รายงานวิจัยฉบับสมบูรณ์

โครงการ

วิธีการออกกำลังกายแบบที่กล้ามเนื้อยืดยาวออก ในระดับเบา ปานกลาง และหนัก มีผลช่วยในการป้องกันผลเสียจากการออกกำลังกายในระดับหนัก ในแง่ของการบาดเจ็บของกล้ามเนื้อ และการเปลี่ยนแปลงของระบบประสาทได้อย่างไร

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31 มีนาคม 2554

สัญญาเลขที่ MRG5280246

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สนับสนุนโดยสำนักงานคณะกรรมการอุดมศึกษาและสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกอ. และสกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิตติกรรมประกาศ

คณะผู้วิจัยถือเป็นเกียรติอย่างสูงที่ได้รับการสนับสนุนการทำวิจัย ในด้านงบประมาณการดำเนินการ การนำเสนอผลงานเพื่อติดตามความก้าวหน้าของงานวิจัย และการเผยแพร่ผลงานวิจัยในระดับนานาชาติ ในการนี้คณะผู้วิจัยใคร่ขอขอบคุณ สำนักงานคณะกรรมการอุดมศึกษาและสำนักงานกองทุนสนับสนุนการวิจัย ที่มีส่วนผลักดันให้เกิดการสร้างองค์ความรู้ใหม่ ซึ่งจะเป็นประโยชน์ต่อการเรียนการสอนในระดับอุดมศึกษาต่อไป ซึ่งผลงานวิจัยนี้สามารถนำไปประยุกต์ใช้ให้เกิดประโยชน์ต่อประชาชนทั่วไปที่สนใจการออกกำลังกายเพื่อเสริมสร้างสุขภาพที่ดี และต่อวงการกีฬาของประเทศชาติต่อไป การดำเนินการวิจัยในครั้งนี้คณะผู้วิจัยเองยังได้พัฒนาทักษะการเรียนรู้และการวิเคราะห์อย่างมีเหตุผลเพิ่มขึ้นด้วย

นอกจากนี้ คณะผู้วิจัยขอขอบคุณ ภาควิชากายภาพบำบัด คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่ ที่ให้การสนับสนุนด้านเวลา สถานที่ และเครื่องมือในการทำวิจัย และที่สำคัญเป็นอย่างยิ่งคือ อาสาสมัครทุกท่านที่สละเวลาอันมีค่ายิ่ง เพื่อเข้าร่วมงานวิจัยในครั้งนี้ ทำให้งานวิจัยประสบความสำเร็จลุล่วงเป็นอย่างดี

บทคัดย่อ

รหัสโครงการ: MRG5280246

ชื่อโครงการ: วิธีการออกกำลังกายแบบที่กล้ามเนื้อยืดยาวออกในระดับเบา ปานกลางและหนักมีผลช่วยในการป้องกันผลเสียจากการออกกำลังกายในระดับหนัก ในแง่ของการบาดเจ็บของกล้ามเนื้อและการเปลี่ยนแปลงของระบบประสาทได้อย่างไร

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ระยะเวลาโครงการ: 2 ปี

การออกกำลังกายแบบกล้ามเนื้อยืดยาวออกซ้ำ สามารถใช้เป็นแนวทางเพื่อลดการบาดเจ็บต่อกล้ามเนื้อ ภายหลังการออกกำลังกายแบบนี้ การบาดเจ็บของกล้ามเนื้อมีส่วนทำให้เกิดการลดลงของแรง อย่างไรก็ตาม การลดลงของกระแสประสาทก็มีส่วนอย่างมากเช่นกัน งานวิจัยนี้มีวัตถุประสงค์คือเพื่อประเมินว่าการออกกำลังกายที่ระดับเบา ปานกลาง และหนักในเซตแรก มีผลต่อการลดลงของแรงจากการออกกำลังกายในเซตที่สองอย่างไร และประเมินกลไกที่เกี่ยวข้องว่าเป็นผลจากการปรับตัวของระบบประสาท และ/หรือ การปรับตัวของระบบกล้ามเนื้อ อาสาสมัครสุขภาพดี 30 คน ถูกจัดให้เข้ากลุ่มเพื่อไม่ให้เกิดความแตกต่างของระดับแรงสูงสุด โดยแบ่งกลุ่มออกเป็น 3 กลุ่มๆ ละ 10 คน อาสาสมัครออกกำลังกายของกลุ่มกล้ามเนื้องอข้อศอก โดยให้กล้ามเนื้อทำงานแบบยืดยาวออก 30 ครั้ง ที่ระดับความหนัก 10%, 20% และ 40% ของแรงสูงสุดในเซตแรก จากนั้น 2 สัปดาห์ ทุกกลุ่มทำการออกกำลังกายซ้ำ ที่ระดับความหนัก 40% ของแรงสูงสุด วัดระดับแรงสูงสุด ระดับพลังประสาท และแรงกระตุกขณะพัก โดยวัดก่อนและทันทีหลังการออกกำลังกาย วันที่ 1 และ 4 หลังการออกกำลังกายในเซตที่ 1 และ 2 ผลการศึกษาพบว่าหลังการออกกำลังกายเซตแรก ทั้งสามกลุ่มมีการลดลงอย่างมีนัยสำคัญทางสถิติของแรงสูงสุด ระดับพลังประสาท และแรงกระตุกขณะพัก ทันทีหลังการออกกำลังกายเซตที่ 2 ทั้งสามตัวแปรของทั้งสามกลุ่มไม่มีความแตกต่างกัน อย่างไรก็ตามวันที่ 1 และ 4 หลังการออกกำลังกาย กลุ่มที่ออกกำลังกายระดับหนัก มีการฟื้นตัวของแรงดีกว่ากลุ่มอื่นๆ แสดงให้เห็นว่าเป็นผลจากการลดการบาดเจ็บของการออกกำลังกายซ้ำ การฟื้นตัวของแรงที่ดีขึ้นนี้สอดคล้องกับการเปลี่ยนแปลงของระดับพลังประสาท แสดงให้เห็นว่าเกิดการปรับตัวของระบบประสาทในช่วงแรก ในขณะที่แรงกระตุกขณะพักของกลุ่มที่ออกกำลังกายระดับหนัก แตกต่างจากลุ่มอื่นๆ ในวันที่ 4 แสดงให้เห็นว่ามีการปรับตัวของระบบกล้ามเนื้อ สรุปการฟื้นตัวของแรงจากการออกกำลังกายแบบกล้ามเนื้อยืดยาวออกซ้ำในช่วงแรกเป็นผลจากการปรับตัวของระบบประสาท และจากนั้นเป็นผลการปรับตัวของระบบกล้ามเนื้อร่วมด้วย

คำหลัก: การบาดเจ็บของกล้ามเนื้อ, การออกกำลังกายซ้ำ, การออกกำลังกายแบบกล้ามเนื้อยืดยาวออก

ABSTRACT

Project Code:	MRG5280246
Project Title:	How do the low, moderate and high loads of eccentric exercises affect muscular damage and neural changes against high load eccentric exercise?
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E-mail Address:	oprasa@chiangmai.ac.th
Project Period:	2 years

Repeated bout effect (RBE) is known as a protective way to reduce the eccentric damaging exercise. After eccentric exercise (ECC), muscle damage reduces maximal voluntary force; however, impaired neural drive to the muscle has also primarily contributed. We aimed to investigate whether three sub-maximal loads could contribute differently to force loss in the repeated bout effect and to clarify the underlying mechanisms in term of neural and muscular adaptations. Thirty healthy subjects were selectively placed into three groups to match for maximal voluntary contraction (MVC) (n = 10 per group), performing 30 eccentric actions of the elbow flexors of 10%, 20% and 40% of MVC for ECC1, followed 2 weeks later by a similar exercise (ECC2) that used 40%MVC load. MVC, voluntary activation and resting twitch were measured before, immediately, day 1 and day 4 after exercise following ECC1 and ECC2. The results showed that for the first eccentric bout, MVC, voluntary activation and the resting twitch were significant ($p < 0.0001$) interaction (group x time). Following the second eccentric bout immediately after exercise, there were no significant (group x time) interaction in all outcome variables. However, at day 1 and 4, only high-load group demonstrated significantly ($p < 0.01$) greater improvement in maximal voluntary force compared with the first high-load eccentric bout, indicating greater recovery in force generating capacity as RBE. This force recovery at day 1 and 4 corresponded with a significant ($p < 0.01$) improvement in voluntary activation, indicating better the level of neural drive to the muscle or neural adaptations in the early stage. In contrast, the resting twitch in high-load group demonstrated a significant ($p < 0.01$) improvement only at day 4, suggesting muscular adaptations. In conclusion, greater force recovery in the second eccentric damaging exercise at day 1 and 4 measured in high-load group could primarily be the neural adaptations. There is a contribution of muscular adaptations at day 4 as well.

Keywords: Muscle damage, Repeated bout effect, Eccentric exercise

เนื้อหางานวิจัย

Introduction

High force eccentric exercise induces prolonged and marked muscle fatigue in unfamiliar persons. Immediately after exercise, muscle becomes stiffness causing limitation in joint range of motion. Muscle soreness also pronounced lately at day 1-2 (1-6). These changes have well-investigated demonstrating cellular and muscular changes (7). For cellular changes, an increase in muscle-specific proteins in the blood e.g. creatine kinase or myoglobin indicated damages in muscle fibers(8). These damages can also be visualized by electron microscopy, ultrasound and magnetic resonance imaging illustrating disruption in contractile apparatus (9-13). Connective tissues (perimysium or endomysium) could also be damaged, illustrating an increase in urine hydroxyproline (14) and serum type I collagen concentration (15). However, researches in the contribution of neural control are limited and the results remain controversial. With tetanic stimulation, there were no increases in the superimposed twitch elicited by elbow flexors during maximal effort (16). In contrast, single pulse stimulation confirmed a reduction in voluntary action or an impaired neural drive to the muscle (6, 17). This discrepancy might be related to technical sensitivity using tetanic and single twitch stimulation. Therefore, underlying mechanisms in force deficit could be primarily attributed to neural and secondarily to muscular contribution.

Once performed exercise again or repeated bout exercise, it could reduce prolonged and marked force loss, muscle stiffness and muscle soreness (1, 18, 19). These improvements are referred as the protective adaptation or repeated bout effect

(RBE) (20). Similar to the first bout, the cellular and muscular adaptations are well-documented involving blunted inflammatory responses (21, 22), addition of series sarcomeres (23-26) and rightward shift in length-tension curve (17, 27, 28). On the other hand, limited evidence supports the neural adaptations in RBE. Even in resistance training, any improvement in muscle force which could not be explained by changes in muscle size has been addressed to neural adaptations. In addition, changes in cross-training to contralateral muscles (29-34) have been used to confirm neural adaptations. For the repeated bout effect, contralateral eccentric training appeared predominantly indicating neural adaptations (35).

Several lines of the evidence for neural adaptations in RBE have been drawn from surface electromyography (EMG) recording. During the second bout of eccentric contractions, a decrease in the median frequency of EMG activity was found indicating an increased activation of slow motor unit and a concomitant decrease in activation of fast units (36, 37). However, the questions for methodological reasons (38-40), have been addressed as a result of motor unit synchronization (7) and signal cancellation (41). Therefore, another possible way to identify neural adaptations includes changes in the pattern of neural activity associated with motor drive. Adaptive modifications in neural circuits could involve either in descending command at the motor cortex or in the final neural drive to the muscle at the spinal cord. Even though no previous research reported these modifications as a result of RBE, resistance training consisting concentric and eccentric muscle actions could alter the functional properties of spinal circuitry, but less affect the organization of the motor cortex (42). With eccentric muscle actions, there is

an evidence of lower excitability of the corticospinal tract (43). By defining the possible sites, we firstly need to confirm whether there is neural adaptation in RBE or not.

As limited research has been conducted to reveal the neural adaptations in RBE, therefore the underlying mechanisms regarding to neural adaptations to RBE is still unclear. Recently, a research has reported no neural adaptations in RBE in the knee extensors by using tetanic electrical stimulation (44). However, base on our previous research of the first eccentric bout, neural contribution primarily plays an important role. Therefore, further researches are still needed and the knowledge will add our understanding in neural adaptations of RBE. Our first aim was to investigate whether RBE is the neural contribution or not. We hypothesized that neural adaptations could be identified with an improvement in voluntary activation in the RBE.

In addition, the magnitude of RBE does not appear to depend on the number of eccentric contractions, but depends on the magnitude of load in the first bout. For example, Chen et al 2009 reported that the magnitude of the repeated bout effect was smaller for 40% or 60% MVC load than 80% or 100% MVC load. In contrast, a recent study has shown that submaximal eccentric exercise (40% MVC load) conferred the same magnitude of RBE as that produced by one maximal eccentric exercise bout. Although it seems likely that the different loads in the initial bout of eccentric exercise could attribute to minimize the magnitude of muscle damage, we still do not know that low-, moderate- or high-load could be recommended to use as the best repeated bout effect. Revealing this information could be benefit to injury prevention in athletes during training, competition and rehabilitation. Therefore, this study was also designed to

investigate whether the three different levels of eccentric load in the first bout could confer the different changes in RBE. Our second aim was to determine whether the low-, moderate- or high-load of the initial eccentric exercise could affect to the degree of subsequent protection effect. We hypothesized that high-load of the initial eccentric exercise could confer the best RBE.

Methods

Subjects and study design

Thirty healthy volunteers who had not performed regular resistance training gave written informed consent. Subjects were selectively placed into one of three groups (n = 10 per group) based on the baseline maximal voluntary isometric contraction torque (MVC) of the elbow flexors at an elbow joint angle 90^0. The groups were performed; low-load at 10%ECC, moderate-load at 20%ECC and high-load at 40%ECC as explained below, and all groups had similar mean baseline MVC, with no significant differences in age, height or body mass across groups (Table 1). All subjects were asked to refrain from unaccustomed exercise or vigorous physical activity for at least 6 months before the experiment and to not to take any anti-inflammatory drugs or nutritional supplements during the experimental period. All subjects performed the second bout at the same high-load (40%ECC) of elbow flexor eccentric exercise with the same arm separated by 2 weeks. A number of measurements were taken before, immediately after, 1 and 4 days after eccentric exercise for the first and second bouts. The main dependent variables include MVC, voluntary activation, resting twitch, elbow angles and degree of muscle soreness.

Eccentric exercise

All subjects performed the first eccentric exercise (ECC1) of the elbow flexors with the non-dominant arm using a dumbbell adjusted to either 10%, 20% or 40% of individual's MVC at an elbow angle of 90^0 (Figure 1). The subjects were instructed to lower the dumbbell from an elbow flexed ~90^0 to an elbow extended position (0^0) in 4-5

second, keeping the velocity as constant as possible by following the examiner's counting "start" and "1, 2, 3, 4 and 5" for the movement. After each eccentric contraction, the examiner lifted the load up while subjects relaxed and the arm was passively returned to the starting position. The exercise consisted of 3 sets 10 repetitions with a 2-minute rest between sets.

Figure 1 Subject performed eccentric exercise using dumbbell.

Two weeks after ECC1, all subjects performed the second eccentric exercise bout (ECC2) with the same arm using a dumbbell adjusted to 40% of individual's MVC at an elbow angle of 90º. The exercise protocol for ECC2 was the identical to that of ECC1 (Figure 2-4).

Group I: Low load of eccentric exercise

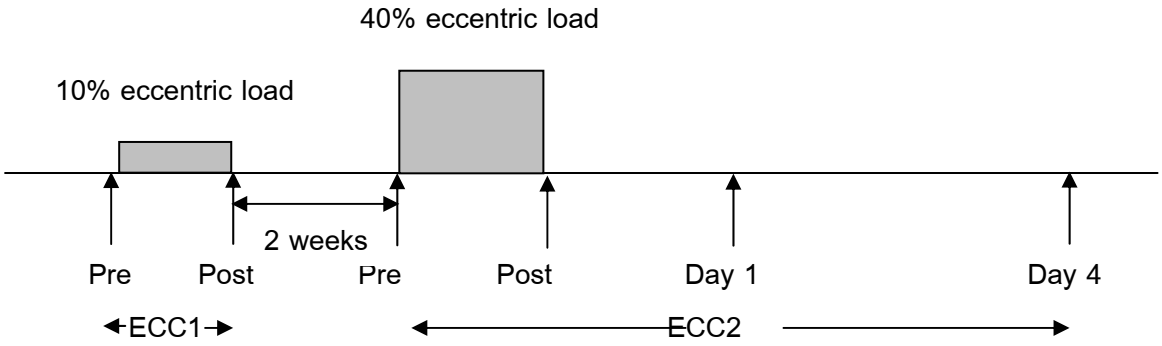

Figure 2 Schematic drawing represents low load group of eccentric exercise (group I). Low load was set at 10% of maximal voluntary isometric contraction for the first bout of eccentric exercise and high load was set at 40% of maximal voluntary isometric contraction for the second bout.

Group II: Moderate load of eccentric exercise

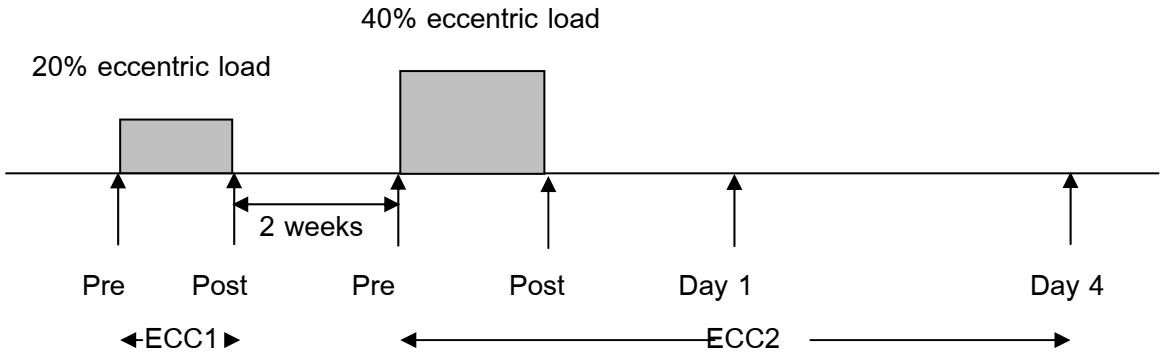

Figure 3 Schematic drawing represents moderate load group of eccentric exercise (group II). Moderate load was set at 20% of maximal voluntary isometric contraction for the first bout of eccentric exercise and high load was set at 40% of maximal voluntary isometric contraction for the second bout.

Group III: High load of eccentric exercise

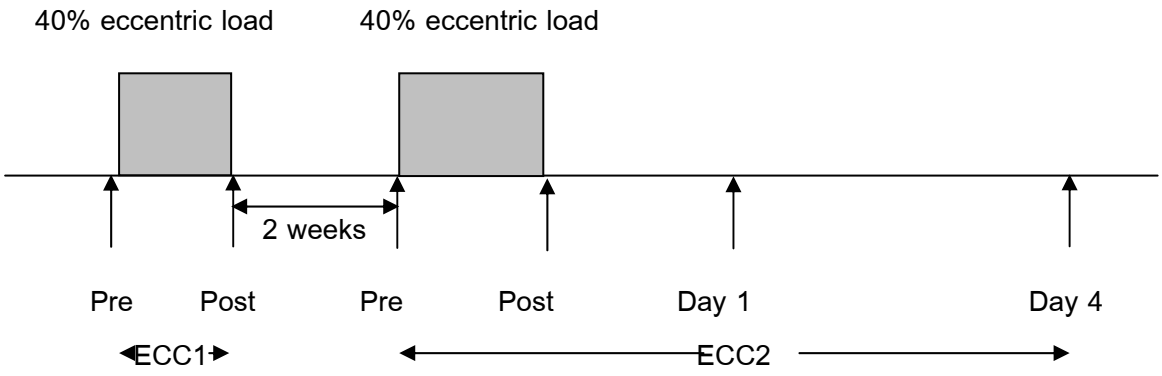

Figure 4 Schematic drawing represents high load group of eccentric exercise (group III). High load was set at 40% of maximal voluntary isometric contraction for both bouts of eccentric exercise.

Motor nerve stimulation

For stimulation of the motor nerve, single electrical stimuli (100 μs duration, constant current, DS7, Digitimer) (Figure 5) were delivered to intramuscular nerve fibres innervating biceps brachii via a surface cathode located midway between the anterior edge of the deltoid and the elbow crease and a surface anode positioned over the distal biceps tendon (Figure 6). The stimulation intensity was set at 10% above the level required to produce a resting twitch of maximal amplitude. The stimulus intensity was set at each measurement session. The sites of stimulation were marked on the skin to ensure consistent placement throughout the 3 testing sessions.

Figure 5 Peripheral Electrical Stimulator “Digitimer DS7”

Figure 6 Electrode position to stimulate intramuscular nerve fibres innervating biceps brachii

Dependent variables

MVC

Subjects sat with the arm in an arm bar attached with a force gauge (2KN, A&D CO, Ltd, Japan) connected to a digital recorder (Figure 6). This arm bar was used to measure maximal isometric flexion torque at 90 degree of elbow flexion. Three measurements of maximal effort were made and the maximal value was chose to be a peak torque. Verbal encouragement was provided during MVC measurements.

Voluntary activation and resting twitch

During the electrical stimulation of the motor nerve, any increment in elbow flexion torque evoked during a MVC ("superimposed twitch") was expressed as a fraction of the amplitude of the maximal response evoked by the same stimulus in the relaxed muscle immediately after an MVC ("resting twitch") (Figure 7). Voluntary activation was then quantified as a percentage using the formula (45):

Voluntary activation (%) = (1-superimposed twitch/resting twitch) x 100

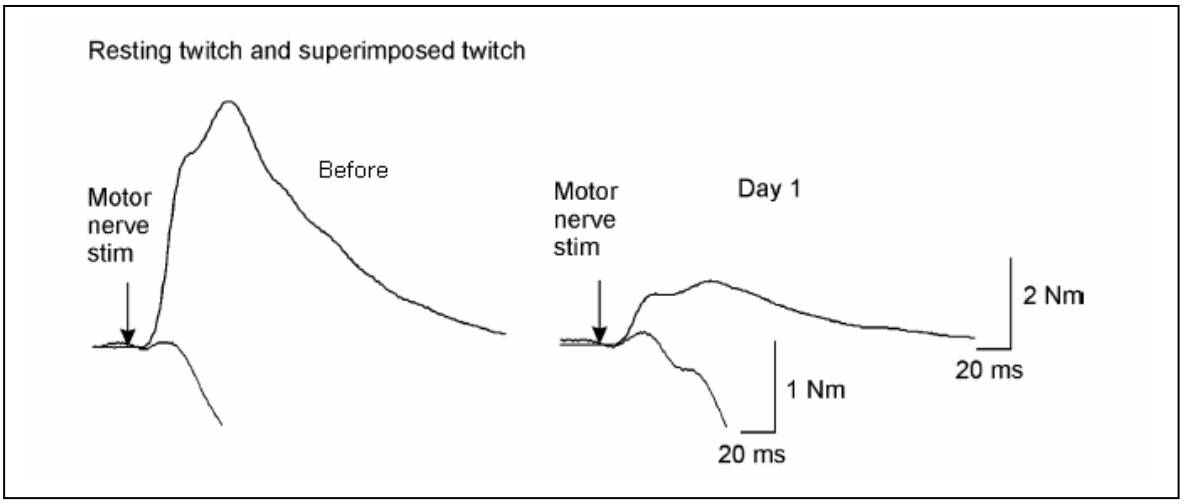

Figure 7 Typical traces from one subject of the resting twitch (longer twitch) and superimposed twitch (shorter twitch) evoked by motor nerve stimulation

Elbow joint angle and range of motion

Elbow angle was measured in three different positions: relaxed (RANG), full flexed (FANG) and full extended (EANG). Subjects stood with the arm hanging vertically and were asked to relax, fully flex and fully extend at the elbow joint angle for the measures. Two measurements of the angle were taken by using an electrical goniometer (Figure 8) and range of motion (ROM) was defined as the angle subtracting the mean FANG from the mean EANG.

Figure 8 Electrical Goniometer

Muscle soreness

Degree of muscle soreness was measured in two ways: pressure pain threshold (PPT) using a pressure algometer (Figure 9) and pain rating scale using a visual analog scale (VAS). For both measurements, subjects sat with the elbow joint at 90º in flexion, and assessments were taken from the three marked spots; 5 cm, 8 cm and 11 cm from the elbow crease over the biceps brachii. A pressure algometer with a circular disc (10 mm-diameter) was applied perpendicular to the skin over the three reference spots to measure the force at which subjects reported any discomfort or pain. A visual analog scale consists of a 100-mm continuous line representing “no pain” at one end (0mm) and “very, very painful” at the other (100 mm). Subjects were asked to indicate the soreness level on the line when the examiner applied pressure with the algometer at 2 kg.

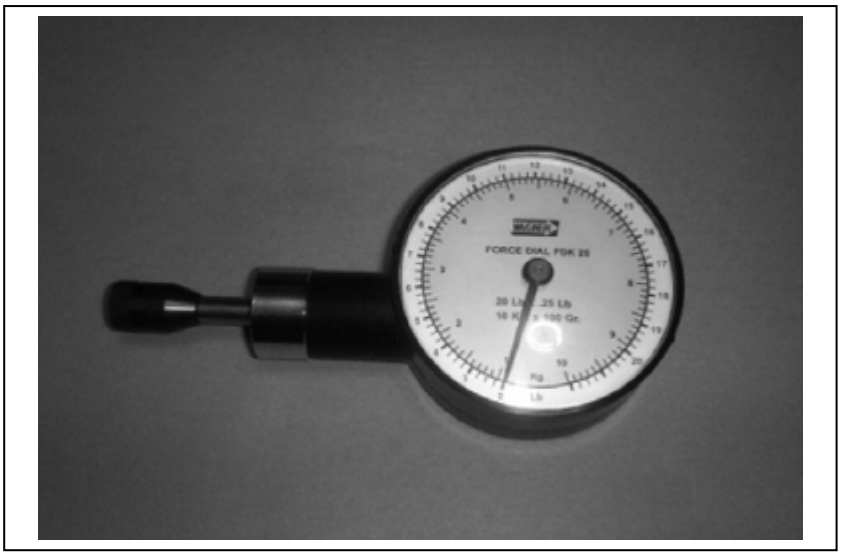

Figure 9 Pressure Algometer

Statistical analysis

The results are presented as means ± SD. Changes in the dependent variable over time were compared amongst the groups for the first bout (ECC1) and the second bout (ECC2) separately by two-way repeated measures ANOVA. The ANOVA was performed for both raw and normalized data for all variables except for voluntary activation. For the ANOVA using the normalized data, the pre-exercise values (100 for MVC, RT, PPT and VAS) were excluded. The ANOVA also compared between the first 40% bout performed by the 40-40%ECC group and the second eccentric exercise bout performed by all groups in which the 40% load was used. When the ANOVA showed a significant interaction (group x time) effect, a Tukey's HSD test was employed as post hoc analysis to locate the time points of significant differences between groups. One-way ANOVA were also used to compare between groups for each time. Statistical significance was set at $P<0.05$.

RESULTS

We hypothesized that the underlying mechanisms of the repeated bout effect could be due to the neural adaptations. To test this hypothesis, the improvements in the voluntary activation in the second bout were used to indicate the neural adaptations. With the same technique of twitch interpolation, the improvements in the resting twitch could also be indicated muscular adaptations. In addition, we would like to investigate whether the different loads of eccentric exercise in the first bout as different stimuli may induce different responses of protective effect in the second bout. To test this research question, subjects were chosen into three exercise groups i.e. low-load (10%ECC), moderate-load (20%ECC) and high-load (40%ECC) to have a similar baseline MVC. The subject characteristics of each group showed no significant difference in age, height and body mass ($P>0.05$) (Table 1).

Table 1 The anthropometric characteristics of 30 subjects who completed the eccentric exercise and 10 subjects for each group (values are mean ± SD)

	Low-load group	Moderate-load group	High-load group
Age (yr)	29.0 ± 8.1	25.5 ± 4.5	26.8 ± 4.7
Weight (Kg)	59.3 ± 13	57.0 ± 11.2	64.7 ± 33.8
Height (cm)	161.1 ± 9.3	162.9 ± 6.2	150.0 ± 34.8
Gender (M:F)	2 : 8	3 : 7	2 : 8

FIRST ECCENTRIC EXERCISE

Deficit in force production

For the first bout of eccentric exercise, with the low- and the moderate-load, the maximal voluntary isometric torques decreased by ~7% and ~13% of the pre-exercise value immediately after eccentric exercise respectively and recovered back to normal at day 4. In contrast, with the high-load, the maximal voluntary isometric torques decreased by ~46% of the pre-exercise value immediately after eccentric exercise. It only recovered to ~76% of the initial value at day 4 (Table 2). When compared between groups, changes in raw and normalized maximal voluntary isometric torque in all groups over time (group x time) were significantly ($p<0.0001$) different. Post hoc analysis (Tukey's HSD test) found significantly ($p<0.0001$) different between low- and high-load groups, low- and moderate-load groups, and moderate- and high-load groups.

Table 2 Mean values ± SD for isometric maximal voluntary contraction (MVC) at pre-exercise, post-exercise and day 4 and percentage force loss in the first eccentric bout

	MVC for 1st ECC bout (N)				
Group	Pre-exercise	Post-exercise	% force loss immediately after exercise	At day 4	% force loss at day 4
Low-load group	167.9 ± 63.8	153.9 ± 53.1	7.12	170.4 ± 55.7	0
Moderate-load group	170.02 ± 59.4	147.8 ± 54.7	12.76	187.7 ± 80.6	0
High-load group	155.0 ± 61.8	81.4 ± 31.5	45.61	105.4 ± 35.4	23.75

Voluntary activation

With the low-load, voluntary activation remained the same after the exercise up to 4 day. While the moderate-load, voluntary activation slightly decreased by 7% (from ~99% to ~ 92%) immediately after exercise and returned back to normal at day 4. With the high-load, voluntary activation markedly decreased by 33% (from ~97% to ~64%) immediately after exercise and gradually recovered to ~91% at day 4 (Table 8). When compared between groups, changes in voluntary activation in all groups over time (group x time) were significantly ($P<0.001$) different. There were significantly ($p<0.001$) different between low- and high-load groups, low- and moderate-load groups and moderate- and high-load groups.

Table 3 Mean values (± SD) for voluntary activation at pre-exercise, post-exercise and day 4 and percentage decrease in the first eccentric bout

Group	Voluntary activation for 1st ECC bout (%)				
	Pre-exercise	Post-exercise	% decrease immediately after exercise	At day 4	% decrease at day 4
Low-load group	96.85± 1.87	96.43± 2.73	0	96.76 ± 0.33	0
Moderate-load group	99.26± 0.84	92.44± 4.79	6.82	98.58 ± 1.20	0.68
High-load group	96.83± 2.64	64.21± 18.35	32.62	91.92 ± 6.06	4.91

Resting twitch

The resting twitches produced by motor nerve stimulation decreased by ~27%, ~64% and ~86% of their pre-exercise values with the low-, moderate- and high-load of the eccentric exercise respectively. They then gradually recovered to ~88%, ~73% and ~50% of their pre-exercise values at day 4 with the low-, moderate- and high-load of the eccentric exercise respectively (Table 4). Changes in the resting twitches in all groups over time (group x time) were significantly ($p=0.0001$) different. When compared the resting twitches between low- and moderate-load group, low- and high-load group, there were significantly ($p=0.01$, $p=0.0001$ respectively) different. However, there was no a significant ($p=0.095$) difference between moderate- and high-load group.

Table 4 Mean values (± SD) for the rest twitches at pre-exercise, post-exercise and day 4 and percentage decrease in the first eccentric bout

Group	Resting twitch for 1st ECC bout (N)				
	Pre-exercise	Post-exercise	% force loss immediately after exercise	At day 4	% force loss at day 4
Low-load group	12.12 ± 6.21	8.80 ± 4.27	26.7	10.66 ± 4.71	12.05
Moderate-load group	12.36 ± 3.14	4.41 ± 1.80	63.91	8.73 ± 1.77	26.55
High-load group	12.97 ± 6.26	1.98 ± 2.40	85.68	6.40 ± 4.74	49.88

SECOND ECCENTRIC EXERCISE

Deficit in force production

For the second bout of eccentric exercise, each group performed the eccentric exercise with the same high-load (40%MVC). Immediately after the exercise, the maximal voluntary isometric torques in all groups decreased by ~38-48% of their initial values. At the 4 day, the maximal voluntary isometric torques in low- and moderate-load group gradually recovered to ~90% of their pre-exercise values. Interestingly, the maximal voluntary isometric torques in high-load group returned back to normal (Table 5).

Table 5 Mean values (± SD) for isometric maximal voluntary contraction (MVC) at pre-exercise, post-exercise and day 4 and percentage force loss in the second eccentric bout

Group	Resting twitch for 1st ECC bout (N)				
	Pre-exercise	Post-exercise	% force loss immediately after exercise	At day 4	% force loss at day 4
Low-load group	171.3 ± 57.7	99.3 ± 28.2	39.06	156.9 ± 61.2	8.29
Moderate-load group	194.1 ± 84.0	101.6 ± 46.0	47.70	172.8 ± 75.9	10.94
High-load group	145.6 ± 50.4	90.9 ± 39.2	37.99	146.8 ± 51.2	0

Comparison of MVC between all groups in 2nd bout and high-load group in 1st bout as baseline

With repeated measures ANOVA, the normalized data showed a significant main effect for time ($F=145.723$, $p=0.0001$). Comparison between groups, there were also significant differences ($F=3.885$, $p=0.017$). There was also a significant interaction effect (group x time) ($F=3.250$, $p=0.007$). One-way ANOVA found significant differences

between high-load group and the baseline (the first bout of ECC exercise) at day 1 and 4 ($p=0.014$, $p=0.003$, respectively) (Figure 10).

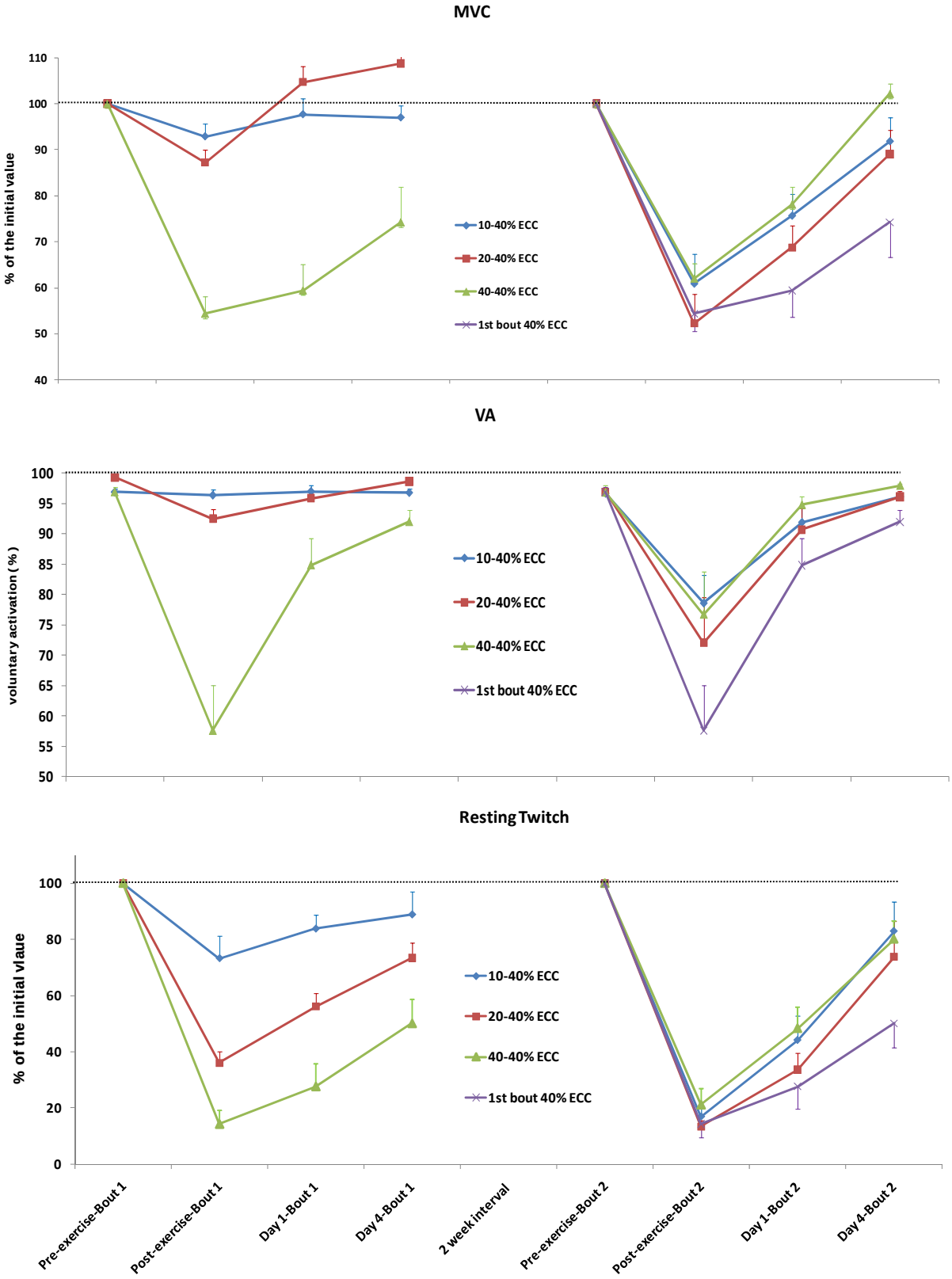

Figure 10 Comparison between the first and second eccentric bout, performed 2 weeks apart in three different loads (low-, moderate- and high-load). Top panel represents

changes in MVC. Middle panel represents changes in voluntary activation. Lower panel represents changes in the resting twitch.

Voluntary activation

For the second bout of the eccentric exercise, all groups showed the similar changes and recovery patterns as the exercise load was equal for each group (40%MVC). Voluntary activation dropped in the similar amount after exercise (~70-80%). At day 4, all groups recovered back to pre-exercise value (~96%) (Table 6).

Table 6 Mean values (± SD) for voluntary activation at pre-exercise, post-exercise and day 4 and percentage decrease in the second eccentric bout

Group	Voluntary activation for 1st ECC bout (%)				
	Pre-exercise	Post-exercise	% decrease immediately after exercise	At day 4	% decrease at day 4
Low-load group	96.60 ± 2.49	78.56 ± 14.73	19	96.50 ± 1.49	0
Moderate-load group	96.85 ± 1.83	71.56 ± 23.08	26	96.04 ± 3.08	0
High-load group	96.75 ± 3.64	76.70 ± 22.46	21	97.88 ± 1.26	0

Comparison of voluntary activation between all groups in 2nd bout and high-load group in 1st bout as baseline

With repeated measures ANOVA, the voluntary activation showed a significant main effect for time (F=40.99, p=0.0001). Comparison between groups, there were no significant differences (F=2.313, p=0.093). There was also no significant interaction effect (group x time) (F=0.638, p=0.762). With this absence of a significant interaction effect,

one-way ANOVA was applied and revealed that there were significant differences between high-load group and the baseline at day 1 and 4 (Figure 1).

Resting twitch

Similar to the voluntary activation, all three groups showed similar changes after exercise and similar recovery rate. With normalized data, the resting twitches decreased by ~81% (from 100 to 19%) after exercise and return back by ~ 26% (from 100 to 74%) at day 4 (Table 7).

Table 7 Mean values (± SD) for the resting twitches at pre-exercise, post-exercise and day 4 and percentage decrease in the second eccentric bout

Group	Resting twitch for 1st ECC bout (N)				
	Pre-exercise	Post-exercise	% force loss immediately after exercise	At day 4	% force loss at day 4
Low-load group	13.71 ± 8.11	2.31 ± 2.09	83	10.79 ± 6.35	21
Moderate-load group	12.22 ± 4.35	1.69 ± 1.15	86	8.11 ± 3.07	34
High-load group	13.34 ± 5.64	3.37 ± 4.32	74	10.45 ± 4.36	22

Comparison of the resting twitches between all groups in 2nd bout and high-load group 1st bout as baseline

With repeated measures ANOVA, the raw and normalized data showed a significant main effect for time (F=117.27, p=0.0001). Comparison between groups, there were no significant differences (F=1.103, p=0.367). There was also no significant

interaction effect (group x time) (F=0.756, p=0.526). With this absence of a significant interaction effect, one-way ANOVA was applied and revealed that there was only a significant difference between high-load group and the baseline at day 4 (Figure 1).

Changes in elbow joint angle and pain measurement

No interaction (group x time) in changes in elbow joint angle in flexed position, extended position, relax position and range of motion as well as changes in muscle soreness measured by VAS and PPT were found among all three groups. When compared with the baseline (1st bout), only changes in elbow joint angle in flexed position and range of motion showed time and group interaction (F=2.264, p=0.048, F=2.576, p=0.027 respectively). Post Hoc analysis found significantly (p=0.04) only in changes in flexed angle between low-load group and baseline.

DISCUSSION

Our main purposes of this study were to determine whether the underlying mechanisms of RBE could be related to neural adaptations and to investigate whether three different loads could confer different protective effects. This study has illustrated that with the second eccentric bout, maximal isometric force (MVCs) demonstrated significant interaction effect (Group x Time) in the high-load eccentric group. One-way ANOVA revealed at day 1 and 4, MVCs in high-load eccentric group were significant recovery compared to the baseline (First high-load bout of eccentric exercise). Therefore, RBE in this study was not an improvement of force generating capacity immediately after exercise. Our exercise protocol failed to produce this protective effect immediately after exercise but confirmed better force recovery. Our results were in the same line with previous studies (44, 46, 47).

MVC, RT and VA for the first eccentric bout

Low-, moderate- and high-load of eccentric exercise caused the decreases in maximal isometric forces by ~7%, ~13% and ~46% of their pre-exercise values, respectively. With low- and moderate-load of exercise, maximal isometric forces were recovery back to normal at day 4, whereas with high-load exercise, it still remained at ~76% of the initial value.

Corresponding with force loss, the amplitude of the resting twitches decreased by ~27%, ~64% and ~86% of their pre-exercise values for low-, moderate- and high-load of exercise, respectively. These changes in the resting twitch in high-load exercise were found in the same line with previous studies (6, 17). Using the same twitch interpolation

technique to assess the resting twitch, force loss by ~35-40% was associated with the decrease in the resting twitch by ~70-85%. However, with different frequency i.e. tetanic stimulation, force loss by ~42% was associated with the decrease in the resting twitch by ~44% (44). Less decrease in the resting twitch from tetanic stimulation could be due to an alteration of the relationship between local intracellular Ca^{++} and force (48, 49) and possibly relate to low- and high-frequency fatigue (50).

With voluntary activation, force loss by ~7%, ~13% and ~46% corresponded with 0%, ~7% and ~33% loss of level of neural drive for low-, moderate- and high-load of eccentric exercise respectively. These results suggested that impairments in voluntary activation played a role in moderate- and high-load of exercise. With high-load of exercise, previous work (6) found similar finding, however, other recent research reported different results (44). Impaired voluntary activation found after eccentric exercise in elbow flexors at 90^{0} up to day 1 (6). In contrast, Kamandulis et al 2010 (44) reported that voluntary activation remained the same at the pre-exercise (~94%) up to day 1 in knee extensors at the same angle. The discrepancy results need to be mentioned and interpreted with cautions. Possible explanation might be related to different types of stimuli (single and tetanic stimulation). Recruitment of synergist muscles might involve with tetanic stimulation and blind the impaired voluntary activation.

In this study, when force recovery especially with low- and moderate-load of exercise at day 4 returned back to pre-exercise, voluntary activation also returned back to pre-exercise. With high-load of exercise, maximal force remained at ~76% of the pre-exercise value at day 4, voluntary activation remained at ~92%. Changes in maximal force corresponded with changes in voluntary activation, suggesting the contribution of

neural drive could be responsible. Therefore, the contribution of voluntary activation could potentially prevent further injury to muscles.

MVC, RT and VA in the second eccentric bout compared to the first bout as the baseline

When performed high-load of eccentric exercise in the subsequent bout, force deficits immediately after exercise were the same in all three loads of eccentric exercise compared to the baseline. Our results were in the same line with previous studies (8, 19, 44, 46, 47, 51, 52). These findings suggested no protective effect from all different loads in the first bout on maximal isometric forces immediately after exercise. Force deficits depend primarily on the extent of performed current load but they do not use any benefits of previous experiences. Therefore, force deficits could primarily be load-dependent. However, an enhanced recovery of force at day 1 and 4 found especially only in high-load of eccentric exercise. As mentioned before, in the first bout, an improved neural drive was attributed to force recovery in high-load of eccentric exercise, this finding might indirectly imply that a better in force recovery in the second bout in high-load of exercise could also attribute to neural adaptations.

In addition, direct comparisons between the second bout of all three different loads and the baseline from the high-load of the first eccentric bout were applied to reveal neural (Voluntary activation) or muscular (Resting twitch) adaptations. At day 1, maximal force significantly recovered from ~60% to ~80% of pre-exercise while voluntary activation significantly improved from ~76% to ~93%. At day 4, maximal force significantly recovered back to pre-exercise while voluntary activation also recovered back

to pre-exercise whereas the resting twitch was still significantly depressed at ~78% of pre-exercise. With high-load of exercise, voluntary activation revealed significant recovery compared to the baseline at day 1 and 4 whereas the resting twitch only showed significant recovery at day 4. These important findings suggest that neural adaptations could play a primarily role in the protective effect in the early phase of the second bout, muscular adaptations then could take a responsible coupling with neural adaptations in force recovery. Only one recent study (44) investigated changes in voluntary activation and reported the opposite findings. This discrepancy could be different technique used as in our research, we used single twitch whereas in other previous study, they used tetanic stimuli, as well as different calculations to assess neural contribution, we used voluntary activation but they used central activation ratio (CAR) and voluntary activation. Interesting observation, using their CAR and VA, even in the first eccentric bout there were no significant changes in CAR and VA for knee angles at 90^0 (compared to our study for elbow angles at 90^0), suggesting no neural adaptations. Thus, in the second bout, there is not surprising that the repeated bout is not associated with changes in voluntary activation.

Our possible explanations of neural adaptations could be that as our high-load of exercise in unaccustomed eccentric actions involves preferably fast-twitch motor units. As fast-twitch motor units recover faster than slow-twitch motor units, when performed the repeated high-load of exercise bout, greater and faster recovery occurred predominantly in high-load group (Warren et al 2000). The underlying mechanisms could involve recruitment strategy and/or modulation of firing rates. Another possible explanation might be that level of neural drive or voluntary activation at pre-exercise of high-load eccentric

exercise especially in the second bout did not fully activate (~96%), therefore it is possible that reserve or inactive motor units could be recruited to have a better and faster force recovery. Similarly, incomplete voluntary activation (~93%) at pre-exercise in the second eccentric bout was found in a recent research (44) although the different techniques (Single pulse VS Tetanic stimulation) were used and different sites (Elbow flexors VS Knee extensors). Taken together both possible explanations might be related to learning process in brain. This process could influence to the response of motoneurons via altering descending drive from motor cortex. Changes from feedback inputs (i.e. muscle soreness, joint stiffness) could also influence as a fine tune (39). Moreover, the alterations in intrinsic motoneuron properties could affect to synaptic inputs (53). In this study, we actually could not prove that neural adaptations depend on the influence at motor cortex and /or at spinal control. Although, there is a limited research supporting in this notion, direct evidence investigated by transcranial magnetic stimulation and electrical brain stimulation during eccentric muscle contractions in elbow flexors showed depressed corticospinal neuron excitability which then could set or tune motoneurone excitability (43, 54). As cortical excitability generated by high force task might attribute to increase performance gains compared to controls (55). Indirect investigations in resistance training (both concentric and eccentric muscle contractions) reveal that the site of neural adaptation might be in spinal circuitry whereas the contribution of motor cortex might be small (29, 42). Similar findings found that neural adaptations may make the greatest contribution during the early stages of a high-resistance strength training program (56). In addition, during ballistic (fast speed) motor performance, the contralateral motor cortex contributes to the initial improvements (55,

57, 58). These results suggest adaptations in the untrained motor cortex contribute to the early retention of ballistic performance gains for the untrained limb. Therefore, crossed adaptations are associated with the extensive bilateral cortical activity generated by unilateral high-force task (55, 57, 58).

In summary, this is the first investigation to demonstrate neural adaptations in repeated bout effect of eccentric exercise at the early phase (day 1 and 4). Muscular adaptations also reported jointly at day 4.

Clinical implications

RBE is important because it could be used as a potential ways of protecting athletes against muscle injuries. It also has some benefits for other clinical conditions in patients.

REFERENCES

1. Clarkson PM, Nosaka K, Braun B. Muscle function after exercise-induced muscle damage and rapid adaptation. Med Sci Sports Exerc. 1992 May;24(5):512-20.
2. Schwane JA, Johnson SR, Vandenakker CB, Armstrong RB. Delayed-onset muscular soreness and plasma CPK and LDH activities after downhill running. Med Sci Sports Exerc. 1983;15(1):51-6.
3. Ebbeling CB, Clarkson PM. Exercise-induced muscle damage and adaptation. Sports Med. 1989 Apr;7(4):207-34.
4. Jones DA, Newham DJ, Torgan C. Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. J Physiol. 1989 May;412:415-27.
5. Cleak MJ, Eston RG. Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. Br J Sports Med. 1992 Dec;26(4):267-72.
6. Prasartwuth O, Taylor JL, Gandevia SC. Maximal force, voluntary activation and muscle soreness after eccentric damage to human elbow flexor muscles. J Physiol. 2005 Aug 15;567(Pt 1):337-48.
7. McHugh MP. Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. Scand J Med Sci Sports. 2003 Apr;13(2):88-97.
8. Nosaka K, Sakamoto K, Newton M, Sacco P. How long does the protective effect on eccentric exercise-induced muscle damage last? Med Sci Sports Exerc. 2001 Sep;33(9):1490-5.
9. Friden J, Sjostrom M, Ekblom B. A morphological study of delayed muscle soreness. Experientia. 1981 May 15;37(5):506-7.
10. Nosaka K, Clarkson PM. Changes in indicators of inflammation after eccentric exercise of the elbow flexors. Med Sci Sports Exerc. 1996 Aug;28(8):953-61.
11. Lieber RL, Friden J. Morphologic and mechanical basis of delayed-onset muscle soreness. J Am Acad Orthop Surg. 2002 Jan-Feb;10(1):67-73.
12. Yu JG, Thornell LE. Desmin and actin alterations in human muscles affected by delayed onset muscle soreness: a high resolution immunocytochemical study. Histochem Cell Biol. 2002 Aug;118(2):171-9.
13. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol. 2001 Dec 1;537(Pt 2):333-45.

14. Abraham WM. Factors in delayed muscle soreness. Med Sci Sports. 1977 Spring;9(1):11-20.
15. Brown S, Day S, Donnelly A. Indirect evidence of human skeletal muscle damage and collagen breakdown after eccentric muscle actions. J Sports Sci. 1999 May;17(5):397-402.
16. Saxton JM, Donnelly AE. Length-specific impairment of skeletal muscle contractile function after eccentric muscle actions in man. Clin Sci (Lond). 1996 Feb;90(2):119-25.
17. Prasartwuth O, Allen TJ, Butler JE, Gandevia SC, Taylor JL. Length-dependent changes in voluntary activation, maximum voluntary torque and twitch responses after eccentric damage in humans. J Physiol. 2006 Feb 15;571(Pt 1):243-52.
18. McHugh MP. Can exercise-induced muscle damage be avoided? West J Med. 2000 Apr;172(4):265-6.
19. Nosaka K, Newton M. Repeated eccentric exercise bouts do not exacerbate muscle damage and repair. J Strength Cond Res. 2002 Feb;16(1):117-22.
20. Nosaka K, Clarkson PM. Muscle damage following repeated bouts of high force eccentric exercise. Med Sci Sports Exerc. 1995 Sep;27(9):1263-9.
21. Pizza FX, Koh TJ, McGregor SJ, Brooks SV. Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. J Appl Physiol. 2002 May;92(5):1873-8.
22. Koh TJ, Brooks SV. Lengthening contractions are not required to induce protection from contraction-induced muscle injury. Am J Physiol Regul Integr Comp Physiol. 2001 Jul;281(1):R155-61.
23. Brockett CL, Morgan DL, Proske U. Human hamstring muscles adapt to eccentric exercise by changing optimum length. Med Sci Sports Exerc. 2001 May;33(5):783-90.
24. Lynn R, Morgan DL. Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. J Appl Physiol. 1994 Sep;77(3):1439-44.
25. Lynn R, Talbot JA, Morgan DL. Differences in rat skeletal muscles after incline and decline running. J Appl Physiol. 1998 Jul;85(1):98-104.
26. Morgan DL. New insights into the behavior of muscle during active lengthening. Biophys J. 1990 Feb;57(2):209-21.

27. Child RB, Saxton JM, Donnelly AE. Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. J Sports Sci. 1998 May;16(4):301-8.
28. Newham DJ, Jones DA, Ghosh G, Aurora P. Muscle fatigue and pain after eccentric contractions at long and short length. Clin Sci (Lond). 1988 May;74(5):553-7.
29. Carroll TJ, Herbert RD, Munn J, Lee M, Gandevia SC. Contralateral effects of unilateral strength training: evidence and possible mechanisms. J Appl Physiol. 2006 Nov;101(5):1514-22.
30. Hortobagyi T, Lambert NJ, Hill JP. Greater cross education following training with muscle lengthening than shortening. Med Sci Sports Exerc. 1997 Jan;29(1):107-12.
31. Hortobagyi T, Scott K, Lambert J, Hamilton G, Tracy J. Cross-education of muscle strength is greater with stimulated than voluntary contractions. Motor Control. 1999 Apr;3(2):205-19.
32. Munn J, Herbert RD, Hancock MJ, Gandevia SC. Training with unilateral resistance exercise increases contralateral strength. J Appl Physiol. 2005 Nov;99(5):1880-4.
33. Shima N, Ishida K, Katayama K, Morotome Y, Sato Y, Miyamura M. Cross education of muscular strength during unilateral resistance training and detraining. Eur J Appl Physiol. 2002 Feb;86(4):287-94.
34. Weir JP, Housh DJ, Housh TJ, Weir LL. The effect of unilateral eccentric weight training and detraining on joint angle specificity, cross-training, and the bilateral deficit. J Orthop Sports Phys Ther. 1995 Nov;22(5):207-15.
35. Howatson G, Van Someren K, Hortobagyi T. Repeated bout effect after maximal eccentric exercise. Int J Sports Med. 2007 Jul;28(7):557-63.
36. McHugh MP, Connolly DA, Eston RG, Gartman EJ, Gleim GW. Electromyographic analysis of repeated bouts of eccentric exercise. J Sports Sci. 2001 Mar;19(3):163-70.
37. Warren GL, Hermann KM, Ingalls CP, Masselli MR, Armstrong RB. Decreased EMG median frequency during a second bout of eccentric contractions. Med Sci Sports Exerc. 2000 Apr;32(4):820-9.
38. Carroll TJ, Riek S, Carson RG. Neural adaptations to resistance training: implications for movement control. Sports Med. 2001;31(12):829-40.

39. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001 Oct;81(4):1725-89.
40. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. J Appl Physiol. 2004 Apr;96(4):1486-95.
41. Keenan KG, Farina D, Maluf KS, Merletti R, Enoka RM. Influence of amplitude cancellation on the simulated surface electromyogram. J Appl Physiol. 2005 Jan;98(1):120-31.
42. Carroll TJ, Riek S, Carson RG. The sites of neural adaptation induced by resistance training in humans. J Physiol. 2002 Oct 15;544(Pt 2):641-52.
43. Sekiguchi H, Kimura T, Yamanaka K, Nakazawa K. Lower excitability of the corticospinal tract to transcranial magnetic stimulation during lengthening contractions in human elbow flexors. Neurosci Lett. 2001 Oct 19;312(2):83-6.
44. Kamandulis S, Skurvydas A, Brazaitis M, Skikas L, Duchateau J. The repeated bout effect of eccentric exercise is not associated with changes in voluntary activation. Eur J Appl Physiol. 2010 Apr;108(6):1065-74.
45. Herbert RD, Gandevia SC. Muscle activation in unilateral and bilateral efforts assessed by motor nerve and cortical stimulation. J Appl Physiol. 1996 Apr;80(4):1351-6.
46. Chen TC, Chen HL, Lin MJ, Wu CJ, Nosaka K. Muscle damage responses of the elbow flexors to four maximal eccentric exercise bouts performed every 4 weeks. Eur J Appl Physiol. 2009 May;106(2):267-75.
47. Chen TC, Nosaka K, Sacco P. Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. J Appl Physiol. 2007 Mar;102(3):992-9.
48. Gandevia SC. Twitch interpolation a valid measure with misinterpreted meaning. J Appl Physiol. 2009 Jul;107(1):363-4; discussion 7-8.
49. Place N, Yamada T, Bruton JD, Westerblad H. Interpolated twitches in fatiguing single mouse muscle fibres: implications for the assessment of central fatigue. J Physiol. 2008 Jun 1;586(Pt 11):2799-805.
50. Jones DA. High-and low-frequency fatigue revisited. Acta Physiol Scand. 1996 Mar;156(3):265-70.
51. Nosaka K, Sakamoto K, Newton M, Sacco P. The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. Eur J Appl Physiol. 2001 Jul;85(1-2):34-40.

52. Paschalis V, Nikolaidis MG, Giakas G, Jamurtas AZ, Owolabi EO, Koutedakis Y. Position sense and reaction angle after eccentric exercise: the repeated bout effect. Eur J Appl Physiol. 2008 May;103(1):9-18.
53. Rekling JC, Funk GD, Bayliss DA, Dong XW, Feldman JL. Synaptic control of motoneuronal excitability. Physiol Rev. 2000 Apr;80(2):767-852.
54. Abbruzzese G, Morena M, Spadavecchia L, Schieppati M. Response of arm flexor muscles to magnetic and electrical brain stimulation during shortening and lengthening tasks in man. J Physiol. 1994 Dec 1;481 (Pt 2):499-507.
55. Lee M, Hinder MR, Gandevia SC, Carroll TJ. The ipsilateral motor cortex contributes to cross-limb transfer of performance gains after ballistic motor practice. J Physiol. 2010 Jan 1;588(Pt 1):201-12.
56. Folland JP, Williams AG. The adaptations to strength training : morphological and neurological contributions to increased strength. Sports Med. 2007;37(2):145-68.
57. Lee M, Carroll TJ. Cross education: possible mechanisms for the contralateral effects of unilateral resistance training. Sports Med. 2007;37(1):1-14.
58. Lee M, Gandevia SC, Carroll TJ. Unilateral strength training increases voluntary activation of the opposite untrained limb. Clin Neurophysiol. 2009 Apr;120(4):802-8.

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ภาคผนวก

Protective effect in the repeated eccentric bout: is it neural or muscular adaptations?

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ABSTRACT

Repeated bout effect (RBE) is known as a protective way to reduce the eccentric damaging exercise. After eccentric exercise, muscle damage reduces maximal voluntary force; however, impaired neural drive to the muscle has also primarily contributed. To determine the underlying mechanisms of the repeated bout effect, voluntary activation was assessed to identify the neural adaptations while the resting twitch was used to indicate the muscular adaptations. We also aimed to investigate whether three different loads could differently confer the RBE. Thirty healthy subjects were selectively allocated into 3 groups (low-, moderate- and high-load group) to match for maximal voluntary contraction (MVC) ($n = 10$ per group). Subjects in each group performed 3 sets of 10 eccentric actions of the elbow flexors at 10%, 20% and 40% of MVC for the first eccentric bout (ECC1) respectively, followed 2 weeks later by a similar high-load eccentric exercise at 40% of MVC for the second eccentric bout (ECC2). Maximal voluntary force, voluntary activation, the resting twitch, changes in the elbow angle and pain sensation were measured before, immediately, day 1 and day 4 after exercise following ECC1 and ECC2. The results showed that for the first eccentric bout, MVC, voluntary activation and the resting twitch were significant ($p< 0.0001$) interaction (group x time). Following the second eccentric bout immediately after exercise, there were no significant (group x time) interaction in all outcome variables. However, at day 1 and 4, only high-load group demonstrated significantly ($p< 0.01$) greater improvement in maximal voluntary force compared with the first high-load eccentric bout, indicating greater recovery in force generating capacity as RBE. This force recovery at day 1 and 4 corresponded with a significant ($p< 0.01$) improvement in voluntary activation, indicating better the level of

neural drive to the muscle or neural adaptations in the early stage. In contrast, the resting twitch in high-load group demonstrated a significant ($p < 0.01$) improvement only at day 4, suggesting muscular adaptations. In conclusion, greater force recovery in the second eccentric damaging exercise at day 1 and 4 measured in high-load group could primarily be the neural adaptations. There is a contribution of muscular adaptations at day 4 as well.

INTRODUCTION

High force eccentric exercise induces prolonged and marked muscle fatigue in unfamiliar persons. Immediately after exercise, muscle becomes stiffness causing limitation in joint range of motion. Muscle soreness also pronounced lately at day 1-2 (1-6). These changes have well-investigated demonstrating cellular and muscular changes (7). For cellular changes, an increase in muscle-specific proteins in the blood e.g. creatine kinase or myoglobin indicated damages in muscle fibers(8). These damages can also be visualized by electron microscopy, ultrasound and magnetic resonance imaging illustrating disruption in contractile apparatus (9-13). Connective tissues (perimysium or endomysium) could also be damaged, illustrating an increase in urine hydroxyproline (14) and serum type I collagen concentration (15). However, researches in the contribution of neural control are limited and the results remain controversial. With tetanic stimulation, there were no increases in the superimposed twitch elicited by elbow flexors during maximal effort (16). In contrast, single pulse stimulation confirmed a reduction in voluntary action or an impaired neural drive to the muscle (6, 17). This discrepancy might be related to technical sensitivity using tetanic and single twitch stimulation. Therefore, underlying mechanisms in force deficit could be primarily attributed to neural and secondarily to muscular contribution.

Once performed exercise again or repeated bout exercise, it could reduce prolonged and marked force loss, muscle stiffness and muscle soreness (1, 18, 19). These improvements are referred as the protective adaptation or repeated bout effect (RBE) (20). Similar to the first bout, the cellular and muscular adaptations are well-

documented involving blunted inflammatory responses (21, 22), addition of series sarcomeres (23-26) and rightward shift in length-tension curve (17, 27, 28). On the other hand, limited evidence supports the neural adaptations in RBE. Even in resistance training, any improvement in muscle force which could not be explained by changes in muscle size has been addressed to neural adaptations. In addition, changes in cross-training to contralateral muscles (29-34) have been used to confirm neural adaptations. For the repeated bout effect, contralateral eccentric training appeared predominantly indicating neural adaptations (35).

Several lines of the evidence for neural adaptations in RBE have been drawn from surface electromyography (EMG) recording. During the second bout of eccentric contractions, a decrease in the median frequency of EMG activity was found indicating an increased activation of slow motor unit and a concomitant decrease in activation of fast units (36, 37). However, the questions for methodological reasons (38-40), have been addressed as a result of motor unit synchronization (7) and signal cancellation (41). Therefore, another possible way to identify neural adaptations includes changes in the pattern of neural activity associated with motor drive. Adaptive modifications in neural circuits could involve either in descending command at the motor cortex or in the final neural drive to the muscle at the spinal cord. Even though no previous research reported these modifications as a result of RBE, resistance training consisting concentric and eccentric muscle actions could alter the functional properties of spinal circuitry, but less affect the organization of the motor cortex (42). With eccentric muscle actions, there is an evidence of lower excitability of the corticospinal tract (43). By defining the possible sites, we firstly need to confirm whether there is neural adaptation in RBE or not.

As limited research has been conducted to reveal the neural adaptations in RBE, therefore the underlying mechanisms regarding to neural adaptations to RBE is still unclear. Recently, a research has reported no neural adaptations in RBE in the knee extensors by using tetanic electrical stimulation (44). However, base on our previous research of the first eccentric bout, neural contribution primarily plays an important role. Therefore, further researches are still needed and the knowledge will add our understanding in neural adaptations of RBE. Our first aim was to investigate whether RBE is the neural contribution or not. We hypothesized that neural adaptations could be identified with an improvement in voluntary activation in the RBE.

In addition, the magnitude of RBE does not appear to depend on the number of eccentric contractions, but depends on the magnitude of load in the first bout. For example, Chen et al 2009 reported that the magnitude of the repeated bout effect was smaller for 40% or 60% MVC load than 80% or 100% MVC load. In contrast, a recent study has shown that submaximal eccentric exercise (40% MVC load) conferred the same magnitude of RBE as that produced by one maximal eccentric exercise bout. Although it seems likely that the different loads in the initial bout of eccentric exercise could attribute to minimize the magnitude of muscle damage, we still do not know that low-, moderate- or high-load could be recommended to use as the best repeated bout effect. Revealing this information could be benefit to injury prevention in athletes during training, competition and rehabilitation. Therefore, this study was also designed to investigate whether the three different levels of eccentric load in the first bout could confer the different changes in RBE. Our second aim was to determine whether the low-, moderate- or high-load of the initial eccentric exercise could affect to the degree of

subsequent protection effect. We hypothesized that high-load of the initial eccentric exercise could confer the best RBE.

METHODS

Subjects and study design

Thirty healthy volunteers who had not performed regular resistance training gave written informed consent. Subjects were selectively placed into one of three groups (n = 10 per group) based on the baseline maximal voluntary isometric contraction torque (MVC) of the elbow flexors at an elbow joint angle 90^0. The groups were performed; low-load at 10%ECC, moderate-load at 20%ECC and high-load at 40%ECC as explained below, and all groups had similar mean baseline MVC, with no significant differences in age, height or body mass across groups (Table 1). All subjects were asked to refrain from unaccustomed exercise or vigorous physical activity for at least 6 months before the experiment and to not to take any anti-inflammatory drugs or nutritional supplements during the experimental period. All subjects performed the second bout at the same high-load (40%ECC) of elbow flexor eccentric exercise with the same arm separated by 2 weeks. A number of measurements were taken before, immediately after, 1 and 4 days after eccentric exercise for the first and second bouts. The main dependent variables include MVC, voluntary activation, resting twitch, elbow angles and degree of muscle soreness.

Eccentric exercise

All subjects performed the first eccentric exercise (ECC1) of the elbow flexors with the non-dominant arm using a dumbbell adjusted to either 10%, 20% or 40% of individual's MVC at an elbow angle of 90^0. The subjects were instructed to lower the dumbbell from an elbow flexed ~90^0 to an elbow extended position (0^0) in 4-5 second,

keeping the velocity as constant as possible by following the examiner's counting "start" and "1, 2, 3, 4 and 5" for the movement. After each eccentric contraction, the examiner lifted the load up while subjects relaxed and the arm was passively returned to the starting position. The exercise consisted of 3 sets 10 repetitions with a 2-minute rest between sets.

Two weeks after ECC1, all subjects performed the second eccentric exercise bout (ECC2) with the same arm using a dumbbell adjusted to 40% of individual's MVC at an elbow angle of 90º. The exercise protocol for ECC2 was the identical to that of ECC1.

Motor nerve stimulation

For stimulation of the motor nerve, single electrical stimuli (100 μs duration, constant current, DS7, Digitimer) were delivered to intramuscular nerve fibres innervating biceps brachii via a surface cathode located midway between the anterior edge of the deltoid and the elbow crease and a surface anode positioned over the distal biceps tendon. The stimulation intensity was set at 10% above the level required to produce a resting twitch of maximal amplitude. The stimulus intensity was set at each measurement session. The sites of stimulation were marked on the skin to ensure consistent placement throughout the 3 testing sessions.

Dependent variables

MVC

Subjects sat with the arm in an arm bar attached with a force gauge (2KN, A&D CO, Ltd, Japan) connected to a digital recorder. This arm bar was used to measure maximal isometric flexion torque at 90 degree of elbow flexion. Three measurements of maximal

effort were made and the maximal value was chose to be a peak torque. Verbal encouragement was provided during MVC measurements.

Voluntary activation and resting twitch

During the electrical stimulation of the motor nerve, any increment in elbow flexion torque evoked during a MVC was expressed as a fraction of the amplitude of the maximal response evoked by the same stimulus in the relaxed muscle immediately after an MVC ("resting twitch"). Voluntary activation was then quantified as a percentage using the formula (see 45):

Voluntary activation (%) = (1-superimposed twitch/resting twitch) x 100

Elbow joint angle and range of motion

Elbow angle was measured in three different positions: relaxed (RANG), full flexed (FANG) and full extended (EANG). Subjects stood with the arm hanging vertically and were asked to relax, fully flex and fully extend at the elbow joint angle for the measures. The points over the lateral middle point of humerus, over the lateral epicondyle, over the radial styloid process and over the middle point between radius and ulna were marked on the skin with semi-permanent pen. Two measurements of the angle were taken by using a goniometer and range of motion (ROM) was defined as the angle subtracting the mean FANG from the mean EANG.

Muscle soreness

Degree of muscle soreness was measured in two ways: pressure pain threshold (PPT) using a pressure algometer and pain rating scale using a visual analog scale (VAS). For both measurements, subjects sat with the elbow joint at 90º in flexion, and assessments were taken from the three marked spots; 5 cm, 8 cm and 11 cm from the elbow crease over the biceps brachii. A pressure algometer with a circular disc (10 mm-diameter) was applied perpendicular to the skin over the three reference spots to measure the force at which subjects reported any discomfort or pain. A visual analog scale consists of a 100-mm continuous line representing "no pain" at one end (0mm) and "very, very painful" at the other (100 mm). Subjects were asked to indicate the soreness level on the line when the examiner applied pressure with the algometer at 2 kg.

Statistical analysis

The results are presented as means ± SD. Changes in the dependent variable over time were compared amongst the groups for the first bout (ECC1) and the second bout (ECC2) separately by two-way repeated measures ANOVA. The ANOVA was performed for both raw and normalized data for all variables except for voluntary activation. For the ANOVA using the normalized data, the pre-exercise values (100 for MVC, RT, PPT and VAS) were excluded. The ANOVA also compared between the first 40% bout performed by the 40-40%ECC group and the second eccentric exercise bout performed by all groups in which the 40% load was used. When the ANOVA showed a significant interaction (group x time) effect, a Tukey's HSD test was employed as post hoc analysis to locate the time points of significant differences between groups. One-way

ANOVA were also used to compare between groups for each time. Statistical significance was set at $P<0.05$.

RESULTS

We hypothesized that the underlying mechanisms of the repeated bout effect could be due to the neural adaptations. To test this hypothesis, the improvements in the voluntary activation in the second bout were used to indicate the neural adaptations. With the same technique of twitch interpolation, the improvements in the resting twitch could also be indicated muscular adaptations. In addition, we would like to investigate whether the different loads of eccentric exercise in the first bout as different stimuli may induce different responses of protective effect in the second bout. To test this research question, subjects were chosen into three exercise groups i.e. low-load (10%ECC), moderate-load (20%ECC) and high-load (40%ECC) to have a similar baseline MVC. The subject characteristics of each group showed no significant difference in age, height and body mass ($P>0.05$) (Table 1).

FIRST ECCENTRIC EXERCISE

Deficit in force production

For the first bout of eccentric exercise, with the low- and the moderate-load, the maximal voluntary isometric torques decreased by ~7% and ~13% of the pre-exercise value immediately after eccentric exercise respectively and recovered back to normal at day 4. In contrast, with the high-load, the maximal voluntary isometric torques decreased by ~46% of the pre-exercise value immediately after eccentric exercise. It only recovered to ~76% of the initial value at day 4 (Table 2, Figure 1). When compared between groups, changes in raw and normalized maximal voluntary isometric torque in all groups

over time (group x time) were significantly ($p<0.0001$) different. Post hoc analysis (Tukey's HSD test) found significantly ($p<0.0001$) different between low- and high-load groups, low- and moderate-load groups, and moderate- and high-load groups.

Voluntary activation

With the low-load, voluntary activation remained the same after the exercise up to 4 day. While the moderate-load, voluntary activation slightly decreased by 7% (from ~99% to ~ 92%) immediately after exercise and returned back to normal at day 4. With the high-load, voluntary activation markedly decreased by 33% (from ~97% to ~64%) immediately after exercise and gradually recovered to ~91% at day 4 (Table 8, Figure 1). When compared between groups, changes in voluntary activation in all groups over time (group x time) were significantly ($P<0.001$) different. There were significantly ($p<0.001$) different between low- and high-load groups, low- and moderate-load groups and moderate- and high-load groups.

Resting twitch

The resting twitches produced by motor nerve stimulation decreased by ~27%, ~64% and ~86% of their pre-exercise values with the low-, moderate- and high-load of the eccentric exercise respectively. They then gradually recovered to ~88%, ~73% and ~50% of their pre-exercise values at day 4 with the low-, moderate- and high-load of the eccentric exercise respectively (Table 4, Figure 1). Changes in the resting twitches in all groups over time (group x time) were significantly ($p=0.0001$) different. When compared the resting twitches between low- and moderate-load group, low- and high-load group,

there were significantly (p=0.01, p=0.0001 respectively) different. However, there was no a significant (p=0.095) difference between moderate- and high-load group.

SECOND ECCENTRIC EXERCISE

Deficit in force production

For the second bout of eccentric exercise, each group performed the eccentric exercise with the same high-load (40%MVC). Immediately after the exercise, the maximal voluntary isometric torques in all groups decreased by ~38-48% of their initial values. At the 4 day, the maximal voluntary isometric torques in low- and moderate-load group gradually recovered to ~90% of their pre-exercise values. Interestingly, the maximal voluntary isometric torques in high-load group returned back to normal (Table 5, Figure 1).

Comparison of MVC between all groups in 2nd bout and high-load group in 1st bout as baseline

With repeated measures ANOVA, the normalized data showed a significant main effect for time (F=145.723, p=0.0001). Comparison between groups, there were also significant differences (F=3.885, p=0.017). There was also a significant interaction effect (group x time) (F=3.250, p=0.007). One-way ANOVA found significant differences between high-load group and the baseline (the first bout of ECC exercise) at day 1 and 4 (p=0.014, p=0.003, respectively) (Figure 1).

Voluntary activation

For the second bout of the eccentric exercise, all groups showed the similar changes and recovery patterns as the exercise load was equal for each group

(40%MVC). Voluntary activation dropped in the similar amount after exercise (~70-80%). At day 4, all groups recovered back to pre-exercise value (~96%) (Table 6, Figure 1).

Comparison of voluntary activation between all groups in 2nd bout and high-load group in 1st bout as baseline

With repeated measures ANOVA, the voluntary activation showed a significant main effect for time (F=40.99, p=0.0001). Comparison between groups, there were no significant differences (F=2.313, p=0.093). There was also no significant interaction effect (group x time) (F=0.638, p=0.762). With this absence of a significant interaction effect, one-way ANOVA was applied and revealed that there were significant differences between high-load group and the baseline at day 1 and 4 (Figure 1).

Resting twitch

Similar to the voluntary activation, all three groups showed similar changes after exercise and similar recovery rate. With normalized data, the resting twitches decreased by ~81% (from 100 to 19%) after exercise and return back by ~ 26% (from 100 to 74%) at day 4 (Table 7, Figure 1).

Comparison of the resting twitches between all groups in 2nd bout and high-load group 1st bout as baseline

With repeated measures ANOVA, the raw and normalized data showed a significant main effect for time (F=117.27, p=0.0001). Comparison between groups, there were no significant differences (F=1.103, p=0.367). There was also no significant

interaction effect (group x time) (F=0.756, p=0.526). With this absence of a significant interaction effect, one-way ANOVA was applied and revealed that there was only a significant difference between high-load group and the baseline at day 4 (Figure 1).

Changes in elbow joint angle and pain measurement

No interaction (group x time) in changes in elbow joint angle in flexed position, extended position, relax position and range of motion as well as changes in muscle soreness measured by VAS and PPT were found among all three groups. When compared with the baseline (1st bout), only changes in elbow joint angle in flexed position and range of motion showed time and group interaction (F=2.264, p=0.048, F=2.576, p=0.027 respectively). Post Hoc analysis found significantly (p=0.04) only in changes in flexed angle between low-load group and baseline.

DISCUSSION

Our main purposes of this study were to determine whether the underlying mechanisms of RBE could be related to neural adaptations and to investigate whether three different loads could confer different protective effects. This study has illustrated that with the second eccentric bout, maximal isometric force (MVCs) demonstrated significant interaction effect (Group x Time) in the high-load eccentric group. One-way ANOVA revealed at day 1 and 4, MVCs in high-load eccentric group were significant recovery compared to the baseline (First high-load bout of eccentric exercise). Therefore, RBE in this study was not an improvement of force generating capacity immediately after exercise. Our exercise protocol failed to produce this protective effect immediately after exercise but confirmed better force recovery. Our results were in the same line with previous studies (44, 46, 47).

MVC, RT and VA for the first eccentric bout

Low-, moderate- and high-load of eccentric exercise caused the decreases in maximal isometric forces by ~7%, ~13% and ~46% of their pre-exercise values, respectively. With low- and moderate-load of exercise, maximal isometric forces were recovery back to normal at day 4, whereas with high-load exercise, it still remained at ~76% of the initial value.

Corresponding with force loss, the amplitude of the resting twitches decreased by ~27%, ~64% and ~86% of their pre-exercise values for low-, moderate- and high-load of exercise, respectively. These changes in the resting twitch in high-load exercise were found in the same line with previous studies (6, 17). Using the same twitch interpolation

technique to assess the resting twitch, force loss by ~35-40% was associated with the decrease in the resting twitch by ~70-85%. However, with different frequency i.e. tetanic stimulation, force loss by ~42% was associated with the decrease in the resting twitch by ~44% (44). Less decrease in the resting twitch from tetanic stimulation could be due to an alteration of the relationship between local intracellular Ca^{++} and force (48, 49) and possibly relate to low- and high-frequency fatigue (50).

With voluntary activation, force loss by ~7%, ~13% and ~46% corresponded with 0%, ~7% and ~33% loss of level of neural drive for low-, moderate- and high-load of eccentric exercise respectively. These results suggested that impairments in voluntary activation played a role in moderate- and high-load of exercise. With high-load of exercise, previous work (6) found similar finding, however, other recent research reported different results (44). Impaired voluntary activation found after eccentric exercise in elbow flexors at 90^{0} up to day 1 (6). In contrast, Kamandulis et al 2010 (44) reported that voluntary activation remained the same at the pre-exercise (~94%) up to day 1 in knee extensors at the same angle. The discrepancy results need to be mentioned and interpreted with cautions. Possible explanation might be related to different types of stimuli (single and tetanic stimulation). Recruitment of synergist muscles might involve with tetanic stimulation and blind the impaired voluntary activation.

In this study, when force recovery especially with low- and moderate-load of exercise at day 4 returned back to pre-exercise, voluntary activation also returned back to pre-exercise. With high-load of exercise, maximal force remained at ~76% of the pre-exercise value at day 4, voluntary activation remained at ~92%. Changes in maximal force corresponded with changes in voluntary activation, suggesting the contribution of

neural drive could be responsible. Therefore, the contribution of voluntary activation could potentially prevent further injury to muscles.

MVC, RT and VA in the second eccentric bout compared to the first bout as the baseline

When performed high-load of eccentric exercise in the subsequent bout, force deficits immediately after exercise were the same in all three loads of eccentric exercise compared to the baseline. Our results were in the same line with previous studies (8, 19, 44, 46, 47, 51, 52). These findings suggested no protective effect from all different loads in the first bout on maximal isometric forces immediately after exercise. Force deficits depend primarily on the extent of performed current load but they do not use any benefits of previous experiences. Therefore, force deficits could primarily be load-dependent. However, an enhanced recovery of force at day 1 and 4 found especially only in high-load of eccentric exercise. As mentioned before, in the first bout, an improved neural drive was attributed to force recovery in high-load of eccentric exercise, this finding might indirectly imply that a better in force recovery in the second bout in high-load of exercise could also attribute to neural adaptations.

In addition, direct comparisons between the second bout of all three different loads and the baseline from the high-load of the first eccentric bout were applied to reveal neural (Voluntary activation) or muscular (Resting twitch) adaptations. At day 1, maximal force significantly recovered from ~60% to ~80% of pre-exercise while voluntary activation significantly improved from ~76% to ~93%. At day 4, maximal force significantly recovered back to pre-exercise while voluntary activation also recovered back

to pre-exercise whereas the resting twitch was still significantly depressed at ~78% of pre-exercise. With high-load of exercise, voluntary activation revealed significant recovery compared to the baseline at day 1 and 4 whereas the resting twitch only showed significant recovery at day 4. These important findings suggest that neural adaptations could play a primarily role in the protective effect in the early phase of the second bout, muscular adaptations then could take a responsible coupling with neural adaptations in force recovery. Only one recent study (44) investigated changes in voluntary activation and reported the opposite findings. This discrepancy could be different technique used as in our research, we used single twitch whereas in other previous study, they used tetanic stimuli, as well as different calculations to assess neural contribution, we used voluntary activation but they used central activation ratio (CAR) and voluntary activation. Interesting observation, using their CAR and VA, even in the first eccentric bout there were no significant changes in CAR and VA for knee angles at 90^0 (compared to our study for elbow angles at 90^0), suggesting no neural adaptations. Thus, in the second bout, there is not surprising that the repeated bout is not associated with changes in voluntary activation.

Our possible explanations of neural adaptations could be that as our high-load of exercise in unaccustomed eccentric actions involves preferably fast-twitch motor units. As fast-twitch motor units recover faster than slow-twitch motor units, when performed the repeated high-load of exercise bout, greater and faster recovery occurred predominantly in high-load group (Warren et al 2000). The underlying mechanisms could involve recruitment strategy and/or modulation of firing rates. Another possible explanation might be that level of neural drive or voluntary activation at pre-exercise of high-load eccentric

exercise especially in the second bout did not fully activate (~96%), therefore it is possible that reserve or inactive motor units could be recruited to have a better and faster force recovery. Similarly, incomplete voluntary activation (~93%) at pre-exercise in the second eccentric bout was found in a recent research (44) although the different techniques (Single pulse VS Tetanic stimulation) were used and different sites (Elbow flexors VS Knee extensors). Taken together both possible explanations might be related to learning process in brain. This process could influence to the response of motoneurons via altering descending drive from motor cortex. Changes from feedback inputs (i.e. muscle soreness, joint stiffness) could also influence as a fine tune (39). Moreover, the alterations in intrinsic motoneuron properties could affect to synaptic inputs (53). In this study, we actually could not prove that neural adaptations depend on the influence at motor cortex and /or at spinal control. Although, there is a limited research supporting in this notion, direct evidence investigated by transcranial magnetic stimulation and electrical brain stimulation during eccentric muscle contractions in elbow flexors showed depressed corticospinal neuron excitability which then could set or tune motoneurone excitability (43, 54). As cortical excitability generated by high force task might attribute to increase performance gains compared to controls (55). Indirect investigations in resistance training (both concentric and eccentric muscle contractions) reveal that the site of neural adaptation might be in spinal circuitry whereas the contribution of motor cortex might be small (29, 42). Similar findings found that neural adaptations may make the greatest contribution during the early stages of a high-resistance strength training program (56). In addition, during ballistic (fast speed) motor performance, the contralateral motor cortex contributes to the initial improvements (55,

57, 58). These results suggest adaptations in the untrained motor cortex contribute to the early retention of ballistic performance gains for the untrained limb. Therefore, crossed adaptations are associated with the extensive bilateral cortical activity generated by unilateral high-force task (55, 57, 58).

In summary, this is the first investigation to demonstrate neural adaptations in repeated bout effect of eccentric exercise at the early phase (day 1 and 4). Muscular adaptations also reported jointly at day 4.

Clinical implications

RBE is important because it could be used as a potential ways of protecting athletes against muscle injuries. It also has some benefits for other clinical conditions in patients.

REFERENCES

1. Clarkson PM, Nosaka K, Braun B. Muscle function after exercise-induced muscle damage and rapid adaptation. Med Sci Sports Exerc. 1992 May;24(5):512-20.
2. Schwane JA, Johnson SR, Vandenakker CB, Armstrong RB. Delayed-onset muscular soreness and plasma CPK and LDH activities after downhill running. Med Sci Sports Exerc. 1983;15(1):51-6.
3. Ebbeling CB, Clarkson PM. Exercise-induced muscle damage and adaptation. Sports Med. 1989 Apr;7(4):207-34.
4. Jones DA, Newham DJ, Torgan C. Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. J Physiol. 1989 May;412:415-27.
5. Cleak MJ, Eston RG. Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. Br J Sports Med. 1992 Dec;26(4):267-72.
6. Prasartwuth O, Taylor JL, Gandevia SC. Maximal force, voluntary activation and muscle soreness after eccentric damage to human elbow flexor muscles. J Physiol. 2005 Aug 15;567(Pt 1):337-48.
7. McHugh MP. Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. Scand J Med Sci Sports. 2003 Apr;13(2):88-97.
8. Nosaka K, Sakamoto K, Newton M, Sacco P. How long does the protective effect on eccentric exercise-induced muscle damage last? Med Sci Sports Exerc. 2001 Sep;33(9):1490-5.
9. Friden J, Sjostrom M, Ekblom B. A morphological study of delayed muscle soreness. Experientia. 1981 May 15;37(5):506-7.
10. Nosaka K, Clarkson PM. Changes in indicators of inflammation after eccentric exercise of the elbow flexors. Med Sci Sports Exerc. 1996 Aug;28(8):953-61.
11. Lieber RL, Friden J. Morphologic and mechanical basis of delayed-onset muscle soreness. J Am Acad Orthop Surg. 2002 Jan-Feb;10(1):67-73.
12. Yu JG, Thornell LE. Desmin and actin alterations in human muscles affected by delayed onset muscle soreness: a high resolution immunocytochemical study. Histochem Cell Biol. 2002 Aug;118(2):171-9.

13. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol. 2001 Dec 1;537(Pt 2):333-45.
14. Abraham WM. Factors in delayed muscle soreness. Med Sci Sports. 1977 Spring;9(1):11-20.
15. Brown S, Day S, Donnelly A. Indirect evidence of human skeletal muscle damage and collagen breakdown after eccentric muscle actions. J Sports Sci. 1999 May;17(5):397-402.
16. Saxton JM, Donnelly AE. Length-specific impairment of skeletal muscle contractile function after eccentric muscle actions in man. Clin Sci (Lond). 1996 Feb;90(2):119-25.
17. Prasartwuth O, Allen TJ, Butler JE, Gandevia SC, Taylor JL. Length-dependent changes in voluntary activation, maximum voluntary torque and twitch responses after eccentric damage in humans. J Physiol. 2006 Feb 15;571(Pt 1):243-52.
18. McHugh MP. Can exercise-induced muscle damage be avoided? West J Med. 2000 Apr;172(4):265-6.
19. Nosaka K, Newton M. Repeated eccentric exercise bouts do not exacerbate muscle damage and repair. J Strength Cond Res. 2002 Feb;16(1):117-22.
20. Nosaka K, Clarkson PM. Muscle damage following repeated bouts of high force eccentric exercise. Med Sci Sports Exerc. 1995 Sep;27(9):1263-9.
21. Pizza FX, Koh TJ, McGregor SJ, Brooks SV. Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. J Appl Physiol. 2002 May;92(5):1873-8.
22. Koh TJ, Brooks SV. Lengthening contractions are not required to induce protection from contraction-induced muscle injury. Am J Physiol Regul Integr Comp Physiol. 2001 Jul;281(1):R155-61.
23. Brockett CL, Morgan DL, Proske U. Human hamstring muscles adapt to eccentric exercise by changing optimum length. Med Sci Sports Exerc. 2001 May;33(5):783-90.
24. Lynn R, Morgan DL. Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. J Appl Physiol. 1994 Sep;77(3):1439-44.
25. Lynn R, Talbot JA, Morgan DL. Differences in rat skeletal muscles after incline and decline running. J Appl Physiol. 1998 Jul;85(1):98-104.

26. Morgan DL. New insights into the behavior of muscle during active lengthening. Biophys J. 1990 Feb;57(2):209-21.
27. Child RB, Saxton JM, Donnelly AE. Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. J Sports Sci. 1998 May;16(4):301-8.
28. Newham DJ, Jones DA, Ghosh G, Aurora P. Muscle fatigue and pain after eccentric contractions at long and short length. Clin Sci (Lond). 1988 May;74(5):553-7.
29. Carroll TJ, Herbert RD, Munn J, Lee M, Gandevia SC. Contralateral effects of unilateral strength training: evidence and possible mechanisms. J Appl Physiol. 2006 Nov;101(5):1514-22.
30. Hortobagyi T, Lambert NJ, Hill JP. Greater cross education following training with muscle lengthening than shortening. Med Sci Sports Exerc. 1997 Jan;29(1):107-12.
31. Hortobagyi T, Scott K, Lambert J, Hamilton G, Tracy J. Cross-education of muscle strength is greater with stimulated than voluntary contractions. Motor Control. 1999 Apr;3(2):205-19.
32. Munn J, Herbert RD, Hancock MJ, Gandevia SC. Training with unilateral resistance exercise increases contralateral strength. J Appl Physiol. 2005 Nov;99(5):1880-4.
33. Shima N, Ishida K, Katayama K, Morotome Y, Sato Y, Miyamura M. Cross education of muscular strength during unilateral resistance training and detraining. Eur J Appl Physiol. 2002 Feb;86(4):287-94.
34. Weir JP, Housh DJ, Housh TJ, Weir LL. The effect of unilateral eccentric weight training and detraining on joint angle specificity, cross-training, and the bilateral deficit. J Orthop Sports Phys Ther. 1995 Nov;22(5):207-15.
35. Howatson G, Van Someren K, Hortobagyi T. Repeated bout effect after maximal eccentric exercise. Int J Sports Med. 2007 Jul;28(7):557-63.
36. McHugh MP, Connolly DA, Eston RG, Gartman EJ, Gleim GW. Electromyographic analysis of repeated bouts of eccentric exercise. J Sports Sci. 2001 Mar;19(3):163-70.
37. Warren GL, Hermann KM, Ingalls CP, Masselli MR, Armstrong RB. Decreased EMG median frequency during a second bout of eccentric contractions. Med Sci Sports Exerc. 2000 Apr;32(4):820-9.
38. Carroll TJ, Riek S, Carson RG. Neural adaptations to resistance training: implications for movement control. Sports Med. 2001;31(12):829-40.

39. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001 Oct;81(4):1725-89.
40. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. J Appl Physiol. 2004 Apr;96(4):1486-95.
41. Keenan KG, Farina D, Maluf KS, Merletti R, Enoka RM. Influence of amplitude cancellation on the simulated surface electromyogram. J Appl Physiol. 2005 Jan;98(1):120-31.
42. Carroll TJ, Riek S, Carson RG. The sites of neural adaptation induced by resistance training in humans. J Physiol. 2002 Oct 15;544(Pt 2):641-52.
43. Sekiguchi H, Kimura T, Yamanaka K, Nakazawa K. Lower excitability of the corticospinal tract to transcranial magnetic stimulation during lengthening contractions in human elbow flexors. Neurosci Lett. 2001 Oct 19;312(2):83-6.
44. Kamandulis S, Skurvydas A, Brazaitis M, Skikas L, Duchateau J. The repeated bout effect of eccentric exercise is not associated with changes in voluntary activation. Eur J Appl Physiol. 2010 Apr;108(6):1065-74.
45. Herbert RD, Gandevia SC. Muscle activation in unilateral and bilateral efforts assessed by motor nerve and cortical stimulation. J Appl Physiol. 1996 Apr;80(4):1351-6.
46. Chen TC, Chen HL, Lin MJ, Wu CJ, Nosaka K. Muscle damage responses of the elbow flexors to four maximal eccentric exercise bouts performed every 4 weeks. Eur J Appl Physiol. 2009 May;106(2):267-75.
47. Chen TC, Nosaka K, Sacco P. Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. J Appl Physiol. 2007 Mar;102(3):992-9.
48. Gandevia SC. Twitch interpolation a valid measure with misinterpreted meaning. J Appl Physiol. 2009 Jul;107(1):363-4; discussion 7-8.
49. Place N, Yamada T, Bruton JD, Westerblad H. Interpolated twitches in fatiguing single mouse muscle fibres: implications for the assessment of central fatigue. J Physiol. 2008 Jun 1;586(Pt 11):2799-805.
50. Jones DA. High-and low-frequency fatigue revisited. Acta Physiol Scand. 1996 Mar;156(3):265-70.
51. Nosaka K, Sakamoto K, Newton M, Sacco P. The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. Eur J Appl Physiol. 2001 Jul;85(1-2):34-40.

52. Paschalis V, Nikolaidis MG, Giakas G, Jamurtas AZ, Owolabi EO, Koutedakis Y. Position sense and reaction angle after eccentric exercise: the repeated bout effect. Eur J Appl Physiol. 2008 May;103(1):9-18.
53. Rekling JC, Funk GD, Bayliss DA, Dong XW, Feldman JL. Synaptic control of motoneuronal excitability. Physiol Rev. 2000 Apr;80(2):767-852.
54. Abbruzzese G, Morena M, Spadavecchia L, Schieppati M. Response of arm flexor muscles to magnetic and electrical brain stimulation during shortening and lengthening tasks in man. J Physiol. 1994 Dec 1;481 (Pt 2):499-507.
55. Lee M, Hinder MR, Gandevia SC, Carroll TJ. The ipsilateral motor cortex contributes to cross-limb transfer of performance gains after ballistic motor practice. J Physiol. 2010 Jan 1;588(Pt 1):201-12.
56. Folland JP, Williams AG. The adaptations to strength training : morphological and neurological contributions to increased strength. Sports Med. 2007;37(2):145-68.
57. Lee M, Carroll TJ. Cross education: possible mechanisms for the contralateral effects of unilateral resistance training. Sports Med. 2007;37(1):1-14.
58. Lee M, Gandevia SC, Carroll TJ. Unilateral strength training increases voluntary activation of the opposite untrained limb. Clin Neurophysiol. 2009 Apr;120(4):802-8.

Figure legends

Figure 1 Comparison between the first and second eccentric bout, performed 2 weeks apart in three different loads (low-, moderate- and high-load). Top panel represents changes in MVC. Middle panel represents changes in voluntary activation. Lower panel represents changes in the resting twitch.

Table legends

Table 6 The anthropometric characteristics of 30 subjects who completed the eccentric exercise and 10 subjects for each group (values are mean ± SD)

Table 7 Mean values ± SD for isometric maximal voluntary contraction (MVC) at pre-exercise, post-exercise and day 4 and percentage force loss in the first eccentric bout

Table 8 Mean values (± SD) for voluntary activation at pre-exercise, post-exercise and day 4 and percentage decrease in the first eccentric bout

Table 9 Mean values (± SD) for the rest twitches at pre-exercise, post-exercise and day 4 and percentage decrease in the first eccentric bout

Table 10 Mean values (± SD) for isometric maximal voluntary contraction (MVC) at pre-exercise, post-exercise and day 4 and percentage force loss in the second eccentric bout

Table 11 Mean values (± SD) for voluntary activation at pre-exercise, post-exercise and day 4 and percentage decrease in the second eccentric bout

Table 12 Mean values (± SD) for the resting twitches at pre-exercise, post-exercise and day 4 and percentage decrease in the second eccentric bout

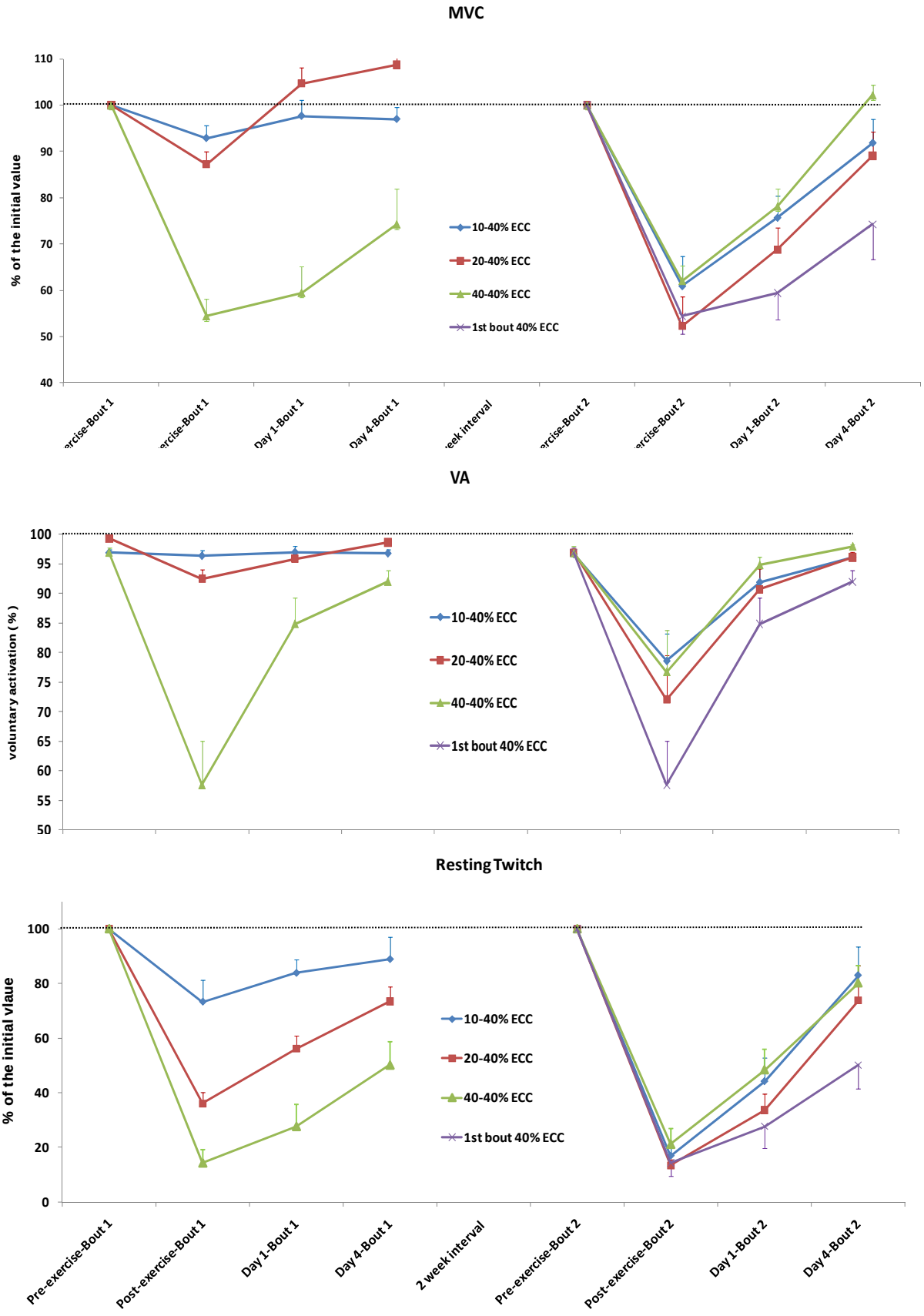
MVC
% of the initial value
110
100
90
80
70
60
50
40
10-40% ECC
20-40% ECC
40-40% ECC
1st bout 40% ECC
Day 1-Bout 1
Day 4-Bout 1
Day 1-Bout 2
Day 4-Bout 2
VA
voluntary activation (%)
100
95
90
85
80
75
70
65
60
55
50
10-40% ECC
20-40% ECC
40-40% ECC
1st bout 40% ECC
Resting Twitch
% of the initial vlaue
100
80
60
40
20
0
10-40% ECC
20-40% ECC
40-40% ECC
1st bout 40% ECC
Pre-exercise-Bout 1
Post-exercise-Bout 1
Day 1-Bout 1
Day 4-Bout 1
2 week interval
Pre-exercise-Bout 2
Post-exercise-Bout 2
Day 1-Bout 2
Day 4-Bout 2

Figure 1

Table 1

	Low-load group	Moderate-load group	High-load group
Age (yr)	29.0 ± 8.1	25.5 ± 4.5	26.8 ± 4.7
Weight (Kg)	59.3 ± 13	57.0 ± 11.2	64.7 ± 33.8
Height (cm)	161.1 ± 9.3	162.9 ± 6.2	150.0 ± 34.8
Gender (M:F)	2 : 8	3 : 7	2 : 8

Table 2

Group	MVC for 1st ECC bout (N)				
	Pre-exercise	Post-exercise	% force loss immediately after exercise	At day 4	% force loss at day 4
Low-load group	167.9 ± 63.8	153.9 ± 53.1	7.12	170.4 ± 55.7	0
Moderate-load group	170.02 ± 59.4	147.8 ± 54.7	12.76	187.7 ± 80.6	0
High-load group	155.0 ± 61.8	81.4 ± 31.5	45.61	105.4 ± 35.4	23.75

Table 3

Group	Voluntary activation for 1st ECC bout (%)				
	Pre-exercise	Post-exercise	% decrease immediately after exercise	At day 4	% decrease at day 4
Low-load group	96.85 ± 1.87	96.43± 2.73	0	96.76 ± 0.33	0
Moderate-load group	99.26± 0.84	92.44± 4.79	6.82	98.58 ± 1.20	0.68
High-load group	96.83± 2.64	64.21 ± 18.35	32.62	91.92 ± 6.06	4.91

Table 4

Group	Resting twitch for 1st ECC bout (N)				
	Pre-exercise	Post-exercise	% force loss immediately after exercise	At day 4	% force loss at day 4
Low-load group	12.12 ± 6.21	8.80 ± 4.27	26.7	10.66 ± 4.71	12.05
Moderate-load group	12.36 ± 3.14	4.41 ± 1.80	63.91	8.73 ± 1.77	26.55
High-load group	12.97 ± 6.26	1.98 ± 2.40	85.68	6.40 ± 4.74	49.88

Table 5

Group	MVC for 2nd ECC bout (N)				
	Pre-exercise	Post-exercise	% force loss (Immediately after exercise)	At day 4	% force loss (4 days after exercise)
Low-load group	171.3 ± 57.7	99.3 ± 28.2	39.06	156.9 ± 61.2	8.29
Moderate-load group	194.1 ± 84.0	101.6 ± 46.0	47.70	172.8 ± 75.9	10.94
High-load group	145.6 ± 50.4	90.9 ± 39.2	37.99	146.8 ± 51.2	0

Table 6

Group	VA for 2nd ECC bout (%)				
	Pre-exercise	Post-exercise	% decrease (Immediately after exercise)	At day 4	% decrease (4 days after exercise)
Low-load group	96.60 ± 2.49	78.56 ± 14.73	19	96.50 ± 1.49	0
Moderate-load group	96.85 ± 1.83	71.56 ± 23.08	26	96.04 ± 3.08	0
High-load group	96.75 ± 3.64	76.70 ± 22.46	21	97.88 ± 1.26	0

Table 7

Group	RT for 2nd ECC bout (N)				
	Pre-exercise	Post-exercise	% force loss (Immediately after exercise)	At day 4	% force loss (4 days after exercise)
Low-load group	13.71 ± 8.11	2.31 ± 2.09	83	10.79 ± 6.35	21
Moderate-load group	12.22 ± 4.35	1.69 ± 1.15	86	8.11 ± 3.07	34
High-load group	13.34 ± 5.64	3.37 ± 4.32	74	10.45 ± 4.36	22