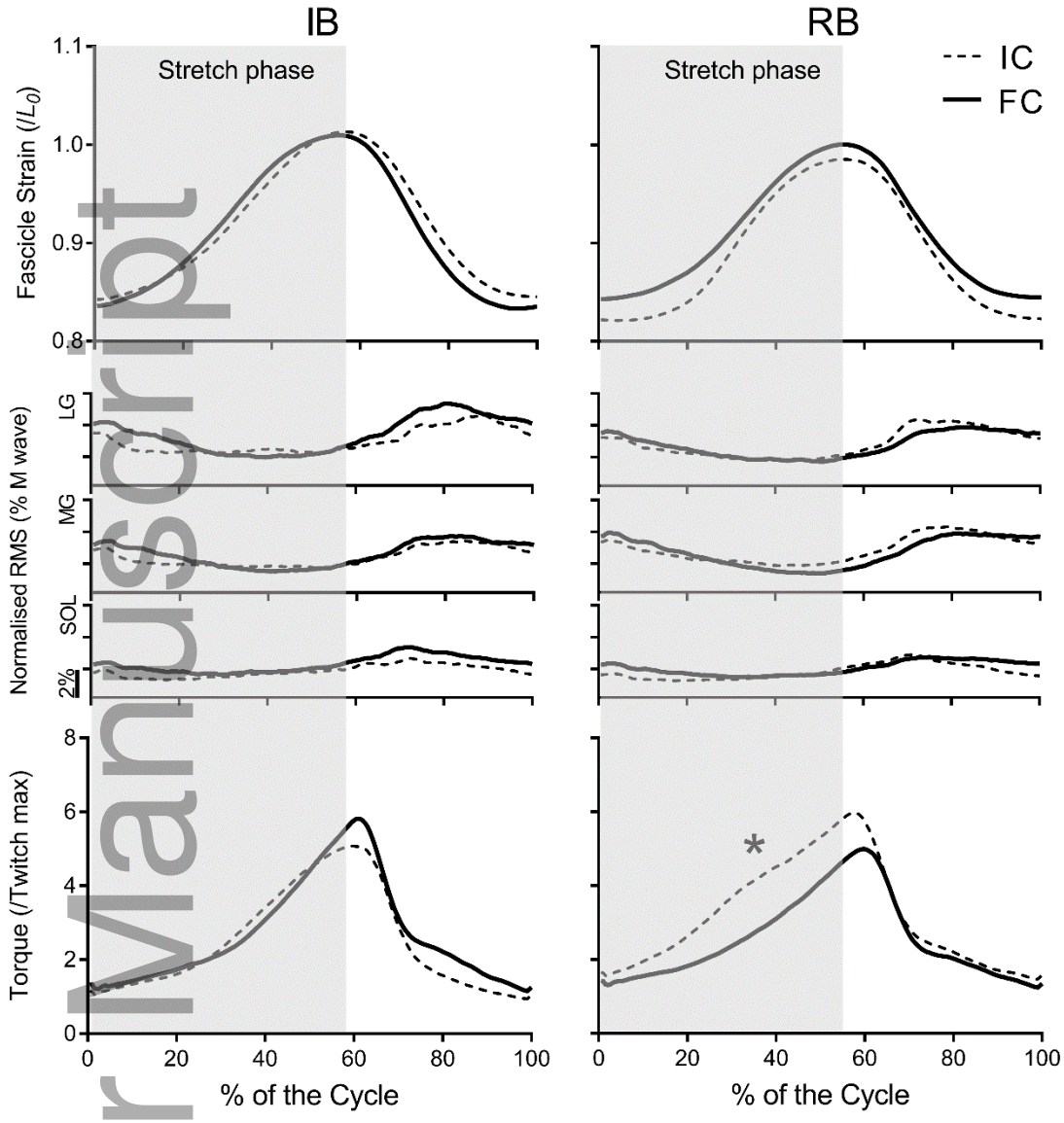

Figure 6. Group mean curves for fascicle strain, EMG RMS and voluntary torque for a time normalised contraction cycle for the initial and repeated bouts (IB and RB, respectively). Comparisons between the initial (IC) and final contractions (FC) for each bout are also presented as solid and dashed lines, respectively. In panels A and B fascicle strain is normalised to optimal length L_0 (calculated during PRE in the IB). Panels C and D, present waveform averages of the EMG RMS (normalised to maximum M-wave amplitude) of the lateral gastrocnemius (LG), medial gastrocnemius (MG) and soleus (SOL) muscles. Panels E and F, show the plantar flexor torque normalised to the maximal value of the twitch recording made before each bout of exercise. The vertical shaded area represents the stretch phase of the exercise cycle, which was used for statistical analyses (see text). *Represents significant difference of the mean of the stretch phase ($P \leq 0.05$).