©Journal of Sports Science and Medicine (2016) **15,** 697-703
http://www.jssm.org

Research article

Low-Frequency Fatigue Assessed As Double to Single Twitch Ratio after Two Bouts of Eccentric Exercise of the Elbow Flexors

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Abstract
The aim of this study was to assess low-frequency fatigue as a double to single twitch ratio after repeated eccentric exercise of the elbow flexors. Maximal isometric torque, single and double twitch responses and low-frequency fatigue were assessed on the elbow flexors in 16 untrained male volunteers before, immediately after, 24 and 48 hours following two bouts of eccentric exercise consisted of 30 repetitions of lowering a dumbbell adjusted to ~75% of each individual's maximal isometric torque. Maximal isometric torque and electrically evoked responses decreased significantly in all measurements after the first bout of eccentric exercise ($p < 0.05$). In measurements performed at 24 and 48 hours after the second bout both maximal voluntary isometric torque and electrically evoked contractions were significantly higher than in measurements performed after the first bout ($p < 0.05$). Although low-frequency fatigue significantly increased up to 48 hours after each bout of eccentric exercise, its values at 24 and 48 hours after the second bout were significantly lower than at respective time points after the first bout ($p < 0.05$). Double to single twitch ratio could be used as a sensitive tool in the evaluation of muscle recovery and adaptation to repeated eccentric exercise.

Key words: Biceps brachii, electrical stimulation, lenghtening action, repeated bout effect.

Introduction

Muscles subjected to unaccustomed eccentric exercise are susceptible to muscle damage that rely on sarcomeres disruption and impairment of the excitation-contraction (E-C) coupling system (Proske and Morgan, 2001). The well known results of this exercise-induced muscle damage are prolonged decrease in maximal force generated by the muscle during both voluntary and electrically evoked contractions and delayed onset muscle soreness (DOMS) (Janecki et al. 2014; Prasartwuth et al. 2005). It is well documented that a prior bout of eccentric exercise triggers a rapid adaptive response that significantly diminishes muscle damage and the muscle force recovery occurs faster after a second bout of the same exercise (Chen et al. 2009; Nosaka et al. 2001). This protection developed after the first bout of eccentric exercise is referred to as repeated bout effect (RBE) and mechanisms responsible for this phenomenon relies on neural, cellural and mechanical factors (McHugh, 2003). Another specific effect that occurs after eccentrically biased exercise is long-lasting increase in low-frequency fatigue (LFF) (Edwards et al. 1981). LFF is defined as the disproportionate decrease in force elicited with electrical stimulation at a low frequency, compared to a high frequency (Bruton et al. 1998). Jones (1996) has proposed that the primary mechanism responsible for LFF is a decrease in calcium release from sarcoplasmic reticulum that affects E-C coupling process. It has been confirmed with direct measure of calcium concentration changes in mouse skeletal muscle in the presence of LFF (Chin et al. 1997). Therefore, LFF reflects changes associated with exercise-induced impairment of the E-C process that affects contractile machinery responsible for the muscle force development. LFF could be measured by calculating the double pulse torque to single pulse torque (DT/ST ratio), which is an effective method to quantify LFF consistent with the literature using trains of high and low frequency stimulation (Ratkevicius et al. 1995; de Ruiter et al. 2005). The advantage of this method of LFF assessment is that DT/ST ratio has less influence on muscle fatigue (Rassier et al. 1999), and because is more comfortable when compared to longer stimulation trains, minimizes volitional reactions to the noxious stimulation and the post stimulus co-activation artifacts (Iguchi and Shields, 2010; Meszaros et al. 2010), that may affect strongly the force curve obtained from the elbow flexors muscles.

The study of Kamandulis et al. (2010) performed on the knee extensors muscles revealed repeated bout effect for the LFF assessed as a stimulation trains. However, it has been reported that elbow flexors muscles, are more susceptible to muscle damage than the knee extensors muscles (Jarmutas et al. 2005; Paschalis et al. 2010) what could effect on their time course of recovery and susceptibility to repeated damage. Because of their function (fast and accurate movements), prolonged low frequency force deficit could affect strongly performance of the daily task at low force level (Dundon et al. 2008; Smith and Newham, 2007).

To the best of our knowledge, there is no study that has measured LFF after repeated ECC of the elbow flexors using DT/ST ratio. Because this mode of stimulation minimizes potential artifacts, it could be a sensitive tool for indirect assessment of muscle damage, recovery and adaptation to ECC. Therefore, the aim of this study was to assess modifications of DT/ST ratio after two bouts of eccentric exercise of the elbow flexors. We hypothesized that the changes of DT/ST ratio will decrease and/or disappear faster after the second bout of ECC when compared to the first bout.

Methods

Sixteen untrained, right handed male volunteers (age 24 ±

Received: 30 June 2016 / Accepted: 08 November 2016 / Published (online): 01 December 2016

4 yr, height 1.79 ± 0.09 m, body mass 75 ± 8 kg) took part in this study and gave their written consent, the study was approved by the local Ethics Committee and complied with the Helsinki Declaration. Some of the subjects were familiarized with resistance training but had not perform any resistance exercise for at least 6 months before this study. The participants did not have any neuromuscular disorders and were free from any injuries of the upper limbs. All subjects were instructed to keep their normal diet and not to take any anti-inflammatory drugs as they could affect recovery mechanisms associated with repeated bout effect (Kyparos et al., 2012; Lapointe et al., 2002).

A within-group repeated measures design was used to determine changes in LFF after two bouts of eccentric exercise separated by 2-3 weeks. Maximal voluntary isometric torque of the right elbow flexors during maximal voluntary contraction (MVC), electrically evoked responses to single pulse (single twitch - ST) and double pulse (double twitch - DT) and pain assessment were collected before (pre), immediately after (post), 24 and 48 hours (h) following each bout of ECC. The sequence of measurements was always in the same order: pain assessment, single twitch, double twitch and MVC tasks. This order was chosen to avoid the potential influence of maximal torque development on subsequent measurements (e.g. MVC effect on twitch potentiation) (Vandervoort et al., 1983).

The measurements of MVC and electrically evoked contractions of the elbow flexors of the right arm at 90° elbow joint angle were described previously (Janecki et al. 2014). Briefly, the MVC's were measured with the BIODYNA dynamometer, designed and built by Warsaw Technical University in Poland (Kędzior et al. 1987) (Figure 1). The device consists of: a chair with seat belts for stabilization, a column, and a moveable arm with a wrist handle. The wrist handle has a high sensitivity force transducer (SML-200, Interface, Scottsdale, Arizona, USA) inbuilt on one side and movable plate for the wrist stabilization from the other side. The adjustable wrist handle allows setting the lever arm individually to ensure that during maximal voluntary contraction measurements force is exerted by the subject's wrist against the force transducer at the level of styloid processes of the radius and ulna. The wrist handle placement on the lever arm was recorded before the first measure, and used as the site for the wrist handle setting on each subsequent measurement. The torque applied at the elbow joint was calculated by multiplying the measured force by the perpendicular distance between the force transducer and the center of rotation of the elbow. Before the study began the MSL-200 force transducer was calibrated on two different lever arms of the dynamometer using weights of known mass (0.5, 1, 2, 5 and 10 kg) and the calibration was linear within the tested range. An angle was measured with a precision potentiometer connected to the lever arm of the dynamometer.

Tests were conducted with the participants seated with their back supported and their shoulders and torso were stabilized by the seatbelts. The right arm was positioned perpendicular to the trunk in the long axis of the shoulder. The arm and forearm were flexed at 90° of the elbow joint, and held in a horizontal plane with the forearm in a supinated position. The forearm was held immobile between force transducer and the stabilizing plate within the wrist handle. The rotation axis of the elbow joint was always at the rotation axis of the equipment. Participants were instructed to exert the maximal elbow flexion as fast and hard as possible when a sound signal was emitted by a computer and to release (relax) the force as fast as they could at the second signal (3 s later) (Jaskólska et al., 2003). The peak force of each three-second test was determined. Subjects developed the MVC three times with 120 s rest between attempts. To ensure that the participants maximally activated their muscles, they had to achieve MVC within a 10% difference on three consecutive trials. If the subject did not achieve the required force, the trial was disregarded and the measurement was repeated after 180 s. The mean force from three appropriate attempts was calculated as a representative score. The average value of the three trials was used for the MVC because the average value is more reproducible and reliable than the maximal value of a single trial (Heinonen et al., 1994).

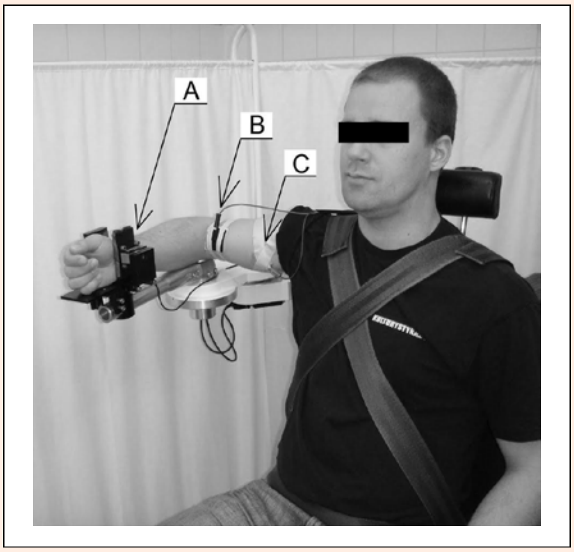

Figure 1. Experimental setup for the BIODYNA system to measure elbow flexion torque. (A) Force transducer; (B) anode; (C) cathode.

Single and double twitch responses were collected to quantify the amount of LFF, by calculating a ratio of the DT to ST force (DT/ST ratio) using the same force transducer used for MVC assessment. Electrical stimulations (0.1 ms, constant current, Model S88K, Astro-Med, Inc., Grass Instrument Division, Warwick, USA) were delivered to the motor point of the biceps brachii muscle by the surface gel pad cathode located at previously determined motor point between the anterior edge of the deltoid muscle and the elbow crease and a surface carbon rubber anode placed over the distal tendon of the biceps brachii muscle. Intensity of stimulation was set 10% above the level required to evoke a resting twitch of maximal amplitude. The internal frequency of the double twitch was 100 Hz (10 ms pause between pulses). A series of three single and three double stimuli at 5 s intervals were delivered to the relaxed muscle before the first MVC trial (a total of 6 stimuli were delivered). The torques for single and double twitch were recorded and averaged from three traces (three for single and three for double twitch separately). Previous studies have confirmed that DT/ST ratio is an effective method to quantify LFF (de Ruiter et al. 2005; Iguchi et al. 2010; Meszaros et al. 2010).

Pain assessment was performed in the same position as MVC assessment before strength measurements were performed. The device used to assess pain consists of a probe with pressure transducer (that allows to obtain the pressure in the range from 0.25 – 2 kg) and a handle. Investigator grasped the handle and put the testing end of the probe on the central part of the biceps brachii muscle and pressed until the pressure of 2 kg was achieved. The maximal value of pressure was chosen to ensure the exposure of even minimal pain sensation that could occur after ECC. After that the investigator released the device and the subjects were asked to indicate the level of soreness on a visual analog scale consisting of 100 mm continuous line representing "no pain" at one end (0 mm) and "very painful" at the other (100 mm). The point where testing end of the probe was placed was marked by permanent, waterproof marker to ensure repeatability during consecutive measurements. Data for muscle soreness are presented in millimeters.

After the first measurement session subjects performed the first bout of eccentric exercise (ECC1) of the right elbow flexors using a dumbbell adjusted to ~ 75% of each individual's MVC recorded at an elbow angle of 90°. This level was chosen because it was the greatest load that allowed finishing exercise protocol for most of the subjects in this experiment. The subjects were instructed to lower the dumbbell from an elbow flexed (~50º) to the full elbow extension (~180º) in 5 s, keeping the velocity as constant as possible, by following the examiner's counting "0" for the beginning and "1, 2, 3, 4, and 5" for the movement. The researcher controlling exercise gave instructions how to perform the exercise correctly and verbally indicated improving movement technique when it was necessary. After each eccentric action, the examiner removed the load, and the arm was returned passively to the start position. The movement was repeated every 45 s for 30 repetitions and this long interval was chosen to minimize the effect of fatigue. The cadence and exercise velocity were controlled using a metronome and a stopwatch. All participants performed the second bout of eccentric exercise (ECC2) with the same arm using the same dumbbell 2–3 weeks after the first bout. The 2–3 week break was used to ensure that MVC has returned to at least 90% of the pre-ECC value (10 subjects performed ECC2 after 2 weeks and 6 subjects after 3 weeks). The protocol for ECC2 was identical to that of ECC1 and performed under the same examiner's supervision.

Statistical analysis

Data were analyzed using SPSS 21.0 software. Dependent variables (MVC, ST, DT, DT/ST and DOMS) were analyzed by two-way ANOVA (2 bout x 4 time, 8 test sessions) for repeated measures, and a Bonferroni post-hoc procedure was used to find a specific difference between bouts and measurement time. The test-retest reproducibility was estimated by intraclass correlations coefficient (ICC) and coefficient of variations (CV). Measurements performed before each bout, were taken to analysis for test vs. retest. A significance level was set at $p < 0.05$. All data are presented as mean ± SD.

Results

Two-factorial ANOVA for repeated measures showed a significant ($p < 0.05$) effect for measurement time, bout and an interaction of both for MVC, ST, DT, and DT/ST ratio.

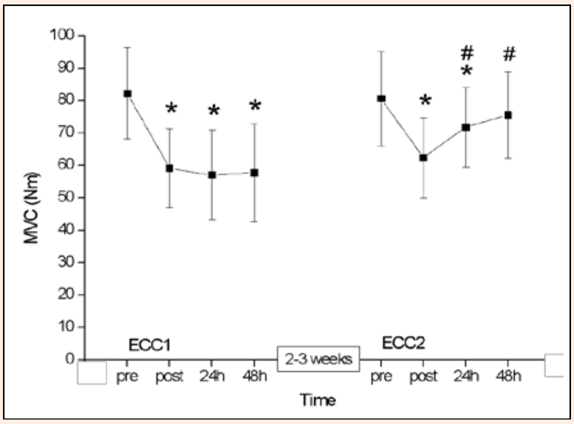

Figure 2. Changes in maximal isometric torque of the elbow flexors muscles before (pre), immediately after (post), 24 and 48 hours following the first (ECC1) and the second bout (ECC2) of eccentric exercise 2-3 weeks later. * indicates significant ($p < 0.05$) difference compared to pre, # indicates significant ($p < 0.05$) difference compared to the first bout, h – hours after exercise. Data are presented as mean and ± SD.

Figure 2 presents data for MVC before and after two bouts of ECC. There was a significant decrease ($p < 0.001$) in MVC (81 ± 16 Nm) immediately post ECC1, 24h and 48h after ECC1 (MVC reduced 28 ± 21%, 30 ± 24% and 30 ± 26% respectively, mean ± SD, $p < 0.05$). The second bout of eccentric exercise caused a significant decrease in MVC (79 ± 19 Nm) immediately post ECC2 and 24h after ECC2 (MVC reduced 23 ± 20% and 11 ± 17 % respectively, mean ± SD, $p < 0.05$). Moreover, the magnitude of the decrease of the MVC in measurements performed at 24h and 48h after ECC2 was significantly smaller when compared to respective time point after ECC1 ($p < 0.05$). There was no significant difference ($p > 0.05$) between mean MVC values for test-retest reproducibility. ICC was R = 0.98, and CV was 17 % before ECC1 vs. 18 % before ECC2.

Figure 3 presents data for ST and DT before and after two separated bouts of ECC. There was a significant decrease ($p < 0.05$) in ST and DT immediately post, 24h and 48h after ECC1 (ST reduced 48 ± 22%, 47 ± 24% and 43 ± 27% whereas DT reduced 25 ± 20%, 25 ± 21% and 24 ± 23%, respectively, mean ± SD, $p < 0.05$). The second bout of eccentric exercise caused a significant decrease in all ST measurements performed after ECC, but the magnitude of the decrease was significantly smaller at 24h and 48h after ECC2 when compared to respective time points after ECC1 (ST reduced 40 ± 25%, 22 ± 16% and 13 ± 16%, mean ± SD, $p < 0.05$). The second bout of eccentric exercise caused a significant decrease in DT in measurements performed immediately post and 24h after ECC2 (DT reduced 21 ± 18% and 12 ± 17% respectively, mean ± SD, $p < 0.05$), and the magnitude of the decrease was significantly smaller at 24h and 48h after ECC2 when compared to respective time points after ECC1 ($p < 0.05$). There was no significant difference ($p > 0.05$) between mean ST and DT values for test-retest reproducibility. ICC for ST was R = 0.97 and ICC for DT was R = 0.98, whereas CV for ST was 19% before ECC1 vs. 16% before ECC2 and CV for DT was 20% before ECC1 vs. 18% before ECC2.

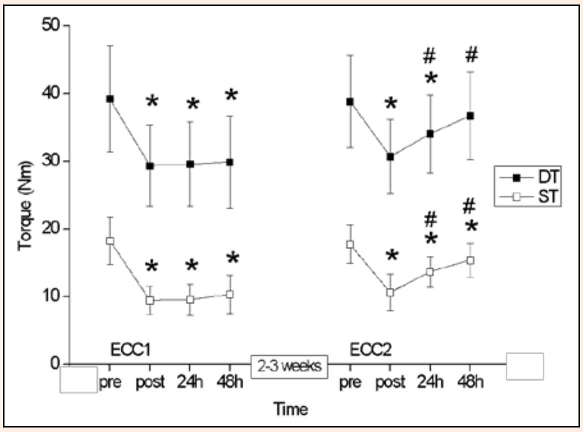

Figure 3. Changes in single (ST - open squares) and double (DT - filled squares) twitch torque before (pre), immediately after (post), 24 and 48 hours following the first (ECC1) and the second bout (ECC2) of eccentric exercise 2-3 weeks later. * indicates significant ($p < 0.05$) difference compared to pre, # indicates significant ($p < 0.05$) difference compared to the first bout, h – hours after exercise. Data are presented as mean and ± SD.

Figure 4 presents DT/ST ratio as a measure of LFF after two separate bouts of ECC. There was a significant increase in DT/ST ratio in all measurements performed after each bout of ECC ($p < 0.05$). However, the values of DT/ST ratio noted 24h and 48h after ECC2 were significantly lower than in corresponding time after ECC1 ($p < 0.05$). There was no significant difference ($p > 0.05$) between mean DT/ST ratio values for test-retest reproducibility. ICC was R = 0.83, and CV was 4 % before ECC1 vs. 3.6 % before ECC2.

Figure 5 presents data for DOMS developed after each exercise bout. Subjective pain sensation significantly increased at 24h and 48h after ECC1 ($p < 0.05$). The second bout resulted in significantly smaller pain perception at 24h and 48h after ECC when compared to ECC1 ($p < 0.05$). There was no significant difference ($p > 0.05$) between mean muscle soreness values for test-retest reproducibility. ICC was R = 0.61, and CV was 130% before ECC1 vs. 139% before ECC2.

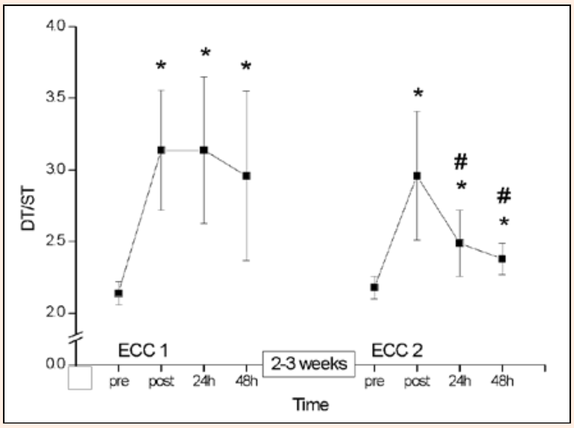

Figure 4. Changes in double to single twitch ratio (DT/ST) before (pre), immediately after (post), 24 and 48 hours following the first (ECC1) and the second bout (ECC2) of eccentric exercise 2-3 weeks later. * indicates significant ($p < 0.05$) difference compared to pre, # indicates significant ($p < 0.05$) difference compared to the first bout, h – hours after exercise. Data are presented as mean and ± SD.

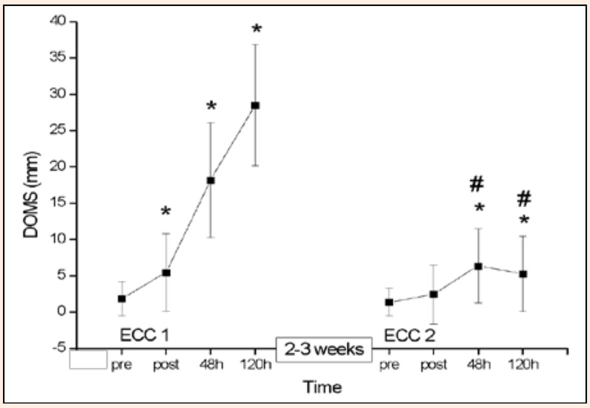

Figure 5. Changes in soreness of the biceps brachii muscle before (pre), immediately after (post), 24 and 48 hours following the first bout of eccentric exercise and the second bout of eccentric exercise 2-3 weeks later. * indicates significant ($p < 0.05$) difference compared to pre; # indicates significant ($p < 0.05$) difference compared to the first bout; h – hours after exercise. Data are presented as mean and ± SD.

Discussion

The new finding of this study is that two separate bouts of ECC of the elbow flexors induced prolonged (up to 48h after exercise) low-frequency fatigue of the biceps brachii muscle assessed with a DT/ST ratio. However, the increase in LFF was significantly lower at 24h and 48h after ECC2 when compared to ECC1. These results confirm our hypothesis that repeated bout effect attenuates LFF, and is accompanied by a faster MVC recovery and smaller DOMS development after ECC2 compared to ECC1.

The first bout of eccentric exercise reduced MVC, ST, DT in all measurements. Long-term decreases of force generating capacity are generally known and occur for both voluntary and stimulated contractions (Janecki et al., 2014; Prasartwuth et al., 2005). One of the explanations of the prolonged reduction in muscle force is impairment in E-C coupling. Takekura et al. (2001) reported damage of the muscle triads system (T-tubule and terminal cisternae of the sarcoplasmic reticulum) after downhill running in rats that probably disturbed the link between excitation of the sarcolemma and calcium release to the sarcoplasm (Prasartwuth et al. 2005). Another explanation of the changes in muscle force after ECC is a mechanical sarcomeres disruption that may to lead to a decrease in the number of the actino-myosin cross-bridges (Proske and Morgan, 2001). Similarly to the contractile properties, the prolonged recovery in LFF could also be attributed to exercise-induced muscle damage that impairs E-C coupling system (Morgan and Allen, 1999). The initial muscle membrane system injury leads to an influx of the extracellular calcium into the sarcoplasm (Takekura et al., 2001). This elevated sarcoplasmic calcium level in turn activates the enzymatic mechanisms that trigger local inflammatory response that may be associated with muscle rebuilding and repair processes (Proske and Allen, 2005). These processes may include: removal of destroyed sarcomeres, longitudinal addition of sarcomeres, cell membrane strengthening (Takekura et al., 2001), and structural protein reorganization (Yu et al., 2004; Lehti et al., 2007). One of the symptoms of inflammatory response is DOMS that significantly increases in measurements performed at 24h and 48h after ECC.

To our best knowledge this is the first report of changes in LFF estimated as a DT/ST ratio after two separated bouts of eccentric exercise of the elbow flexors. We have found similar LFF immediately after both bouts ECC. Smith and Newham (2007) have suggested that LFF during the first 20 min after ECC could be explained by metabolite changes, such as lactate and pH. Therefore, although our protocol was designed to minimize the effect of metabolic fatigue, some changes in the above parameters immediately after our ECC cannot be excluded. The prolonged recovery of LFF that occurs after single bout of eccentric exercise, has been reported on several muscle groups (Iguchi et al., 2010; Meszaros et al., 2010; Power et al., 2010), including elbow flexors (Dundon et al., 2008; Smith and Newham 2007). It is believed that prolonged LFF is the result of uncoupling of E-C process that leads to a reduction in rate of calcium release from the sarcoplasmic reticulum per action potential (Jones, 1996). With the increase of stimulation frequency, there is an increase of sarcoplasmic calcium concentration that diminishes negative effect of lower rate of calcium release on muscle force generating capacity (Hill et al., 2001). During voluntary contraction the central nervous system regulates the firing frequency of the motoneurons by changes in descending neural drive, which could be altered after ECC (Launikonis et al., 2009). It could explain why ECC-induced decreases in MVC reported in our study are smaller than decreases in ST. Moreover LFF occurrence, measured as DT/ST ratio, represents smaller decrease of DT than ST, what is presented on Figure 3. As the values of MVC and DT obtained this study are relatively less reduced by each bout of ECC than the ST, double to single twitch ratio could be used as a more sensitive indicator of the extent of the exercise-induced muscle damage than the MVC. Moreover, as the DT/ST ratio minimizes the influence of volitional reactions to the noxious stimulation that could be evoked by multiple stimulation trains that may affect strongly on the force curve obtained during measurements (Meszaros et al., 2010). Therefore this mode of stimulation could be more appropriate to assess the recovery processes at peripheral level (which exclude the influence of the central nervous system).

Although it was expected that the ECC1 would evoke prolonged LFF, there is limited evidence about changes in this parameter after the second bout of ECC. Kamandulis et al. (2010) have estimated LFF of the quadriceps femoris muscle as the ratio of force evoked by electrical stimulation at 15 and 50 Hz, and revealed that LFF was significantly less apparent 24h after the second bout of stretch-shortening cycle exercise when compared to respective time point after the first bout. In this study we have found that the value of the LFF assessed as DT/ST ratio is significantly greater up to 48h after each bout of ECC of the elbow flexors muscles, although this increase was significantly less apparent after the ECC2 than after ECC1. The results presented by Kamandulis et al. (2010) indicate that LFF of the knee extensors assessed at 24h after exercise recovers faster to baseline values than LFF of the biceps brachii muscle evaluated in our study, independently of the exercise bout. These results are in agreement with previous studies that have proved, that elbow flexors muscles could be more susceptible to ECC induced muscle damage than knee extensors (Jarmutas et al., 2005; Paschalis et al,. 2010). However, the differences of both exercise protocol used and the method of LFF assessment in our investigation and Kamandulis et al. (2010) cannot be excluded.

Significantly attenuated LFF after ECC2 compared to ECC1 provides the evidence (together with faster MVC, ST, DT recovery and smaller DOMS development) that repeated bout effect has occurred, and DT/ST ratio could be used as another indirect marker to evaluate the progress of this phenomenon. The sports trainers and physical therapists often used MVC to assess the recovery of muscle after ECC. However in some cases it seems to be inappropriate because it could not reflect some subtle deficits of force generating capacity which could affect the final training results and/or quality of exercise performance. The single twitch and DT to ST ratios seem to be more susceptible for minimal force deficits that could occur even after repeated ECC where repeated bout effect should confer the protection after subsequent muscle damage. This is because during possible neural adaptation during MVC (McHugh, 2003) which can compensate subtle force deficits. Therefore single twitch recordings and double to single twitch ratio could be used as sensitive tools in the evaluation of muscle recovery and adaptation to repeated eccentric exercise. Future studies incorporating the same muscle group and performing the same exercise protocol, should determine which mode of LFF measure (double to single twitch ratio or multiple stimulation trains) is more appropriate and sensitive in the evaluation of muscle recovery and adaptation to repeated eccentric exercise.

Conclusion

The results of this study revealed that the first bout of eccentric exercise of the elbow flexors muscles induced repeated bout effect that lead to faster recovery of the low-frequency fatigue, the maximal torque of voluntary and electrically evoked contractions, and smaller soreness sensation, when the exercise are repeated 2-3 weeks later. Double to single twitch ratio could be used as a sensitive tool in the evaluation of muscle recovery and adaptation to repeated eccentric exercise.

Acknowledgments

This work was supported by National Science Centre Grant DEC-2011/01/N/NZ7/00012.

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Key points

- First bout of eccentric (ECC) exercise of the elbow flexors muscles induced repeated bout effect that lead to faster recovery of the low-frequency fatigue, the maximal torque of voluntary and electrically evoked contractions, and smaller soreness sensation, when the exercise are repeated 2-3 weeks later.
- Double (DT) to single twitch (ST) ratio could be used as a sensitive tool in the evaluation of muscle recovery and adaptation to repeated eccentric exercise.
- The single twitch and DT to ST ratios seem to be more susceptible for minimal force deficits that could occur even after repeated ECC where repeated bout effect should confer the protection after subsequent muscle damage.

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