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ORIGINAL ARTICLE

Cyclic eccentric stretching induces more damage and improved subsequent protection than stretched isometric contractions in the lower limb

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Abstract

Purpose Controversy remains about whether exercise-induced muscle damage (EIMD) and the subsequent repeated bout effect (RBE) are caused by the stretching of an activated muscle, or the production of high force at long, but constant, muscle lengths. The aim of this study was to determine the influence of muscle fascicle stretch elicited during different muscle contraction types on the magnitude of EIMD and the RBE.

Methods Fourteen participants performed an initial bout of lower limb exercise of the triceps surae. One leg performed sustained static contractions at a constant long muscle length (ISO), whereas the contralateral leg performed a bout of eccentric heel drop exercise (ECC). Time under tension was matched between the ECC and ISO conditions. Seven days later, both legs performed ECC. Plantar flexor twitch torque, medial gastrocnemius (MG) fascicle length and muscle soreness were assessed before, 2 h and 2 days after each exercise bout. MG fascicle length and triceps surae surface electromyography were examined across the bouts of exercise.

Results We found that both ECC and ISO conditions elicited EIMD and a RBE. ISO caused less damage 2 h after the initial bout (14% less drop in twitch torque, $P=0.03$) and less protection from soreness 2 days after the repeated bout (56% higher soreness, $P=0.01$). No differences were found when comparing neuromechanical properties across exercise bouts.

Conclusion For MG, the action of stretching an active muscle seems to be more important for causing damage than a sustained contraction at a long length.

Keywords Exercise-induced muscle damage · Repeated bout effect · Triceps surae · Muscle soreness · Ultrasonography

Abbreviations

ANOVA	Analysis of variance
BW	Backward downhill walking
ECC	Eccentric bout
EIMD	Exercise-induced muscle damage
EMG	Electromyography
IB	Initial bout
ISO	Isometric bout
LG	Lateral gastrocnemius
MG	Medial gastrocnemius
MVC	Maximal voluntary contraction
PRE	Before the exercise bout
RB	Repeated bout
RBE	Repeated bout effect
RMS	Root mean square
SOL	Soleus
2H	2 hours
2D	2 days

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Introduction

It has long been recognised that a bout of unaccustomed exercise involving eccentric (lengthening) muscle contractions can result in muscle soreness and damage to the muscle. A wide variety of both human and non-human experiments have confirmed that eccentric contractions provide the stimulus for this exercise-induced muscle damage (EIMD) that can lead to both weakness and inflammation (Hyldahl and Hubal 2014). Interestingly, after a subsequent bout of the same exercise some days later, the damaging effect is significantly mitigated, suggesting that an adaptive or protective muscle response occurs as a consequence of the initial exercise. This phenomenon has been coined the repeated bout effect (RBE) (Nosaka and Clarkson 1995). Due to the detrimental effects of EIMD on muscle performance, a thorough understanding of the factors that determine and mitigate EIMD severity is critical, for instance, for athletes preparing for competition and for novice populations starting to exercise for health benefits.

An interesting and seemingly unresolved question in understanding the mechanisms of EIMD and the RBE is whether the process of stretching an actively contracting muscle is necessary to cause damage, or whether damage arises due to the production of high force at long muscle lengths. Both of these putative mechanistic processes typically occur in common eccentric muscle contraction protocols that have been used to study EIMD. Although repeated active muscle stretches are often considered the main stimulus for inducing EIMD (Chen et al. 2011; Hyldahl et al. 2017), it has been reported that isometric contractions at long muscle lengths can also induce muscle damage (Philippou et al. 2003; Allen et al. 2017). For instance, Allen et al. (2017) showed that in the elbow flexors, sustained isometric contractions at long muscle lengths (i.e. with the elbow extended) caused significant drops in maximum voluntary torque that persisted for up to 24 h. To explain this finding, the authors suggested that EIMD is predominantly a result of contractile failure arising from the non-uniform lengthening of sarcomeres beyond myofilament overlap, according to the "popping sarcomere hypothesis" (Morgan 1990). While this is a plausible explanation that has been previously discussed and documented (Proske and Morgan 2001), lower limb muscles such as the gastrocnemius are buffered from large strains, even during eccentric contractions, due to the role of tendinous tissue straining with force (Hoffman et al. 2016; Pincheira et al. 2018), and their response to prolonged stretch may differ to upper limb muscles.

The heads of the gastrocnemius muscle operate mainly on the ascending limb of the length tension relationship during most functional tasks (e.g. walking) (Hoffman et al. 2016). Any stretch of the fascicles during cyclic eccentric contractions is considered to be limited due to their buffering by the long and compliant Achilles tendon and the muscle's aponeurosis (Hoffman et al. 2014, 2016; Pincheira et al. 2018). Such buffering is likely to mitigate contractile damage, as per the popping sarcomere hypothesis. However, it could be expected that contractions at long lengths for long periods would result in 'popped' sarcomeres, as such contractions should provide more time for sarcomere inhomogeneity to develop (Morgan 1990) and damage occur. This means that, as seen in elbow flexors (Allen et al. 2017), isometric contractions at a long muscle length in the gastrocnemius may cause significant contractile failure and EIMD evidenced by a reduction in muscle torque output. As such, comparing the degree of muscle damage caused by sustained isometric contractions at long muscle lengths to repeated muscle stretches to similar lengths, should help to shed light on the factors that determine EIMD severity.

The aim of this study was to determine the effect of different muscle contractile conditions (cyclic eccentric contractions and isometric contractions held at long muscle lengths) on the magnitude of EIMD and its subsequent protective effect. We used ultrasound imaging techniques, muscle twitches elicited by peripheral nerve stimulation, measures of muscle activity (EMG) and soreness scores to identify mechanical and perceptual changes that occur in the human medial gastrocnemius (MG) muscle, before and after two separate bouts of exercise containing eccentric and isometric contractions matched for time under tension. We hypothesised that EIMD and the RBE would be elicited by both contraction conditions, and speculated that the magnitude of the effect would be greater after the eccentric contractions due to repeated fascicle lengthening and the associated higher muscles fibre forces that occur during active lengthening (Hill 1938).

Materials and methods

Participants

Based on being able to detect a drop of 15% in plantar flexor twitch torque (Pincheira et al. 2018), which is equivalent to a large effect size ($d = 0.9$), a sample size of 12 subjects was required ($\alpha = 0.05$ and $\beta = 0.15$; repeated measures within-between interaction). Fourteen recreationally active participants (11 male, age 26 ± 4 years, mass 74.6 ± 11 kg, height 178 ± 6 cm; 3 female, age 28 ± 8 years, mass 70.4 ± 6 kg, height 171 ± 7 cm) volunteered to participate in the study. Participants were excluded if they had any pre-existing lower limb injuries, had surgery on the lower limb within the past 5 years and had undertaken eccentric training exercises

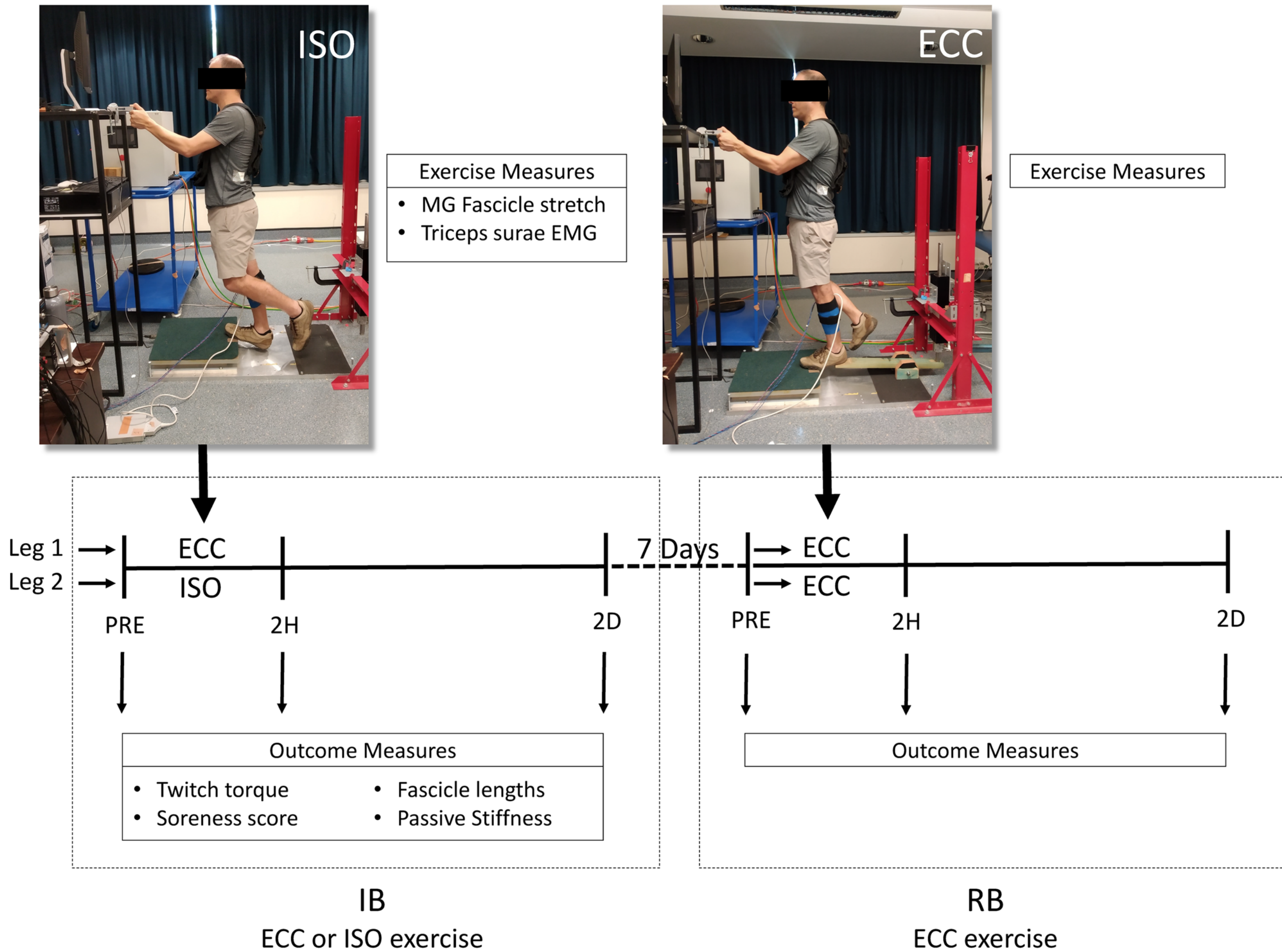

Fig. 1 Diagram showing the study protocol. Outcome measures were taken before (PRE) 2 hours (2H) and 2 days (2D) after an initial bout (IB) of exercise. During the IB, a randomly selected leg of the participants ($N=14$) underwent isometric contractions at a long muscle length (ISO) in one leg while the contralateral leg performed eccentric heel drops (ECC). 7 days later, both legs performed a repeated bout (RB) of ECC, with outcome measures being collected at the same time points. Neuromechanical measurements during the exercise bouts (exercise measures) were also collected from the medial gastrocnemius (MG) and triceps surae muscles

specific to the calf muscle group (e.g. heel drop exercises) within 6 months prior to the first testing session. The study protocol was approved by the local university ethics committee and was conducted according to the Declaration of Helsinki. All participants provided written informed consent.

Experimental design

Two bouts of exercise were utilised to cause EIMD (Fig. 1) and examine the RBE. The initial bout (IB) consisted of two conditions: cyclic eccentric heel drops (ECC) and repeated isometric contractions at a long muscle length (ISO). For the IB, ISO and ECC were performed on contralateral legs of each participant to test the hypothesis that the IB of ECC would cause more EIMD and more protection for the RBE compared to the IB of ISO. The ECC exercising leg was randomly selected, and the contralateral leg assigned to perform the ISO exercise. This was used to assure internal validity of the intervention and to avoid leg dominance as a possible confounding factor. 7 days later, participants performed a repeated bout (RB). During the RB, both legs performed the ECC task, to assess the magnitude of the protection conferred by the different IB stimuli (i.e. magnitude of the RBE conferred by ECC versus and ISO). The ISO and ECC exercise tasks consisted of an equivalent time under tension (600 s). Thus, this experimental design allowed us to elicit the mechanical insult with different types of muscle contraction, but with matched exercise volume between bouts. Details about the characteristics of the ISO and ECC conditions can be found in Table 1.

Neuromechanical data (i.e. plantar flexor twitch torque, medial gastrocnemius (MG) soreness scores, MG fascicle length and passive stiffness) were collected, before (PRE), 2 hours (2H), and 2 days (2D) after the IB and RB. A

Table 1 Characteristics of the exercise bouts utilised to elicit exercise-induced muscle damage and the repeated bout effect

	Eccentric heel drop (ECC)	Isometric contractions at a long muscle length (ISO)
Load magnitude	Body weight + 20% body weight	Body weight + 20% body weight
Contraction type	Eccentric	Isometric
Number of repetitions per set	15	1
Time under tension per repetition	2 s	30 s
Number of sets per bout	20	20
Rest between sets	2 min	2 min
Duration of one bout	~ 50 min	~ 66 min
Total time under tension per bout	600 s	600 s
Bouts per week	1	1
Ankle range of motion	~ 45°	At ~ 20° dorsiflexion
Ankle angular velocity	~ 23°/sec (~ 40 bpm)	0°/sec

significant reduction in plantar flexor twitch torque after the exercise bouts was considered as evidence of EIMD. This variable together with MG soreness scores were collected as the main outcome measures for the RBE. MG fascicle length and passive stiffness were collected as complimentary muscle damage outcomes. During the exercise bouts, MG maximum stretch and triceps surae EMG activity [MG, lateral gastrocnemius (LG) and soleus (SOL)], were collected as exercise measures to detect potential mechanisms that could underpin EIMD and RBE.

Exercise bouts protocol

During the exercise bouts, participants wore a vest equivalent to 20% of their body weight. This weight was selected following preliminary testing where different loads were trialled. It was found that a 20% load helped to increase the exercise load that the triceps surae would experience during the bouts, but avoided interfering with the exercise performance (due to weight-vest induced fatigue in the upper trapezius muscles). For the ECC task, each participant started by standing on the top edge of a force plate which was 15 cm above the floor (AMTI OR6-7-1000, AMTI, MA, USA) with an upright body position, knee straight, ankle joint plantar flexed to approximately 25°, and with full body weight on the forefoot. From this position, the triceps surae was lengthened under load by having the participant dorsiflex the ankle to a point where the heel was beneath the forefoot (~ 20° dorsiflexion) but not in contact with the floor. The speed of the movement was controlled by matching the movement to the frequency to a metronome set to 40 bpm, resulting in a lengthening contraction of approximately 2-s duration (Table 1). To ensure that the triceps surae performed only eccentric contractions, a mechanical lever powered by a magnetically driven servo-controlled actuator (Linmot PS10-70 × 400U-BL-QJ, NTI AG Linmot, Switzerland) lifted the participant to the starting position before each heel drop repetition (Fig. 1). The vertical ground reaction force and triceps surae EMG data were visualised online by the experimenter to ensure that there was little voluntary activity during the lifting phase. For the ISO task, participants dorsiflexed the ankle to a point where the heel was beneath the forefoot (~ 20° dorsiflexion) but above the floor. This point was defined as the start of the ISO condition that lasted 30 s per repetition (Table 1). In this position, triceps surae length was assumed to be similar to the maximum stretch position of the ECC condition (i.e. matched ankle joint angles), which was later confirmed with measures of fascicle length (Fig. 2). During the ECC and ISO tasks, participants were allowed to maintain balance by touching a load cell placed on a surface at elbow level in front of them with two fingers. The force trace from the load cell was used to give feedback to the participants about when a change in load from the fingers might have influenced activity in the triceps surae. Visual feedback of the participant's posture was also provided on a computer screen in front of the participants to ensure that the knee of the exercising leg remained extended, and that their maximal ankle joint range of movement was achieved at the start and end of each repetition. Both legs (ECC and ISO) were tested during the IB and RB testing days, with at least 2 h of rest given before exercising the contralateral leg with the second condition.

Medial gastrocnemius mechanical properties

Ultrasonography was used in conjunction with peripheral nerve stimulation to examine MG mechanical properties before and after the bouts of exercise. The combination of these methods has been shown to be reliable to quantify MG mechanical behaviour (Hoffman et al. 2012). Plantar flexion torque, ankle position, triceps surae EMG, and ultrasound data were synchronised frame by frame utilising analogue pulses sent from an A/D board (Micro 1401–3, Cambridge Electronic Design, Cambridge, UK) to the ultrasound

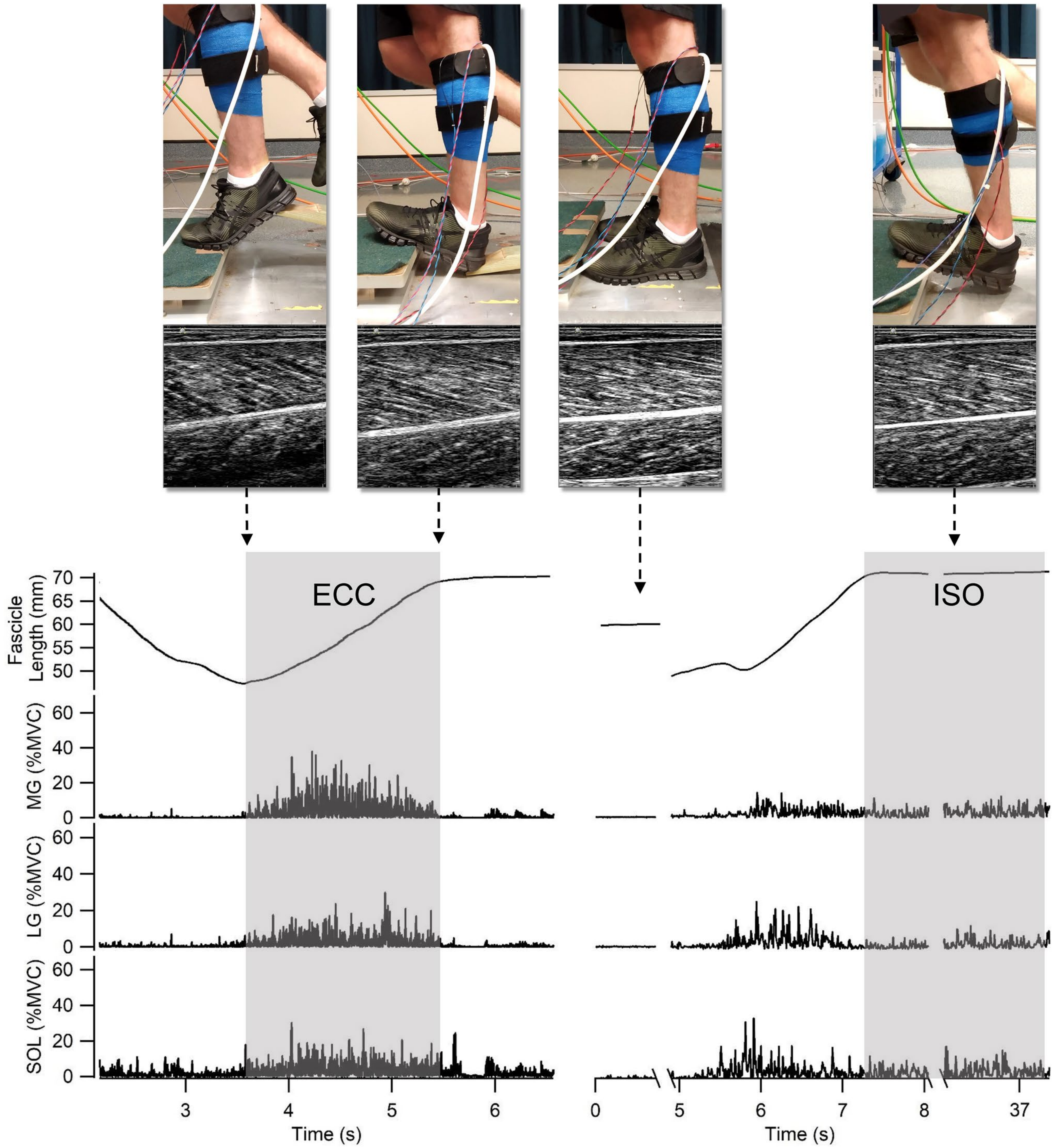

Fig. 2 Fascicle stretch, EMG activity and analysis window definition during exercise bouts utilised to elicit exercise-induced muscle damage and the repeated bout effect. Example data from one representative subject are shown during eccentric heel drops (ECC) and during an isometric contractions at a long muscle length (ISO). The top panels are demonstrative of the limb position and fascicle stretch during each task critical event. During the eccentric heel drop, medial gastrocnemius maximum stretch was estimated within an analysis window (shaded area) defined from maximal plantarflexion to maximal dorsiflexion (dotted arrows, 2 s of duration). Concentric muscle work was avoided with the help of a mechanical lever powered by a servo-controlled actuator that lifted participant's mass. For the isometric task, the participant started in neutral position (standing) to then progress to the isometric stretch position (start of the ISO analysis window). The condition continued for 30 s. Maximum fascicle stretch was estimated within this window

beamformer. That is, every time the beamformer received a pulse from the A/D (135 Hz), one frame of ultrasound data was recorded. This allowed an exact synchronisation between the ultrasound recording and the other signals. Ultrasound images of MG fascicles during maximal muscle twitches were captured using B-mode ultrasonography (6 MHz, 60 mm wide, 50 mm depth field of view) using a 128-element multi-frequency transducer (LV8-5N60-A2; Telemed, Vilnius, Lithuania) attached to a PC-based ultrasound system (ArtUS EXT-1H, Telemed, Vilnius, Lithuania). The flat shaped transducer was strapped over the belly of MG using elastic bandages so that it could not move relative to the leg during testing. The location of the transducer was marked on the skin with an indelible pen for consistent placement of the transducer for subsequent testing sessions.

For peripheral nerve stimulation, subjects laid prone with their knee fully extended (~0°) and their foot tightly attached to the footplate of a dynamometer (Humac Norm, CSMi Computer Sports Medicine, Stoughton, USA). First, MG fascicle length and passive torque were measured during a single passive rotation through the full range of ankle motion at constant angular velocity (15°/s). Then, a series of increasing constant-current stimuli (500 µs width; DS7AH, Digitimer, Welwyn Garden City, UK) each separated by several seconds were applied cutaneously at the popliteal fossa to depolarise the tibial nerve and evoke a twitch of the relaxed triceps surae muscle. The amount of current that elicited the largest twitch was further increased by 20% and subsequently used for all stimulations. Supramaximal stimuli (doublets at 50 Hz) (Fig. 3) were used to evoke resting torque twitches at seven different ankle angles across the range of motion; five stimuli in 5° increments from maximal dorsiflexion, one stimulus at the neutral position (0°) and one stimulus at 15° of plantar flexion. These twitches were then used to calculate the total twitch torque for each angle and ultimately the peak torque generated (see data analysis section for details).

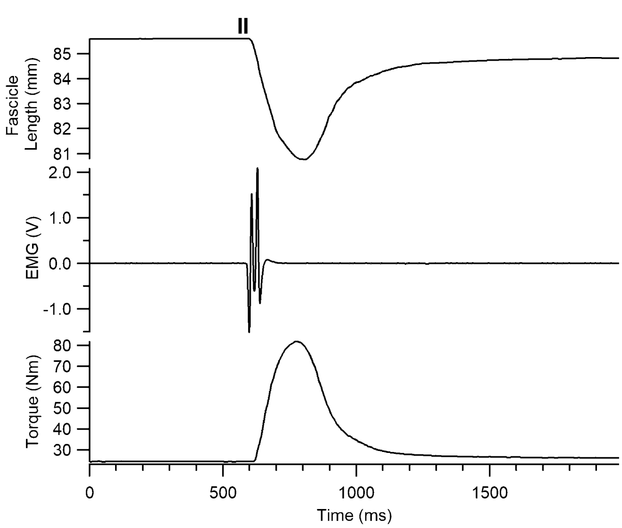

Fig. 3 Example of plantar flexor twitch torque, EMG activity and fascicle length changes elicited by supramaximal electrical stimulation. Medial gastrocnemius (MG) raw data are presented for one participant after a doublet (50 Hz) stimulation (black vertical lines, stimulation intensity 20% above the current that elicited largest twitch)

A small voluntary plantar flexion was performed for ~1 s prior to each stimulation to minimise any thixotropic effect (Proske et al. 1993). Plantar flexion twitch torque and ankle position were directly measured by the dynamometer and collected at a sampling rate of 1 kHz using an A/D board and collection software (Micro 1401–3 and Spike2, Cambridge Electronic Design, Cambridge, UK).

Muscle soreness scores

In the same prone position as described above, the experimenter palpated the relaxed muscle belly of MG to a depth of the width of their thumb. Participant's self-reported muscle soreness was assessed and recorded using a ten-point analogue scale (0 represents no soreness and 10 represents the worst soreness ever felt).

Muscle neuromechanical behaviour during the exercise bouts

Prior to the exercise bouts, maximal voluntary contraction (MVC) data were collected for EMG normalisation purposes. In the prone position described above, on the dynamometer with an ankle angle of 15° dorsiflexion, subjects performed 3 × 3-s isometric MVCs separated by a minimum 120-s rest period. During each isometric contraction, isometric plantar flexor torque and surface EMG of the LG, MG and SOL were collected. EMG signals were 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 distance of 2 cm (centre to centre) according to previously reported guidelines (Hermens et al. 2000). EMG signals were amplified 1000 times and band-passed filtered between 10 and 500 Hz (Neurolog System, Digitimer Ltd., Hertfordshire, UK) prior to being sampled at 2 kHz using the same A/D board and software described above. MG maximum fascicle stretch, and MG, LG and SOL EMG were then measured during the IB and RB tasks, using the methods described above, during the ECC and ISO exercise exercises.

Data analysis

Total twitch torque was calculated as the mean of the peak torque obtained across each of the seven stimuli (doublets) delivered across the full ankle range of motion. The time of peak torque for each contraction was used to measure the MG fascicle length, which corresponds to the shortest muscle length during contraction due to shortening against the elastic tendinous tissue. Fascicle length was estimated automatically utilising a custom script (Simple Muscle Architecture Analysis Software), which has been shown to be accurate in comparison to manual measurement (Seynnes and Cronin 2020). Another custom Matlab (The MathWorks, Natick, MA, USA) script was used to track MG muscle fascicle lengths recorded during the passive ankle rotations (Farris and Lichtwark 2016). MG passive stiffness was determined by fitting a previously described exponential expression (Hoffman et al. 2016) to the relationship between passive MG fascicle length and passive plantar flexion torque.

$$T_{passive} = Ae^{kL}$$

where A is curvature, k is the stiffness of the curve, and L is fascicle length.

Fascicle length parameters and EMG data from the IB and RB trials were utilised to characterise each exercise bout (exercise measures). Ten cycles of heel drops and isometric contractions (taken from the start and the end of each bout) were analysed. The same custom made Matlab software mentioned above was used to track MG fascicle length during the exercise bouts. The definition of the analysis window (i.e. stretch phase) to determine the exercise measures within each cycle and exercise type is presented in Fig. 2 (grey shaded bars). MG maximum stretch length was estimated within this window. For EMG activity estimation, MG, LG and SOL EMG root mean square (RMS) activity (50 ms window) during IB ECC was calculated and averaged over the same ten cycles where fascicle stretch was estimated. Then, the total duration of these ten cycles was used as a temporal parameter to extract RMS data from ISO, during their respective stretch phases. EMG signals were then normalised to the RMS EMG obtained during the MVC with the highest torque value.

Statistical analysis

GraphPad Prism v8.3 (San Diego, CA, USA) was used for all statistical analyses. All variables were tested for normality using the Shapiro–Wilk test. Deviations from sphericity were corrected using the Greenhouse–Geisser method.

The effects of exercise mode on dependent variables (total twitch torque, muscle soreness, MG fascicle length, and MG passive stiffness) were analysed with a three-way repeated-measures analysis of variance (ANOVA) with factors of bout (IB, RB), contraction (ECC, ISO) and time (PRE, 2H, 2D). Differences in exercise measures (i.e. maximum fascicle stretch and triceps surae RMS EMG) between exercise bouts were analysed with a two-way repeated-measures ANOVA with factors of bout (IB, RB) and contraction (ECC, ISO). Further analysis into the exercise bouts is presented as supplementary material, where the initial and final contractions within each exercise bout and the mechanical impulse between exercise bouts were compared. A priori planned comparisons (Tukey's test) were incorporated into the ANOVA design to compare the effects of time and bout over the variables in analysis. Statistical significance was set at $P \leq 0.05$ and all results are presented as means and standard deviations.

Results

Identifying the presence of muscle damage in the initial bout: eccentric vs isometric conditions

Overall, the results suggest the presence of muscle damage during the IB after both the ECC and ISO conditions, with reductions in total twitch torque 2 h after the initial ($P = 0.04$) bout being greater after ECC. A significant main effect of time ($F_{(2, 23)} = 28$, $P < 0.01$) and an interaction effect [time × contraction, ($F_{(2, 24)} = 4$, $P = 0.04$)] was found for total twitch torque. Subsequent planned comparisons indicated a significant drop in total torque from IB PRE to IB 2H after ECC (drop of 18.5%, $P = 0.04$) (Fig. 4A IB, denoted with hash symbol) but not after ISO (drop of 4.3%; $P = 0.81$). When compared at IB 2H, total torque was lower in ECC than in ISO (12.6% lower, $P = 0.03$) (Fig. 4A IB, denoted with asterisk). A significant main effect of time ($F_{(2, 23)} = 47$, $P < 0.01$) contraction ($F_{(0.5, 6)} = 11$, $P = 0.02$) and an interaction of time x bout x contraction ($F_{(1, 13)} = 39$, $P < 0.01$) occurred for muscle soreness. In comparison to IB PRE, the planned comparisons indicated a significant increase in soreness at IB 2H and IB 2D for both ECC (34% of increase from baseline at 2H, $P < 0.01$; 47% of increase from baseline at 2D, $P < 0.01$) and ISO (17% of increase from baseline at 2H, $P = 0.03$; 25% of increase from baseline at 2D $P < 0.01$) (Fig. 4B IB, denoted with hash symbols). When comparing between contraction types, soreness was greater in ECC when compared to ISO at IB 2H (50% greater, $P < 0.04$) and IB 2D (45% greater, $P < 0.01$; Fig. 4B IB, denoted with asterisks). The analysis of the complementary markers of muscle damage (MG fascicle length, Fig. 4C; passive stiffness, Fig. 4D) revealed a main effect of time for MG passive stiffness ($F_{(2, 20)} = 12$, $P < 0.01$), such that when ISO and ECC are considered together, passive stiffness decreased from the PRE IB condition (~ 8% of decrease). No other relevant planned comparisons for identifying the presence of muscle damage were found to be significant.

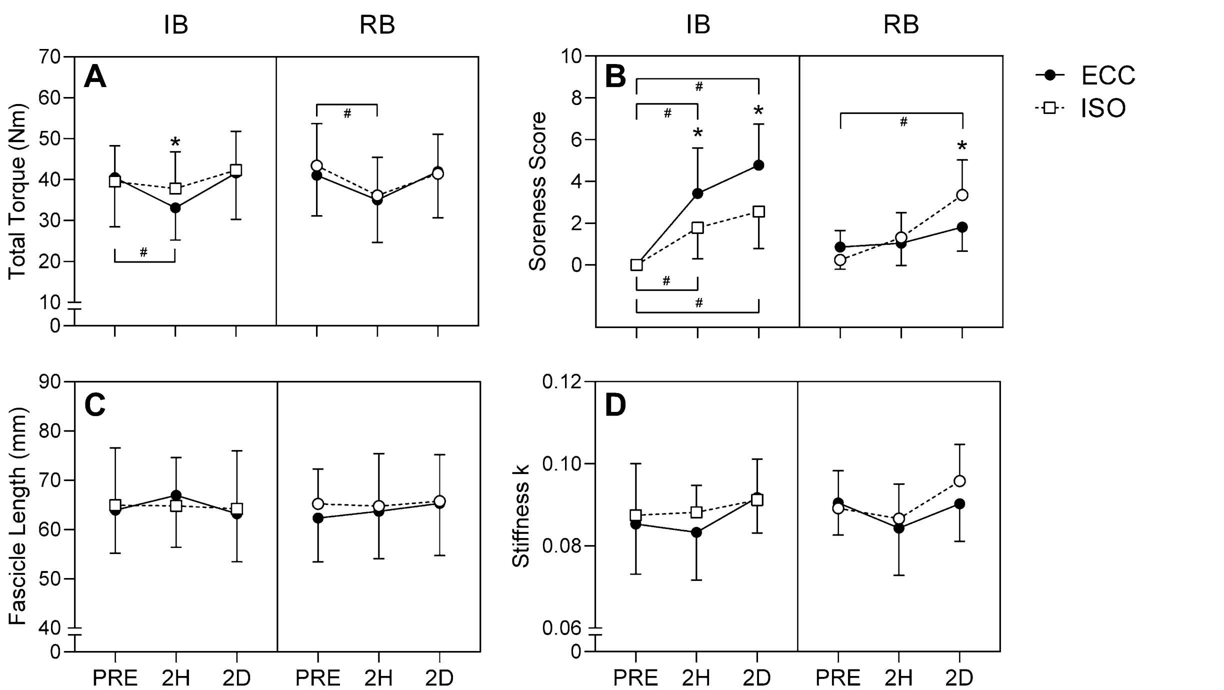

Fig. 4 Medial gastrocnemius markers of muscle damage before and after repeated bouts of exercise with different contraction types. **A** Total torque; **B** soreness scores; **C** fascicle length; **D** passive stiffness (*k*). These variables were analysed before (PRE), 2 hours (2H), and 2 days (2D) after an initial (IB) and a repeated bout (RB) of exercise. In each participant, during the IB, a randomly selected leg performed eccentric heel drop exercise (ECC-ECC) while the contralateral leg performed isometric exercise (ISO-ECC). Both legs performed ECC during RB. For details about the exercise bouts, refer to Table 1. Values are presented as mean and error bars indicate standard deviation. *Significant difference when comparing between the ECC and ISO contraction types ($P < 0.05$). #Significant difference when comparing between timepoints (PRE, 2H, 2D) of the same contraction type ($P < 0.05$)

Identifying the presence of the RBE: eccentric (ECC) versus isometric (ISO) conditions

Our results show that the RBE manifested mainly in the level of muscle soreness experienced by the participants, with higher protection from soreness conferred by ECC in comparison to ISO contractions (Fig. 4A and B, RB). An interaction between time x bout x contraction ($F_{(1, 15)} = 6$, $P = 0.02$) was found for total twitch torque (Fig. 4A RB). The planned comparisons indicated a significant drop in total torque from RB PRE to RB 2H for the ISO condition (drop of 17%, $P < 0.01$) (Fig. 4A RB, denoted with hash symbol) but not for the ECC condition (drop of 14%, $P = 0.13$). No other significant differences were found for total torque. For the soreness scores, a significant main effect of bout ($F_{(0.5, 6)} = 9$, $P = 0.03$), an interaction of time x bout ($F_{(2, 26)} = 13$, $P < 0.01$) and an interaction of time x bout x contraction ($F_{(1, 13)} = 39$, $P < 0.01$) were found. This indicated a significantly reduced level of muscle soreness following the RB for both the ECC and ISO stretch conditions compared to after the IB. Planned comparisons revealed a significant increase in soreness from RB PRE to RB 2D for ISO (31% increase, Fig. 4B RB, denoted with hash symbol). Further, when comparing between the ECC and ISO conditions at RB 2D, soreness was higher after ISO (15% higher, $P = 0.02$, Fig. 4B RB, denoted with asterisk). There were no significant main or interaction effects for fascicle length (Fig. 4C) or passive stiffness (Fig. 4D).

Neuromechanical differences between eccentric (ECC) and isometric (ISO) conditions across the exercise bouts

The two-way ANOVA did not reveal significant interaction or main effects of bout or contraction for any of the variables analysed across the exercise bouts (MG maximum stretch, MG RMS EMG, LG RMS EMG and SOL RMS EMG). This suggests that stretch and EMG variables were similar between the ISO and ECC interventions during the IB or RB. No interaction of bout x contraction ($F_{(1, 24)} = 2.1$, $P = 0.2$), main effect of bout ($F_{(1, 24)} = 3.9$, $P = 0.06$) or contraction ($F_{(1, 24)} = 0.1$, $P = 0.95$), were found for MG maximum fascicle stretch (Fig. 5A). No significant differences in EMG RMS were found for the muscles evaluated (Fig. 5B and C); MG: interaction ($F_{(1, 24)} = 2$, $P = 0.16$), main effect of bout ($F_{(1, 24)} = 3.9$, $P = 0.06$), main effect of contraction ($F_{(1, 24)} = 0.01$, $P = 0.9$); LG: interaction ($F_{(1, 18)} = 1.9$, $P = 0.17$), main effect of bout ($F_{(1, 18)} = 2.3$, $P = 0.14$), main

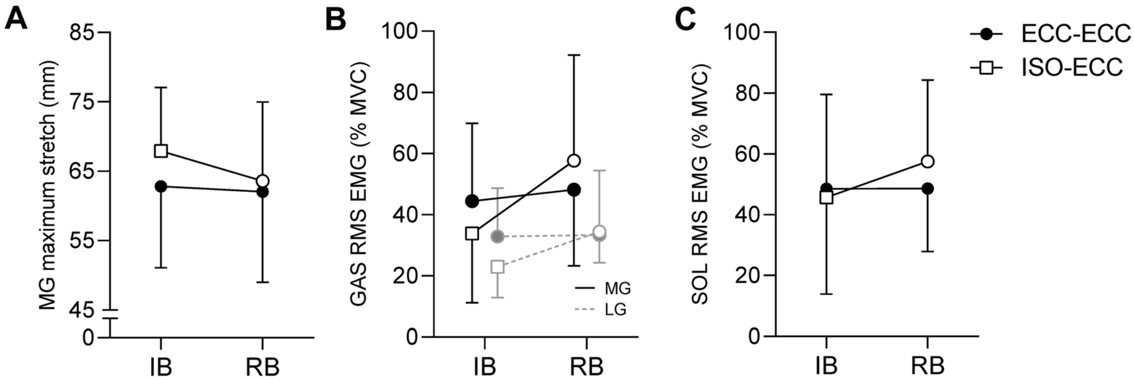

Fig. 5 Medial gastrocnemius fascicle stretch and triceps surae muscle activity during repeated bouts of exercise with the eccentric and isometric conditions. **A** Medial gastrocnemius (MG) maximum stretch; **B** gastrocnemius (GAS) RMS EMG changes for MG and lateral gastrocnemius (LG); **C** Soleus (SOL) RMS EMG. During the initial bout (IB), the participants performed in one leg an eccentric heel drop exercise (ECC-ECC, black circle, 600 s time under tension), whereas the contralateral leg performed an isometric exercise at a long muscle length (ISO-ECC, white square, 600 s time under tension). During the RB, both legs performed the eccentric heel drop task (600 s time under tension). Values are presented as mean and error bars indicate standard deviation. EMG data are presented as % of the maximal voluntary contraction (MVC) obtained during the same session

effect of contraction ($F_{(1, 18)} = 0.7$, $P = 0.4$); SOL: interaction ($F_{(1, 24)} = 1.5$, $P = 0.23$), main effect of bout ($F_{(1, 24)} = 1.57$, $P = 0.22$, main effect of contraction ($F_{(1, 24)} = 0.01$, $P = 0.7$).

Discussion

The results from this study support the hypothesis that EIMD can result from holding muscles under active tension at long lengths for prolonged periods (ISO condition), however, the magnitude of force loss and soreness is less than that from repeated stretches with a similar maximum fascicle length and a similar time under tension. Interestingly, the ISO RBE was only evident using outcomes of muscle soreness, and not in drops of total twitch torque (Fig. 4B RB and Fig. 4A RB, respectively). Furthermore, the protection conferred from the initial bout of ISO for a subsequent bout of eccentric exercise seemed to be less than developed from an initial bout of ECC. Neither of these findings seem to be explained by means of changes in MG contractile mechanics, such as fascicle length changes (Fig. 4C), or by changes in MG muscle activity during the exercise bouts (Fig. 5B).

The effect of ECC and ISO on EIMD

Both ECC and ISO caused EIMD in the triceps surae muscle, as indicated by the significant main effect of time on total torque production. Specifically, the magnitude of this damage was higher after ECC, as demonstrated by a greater force drop at 2H and a greater increase in soreness scores at 2H and 2D following the IB (Fig. 4A IB and 4B IB, respectively). The experimental design was developed so that the triceps surae experienced the same time under tension in both conditions, thereby allowing the same overall force (i.e. the force required to balance the body) to be applied to the gastrocnemius muscle. Thus, the EIMD differences between conditions seen here may be attributable to the act of repeatedly stretching an active muscle, rather sustaining a contraction at a long muscle length.

The drop in total twitch torque seen after ECC (Fig. 4A IB) may in part be due to individual muscle fibres being damaged while being forcibly stretched. However, the drop in total torque was relatively small (18%), and no other mechanical variable was found to be altered by the ECC exercise. An explanation for the low drop in force might be that the mechanical insult to the muscle during ECC was not enough to produce high levels of damage to its fibres. However, the magnitude of the fascicle stretch elicited here (15.4 mm for ECC) was similar to, or much greater than, previous similar studies utilising isokinetic exercise (11.2 mm fascicle stretch; 8% drop in torque) (Pincheira et al. 2018) and backward walking (~ 7 mm fascicle stretch; 13% drop in torque) (Hoffman et al. 2016) as damaging tasks. Furthermore, while the Hoffman et al. (2016) study used a far greater time under tension (~ 1100 s during backward walking), the results presented here showed similar low to moderate level of muscle damage in the triceps surae. Taken together, this may indicate that contractile tissue damage is not preponderant in the MG, as the Achilles tendon and aponeuroses are able to buffer the mechanical stretch, thus avoiding severe muscle fibre damage (Hoffman et al. 2016; Pincheira et al. 2018).

The aim of the ISO exercise was to develop muscle fascicle tension at long muscle fibre lengths, in an attempt to induce sarcomere inhomogeneity and develop contractile damage. However, when time under tension and maximum fascicle length during ISO was matched to ECC, total twitch torque drops were smaller compared to ECC (Fig. 4A IB). There are two likely explanations for this finding. First, the volume of the task (time under isometric tension) might have been insufficient to cause MG muscle damage, as previous studies suggest that the level of EIMD induced by isometric contractions is volume-dependent (Lima and Denadai 2015; Barreto et al. 2019). This may be supported by the supplementary data, where it is shown that the ISO bout has a similar mechanical impulse compared to the stretch phase of the ECC bout, but that body weight support during the shortening phase (heel raised by lever) may also require additional time under tension (see Supplementary Figure S2) which may contribute to higher muscle damage after the ECC bout. However, the calf muscle forces (and EMG activity, see Fig. 2) during the heel raise are likely to be quite low because the lifting force is applied directly to the heel and hence this effect is likely to be rather small. Furthermore, it is noteworthy to mention that the time under tension used here (600 s) was much longer than that used in other studies showing significant torque decrements in elbow flexors (105 s) (Allen et al. 2017) and knee extensors (180 s) (Tseng et al. 2016). This may imply that the mechanisms of eccentric damage are muscle specific. Second, during the ISO condition, the force experienced by the muscle is constant, whereas during the ECC condition, the fascicle is lengthening and shortening repetitively. Due to the force–velocity relationship (Hill 1938) and the fact that fibres generate more force when being actively stretched, it is possible that a smaller population of fibres may have been generating the higher resistance force in the ECC condition compared to the ISO condition and are, therefore, more prone to damage.

The RBE in the MG conferred by ECC and ISO

A RBE was found after both ECC and ISO, with the protection being conferred mainly in terms of muscle soreness. However, the prophylaxis induced by ISO seems to be less, since it was not effective 2D after the RB (Fig. 4B RB). This may be related to the level of EIMD elicited, as ECC damage (torque at 2H and soreness at 2H and 2D) was larger during the IB. This agrees with previous literature suggesting that the magnitude of the protection conferred is proportional to the level of damage that occurs during the IB (Hyldahl et al. 2017). Nevertheless, the RBE in muscle soreness was conferred after ISO, even when no significant detriment in total torque was found during IB. Indeed, a previous study showed that a RBE can occur after submaximal non-damaging eccentric exercise (Lavender and Nosaka 2008), and a recent study showed that isometric preconditioning can protect against eccentric damage of different modalities (e.g. slow–fast EIMD) (Barreto et al. 2019). Overall, this supports the notion that the RBE can occur even when the initial exercise bout (ECC or ISO) does not induce significant contractile damage (Lima and Denadai 2015; Hyldahl et al. 2017).

The current results and previous studies (Hoffman et al. 2016; Pincheira et al. 2018) suggest that neither muscle mechanical adaptations nor fascicle stretch adaptations during the exercise bouts underpin the RBE in the MG. The supplementary material comparing initial and final contractions within each bout also suggest no major neuromechanical adaptations across exercise bouts (see Supplementary Figure S1). Alternative mechanisms such as adaptations in inflammatory processes, adjustments in molecules related to muscle pain, and/or a strengthening of connective tissues surrounding and in series with the fascicles may be related to the protective effect conferred by a low to mild EIMD (Hyldahl et al. 2017). These adaptations, especially changes in connective tissue, have been seen just a few hours after the initial damaging bout (Mackey and Kjaer 2016), and might predominate over muscle fibre adaptations, which usually have a later onset. First, changes in expression of genes related to reactive oxygen species, increased expression of heat shock proteins and greater accumulation of neutrophils following the IB may be related with a more robust inflammatory response during the RBE (Lima and Denadai 2015; Hyldahl et al. 2017). Second, smaller increases in the expression of mRNA molecules related to soreness (i.e. BKB2 receptor, COX-2, and mPGEC-1), may explain decreased levels of soreness during the RB (Nagahisa et al. 2018). Finally, an increased expression of collagen and other components related with matrix compliance may play an important role in the MG RBE (Hyldahl et al. 2015; Mackey and Kjaer 2016). Yet, which mechanism is preponderant in the protective response is still unknown, considering that other factors such as neural adaptations (Dartnall et al. 2011) may still play a role.

Practical implications

In elbow flexor studies (Barreto et al. 2019), isometric preconditioning has been indicated as a strategy to improve the adherence of novice individuals in undertaking different training regimens where soreness and other markers of muscle damage are undesired. However, under the exercise volume utilised here (600 s time under tension), the protection conferred to the MG by ISO is smaller than for ECC, as seen at ISO RB 2H (Fig. 4B RB), where soreness scores are similar to ISO IB 2H (Fig. 4B IB). As mentioned before, this may be due to the lower levels of EIMD elicited by the ISO IB, but may also be because the damage mechanism is not fully expressed when isometric contractions are used (Allen et al. 2017). As such, from a practical perspective, even though a bout of ISO can attenuate some of the MG soreness, is not as beneficial as eccentric contractions to confer protection against further eccentric damage. Fascicle stretch and exercise volume estimated as time under tension have been considered as the most important exercise parameters related to ISO EIMD magnitude (Toigo and Boutellier 2006; Tran et al. 2006; Allen et al. 2014). Since fascicle stretch damage seems difficult to induce in the MG, an increased time under tension might be the key to elicit greater ISO EIMD and thus a higher prophylactic effect conferred by ISO.

Limitations

The results of this study should be interpreted considering certain limitations. One consideration is whether there were crossed effects between the two legs utilised in this study. Although it has been reported that a cross-transfer RBE between the exercised legs can occur (Chen et al. 2018), the IB ECC and ISO bouts were conducted within a range of 3 h, thus making it highly unlikely that any adaptation that underpins the contralateral RBE was established within this time (Chen et al. 2016). Furthermore, as the level of protection of the contralateral RBE is much less than the unilateral RBE, and as both legs in the repeated bout have already undertaken an initial bout, we suspect that any contralateral repeated bout effect is masked by the adaptations that underpin the unilateral repeated bout effect. The mechanical insult to the muscle during ECC may have been not enough to produce high levels of damage to its fibres. Nevertheless, as mentioned before, it seems that extensive damage to the gastrocnemius in vivo is unlikely due to the buffering of stretch by the Achilles tendon. SOL muscle mechanical behaviour (e.g. fascicle length change) was not included in the analysis. Further studies should also include ultrasound images from SOL as this muscle has a larger cross-sectional area than the gastrocnemius (Fukunaga et al. 1992) and may help to buffer, or to compensate, triceps surae function and contractile behaviour. MG and SOL muscles are relatively close to each other, thus there is always a risk of crosstalk when attempting EMG recordings. Furthermore, the changes in twitch torque seen here are likely to also include LG and SOL muscle mechanical adaptations. Our fascicle length estimations considered just one fascicle within each image, which may be considered a limitation. However, MG fascicle architecture was homogeneous through the image, so the fascicle tracked likely represents the dominant orientation of the rest of the fascicles within the region of muscle evaluated (Van Hooren et al. 2020).

Conclusions

In the human MG muscle, eccentric contractions elicit higher levels of muscle damage when compared to isometric contractions at a long muscle length, when exercise volume (i.e. time under tension) and maximum fascicle length is matched between conditions. The process of repeatedly stretching an actively contracting muscle seems to provide a distinct stimulus for damage beyond a sustained contraction at long lengths. Yet, the level of this damage is low to moderate and is expressed mainly as increased levels of soreness rather than changes in muscle mechanical behaviour (i.e. drop in total torque). Overall, this suggests that large levels of muscle damage cannot be elicited in vivo in the MG due to the capacity of muscle's elastic tissue to buffer fascicle stretch. Eccentric and isometric contractions are both able to produce a RBE. Nevertheless, the greater magnitude of muscle damage elicited by eccentric contractions during the IB increases the magnitude of the protective effect during a subsequent bout of eccentric exercise.

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Availability of data and material Data are available upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest None to declare.

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