# Contralateral Repeated Bout Effect of the Elbow Flexors Not Observed In Young Women

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# CONTRALATERAL REPEATED BOUT EFFECT OF THE ELBOW FLEXORS NOT OBSERVED IN YOUNG WOMEN

by Bailey Brown

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford May 2019

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ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. Ling Xin for allowing me to work under her and in the Muscle Biology Lab. She has been so incredibly helpful in creating, executing, and writing this research.

I would also like to thank Dr. Xin Ye and his lab for allowing me to use their equipment and their guidance with the electromyography equipment and data.

I sincerely thank Madison Huffman and Montana Kuzma for lending a hand with everything and working in the lab with me throughout this entire process.

Thank you to Dean Peter Grandjean for being one of my readers.

Finally, I would to thank the Sally Barksdale Honors College for funding my research.

ABSTRACT

The contralateral repeated bout effect (CL-RBE) is known as a protective effect in which an initial bout of eccentric exercise reduces muscle damage in the contralateral limb after a similar bout of eccentric exercise. To our knowledge, previous studies on CL-RBE only recruited males or a mixture of both sexes. **PURPOSE:** To investigate the presence of the CL-RBE of the elbow flexors in women. **METHODS:** Twelve healthy women (20.9 ± 2.5 years) performed two bouts of 45 maximal eccentric contractions (ECC) of the elbow flexors using the opposite arm separated by 14 days. The isokinetic muscle strength (60°/sec) was measured pre-exercise, immediately post-exercise, and 24 and 48 h post-exercise. Limb girth, range of motion (ROM), and muscle soreness were measured pre-exercise, and at 24 and 48 h post-exercise. Surface Electromyography (EMG) was recorded during both exercise bouts from the biceps brachii muscle. Data of all variables were analyzed using two-way repeated measures ANOVA (Bout × Time) except that of EMG amplitude and median frequency (MF), which was analyzed via paired t-test. **RESULTS:** The isokinetic strength was significantly reduced after the eccentric exercise for both bout 1 (-19.3 ± 17.4%, $P < 0.01$) and bout 2 (-15.3 ± 15.2%, $P < 0.01$). Significant main effects of time were also observed for muscle soreness and ROM. Limb girth, EMG amplitude, and MF did not change significantly ($P > 0.05$) after either exercise bout. There were no significant differences between bouts for all the measured variables. **CONCLUSION:** The CL-RBE of elbow flexors was not evident in young healthy women. This was probably because the initial bout of exercise did not induce enough damage to bring about the CL-RBE in the second bout or the CL-RBE in women lasted shorter than two weeks.

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# LIST OF ABBREVATIONS

| | |
|---|---|
| ECC | eccentric |
| EIMD | exercise induced muscle damage |
| ROM | range of motion |
| DOMS | delayed onset muscle soreness |
| CK | creatine kinase |
| RBE | repeated bout effect |
| IL-RBE | ipsilateral repeated bout effect |
| CL-RBE | contralateral repeated bout effect |
| MVC | maximal voluntary contraction |
| EMG | electromyography |
| MF | median frequency |
| PAR-Q | Physical Activity Readiness Questionnaire |
| V0 | initial visit |
| S1V1 | Stage 1 Visit 1 |
| VAS | visual analog scale |
| NMMC | North Mississippi Medical Center |
| RMS | root mean square |
| DFT | Discrete Fourier Transform |
| ML | midluteal |
| MF | midfollicular |

# CHAPTER I

# INTRODUCTION

Eccentric (ECC) exercise is a type of exercise during which a muscle is forced to be lengthened while it is trying to contract to control an outside force. Unaccustomed ECC exercise induces transient muscle damage. Exercise-induced muscle damage (EIMD) is displayed by indirect markers including prolonged loss of muscle strength, decreased range of motion (ROM), delayed onset muscle soreness (DOMS), muscle swelling, and increased blood creatine kinase (CK) activity (1). One bout of eccentric exercise provides a protective effect against muscle damage when the same exercise is conducted later by the same muscle (1, 2). This protective effect causes a faster recovery of the muscle when the same muscle is exposed to the same ECC exercise for the second time and is known as the repeated bout effect (RBE). It is also referred to as the ipsilateral repeated bout effect (IL-RBE) because the second bout is performed by the ipsilateral muscle. IL-RBE is characterized as a reduced magnitude in muscle strength loss, swelling, ROM loss, DOMS, and blood CK activity increase when compared to the first bout (3, 4).

Increasing evidence demonstrated that an initial bout of ECC exercise also produces protective adaptation in the contralateral limb, which is defined as the contralateral repeated bout effect (CL-RBE). Similarly to the IL-RBE, the CL-RBE is manifested by attenuated muscle strength loss, swelling, DOMS, ROM reduction, and less increase in blood CK activity when the second bout of ECC exercise is conducted by the contralateral muscle that was not involved in the initial ECC exercise bout (3, 4). The magnitude of CL-RBE has been shown to be less than that of the IL-RBE (3, 4). The exact mechanisms underlying the CL-RBE are unknown, but it is hypothesized that it may be in part due to neural adaptation. There is evidence of the addition of longitudinal sarcomeres and adaptations in the inflammatory response, which could cause the RBE (5). Although there are theories, the exact mechanism for RBE is unknown.

To our knowledge, the majority of previous studies investigating the IL-RBE and/or CL-RBE only recruited males or a mixture of males and females, so the purpose of this study was to investigate whether the CL-RBE of elbow flexors exists in women. We hypothesized that the CL-RBE would be evident in women which would be characterized as an attenuated decrease in muscle strength and ROM, less DOMS, less increase in blood CK activity and limb girth.

# CHAPTER II

## REVIEW OF LITERATURE

An eccentric (ECC) exercise is an exercise that activates a muscle while the muscle is lengthened under a load. Unaccustomed exercise involving ECC action components can induce transient muscle damage, which is refered to as exercise-induced muscle damage (EIMD). It is hypothesized that during ECC exercise, the sarcomeres of the muscles are stretched too far and become damaged (6). EIMD is often assessed via measuring indirect markers such as muscle strength loss, increased blood CK activity, decreased ROM, increased soreness, and increased limb girth.

One of the most reliable and valid indicators of muscle damage is prolonged muscle strength loss (7). High force ECC exercises have been reported to produce the greatest muscle strength loss (1). One of the major causes of muscle strength loss is the mechanical strain on the muscle fibers during ECC actions. By lengthening the muscle during ECC exercise, an unaccustomed strain is placed on the muscle resulting in microtears in the muscle and thus decreases the force the muscle can produce after ECC exercise (1). Muscle strength loss can last 1 to 2 weeks after ECC exercise (1).

Creatine kinase (CK) is a muscle specific protein whose activity was found increased in the blood after ECC exercise in many studies. For instance, blood CK activity has been seen to increase after high force muscle contractions until around 48 h post exercise (1). It has been suggested that the increase in blood CK activity is due to the

CK leaking out of the damaged muscle (8). Post-exercise CK activity is highly variable among individuals (9). The reason for the high inter-individual variability in CK activity is unknown, but it is probably linked to many different variables including genetic factors and training parameters. An increased level of CK activity is generally accepted as an indicator of exercised-induced muscle damage, but it is unclear to what extent that CK activity in the blood is associated with the magnitude of muscle damage (2).

Delayed onset muscle soreness (DOMS) is believed to depend on the extent of damage in the muscle and seems to peak 24 to 48 hours after exercise (1). When the *tibialis anterior* muscles were biopsied 48 hours after ECC exercise, the biopsy samples were larger and the intramuscular pressure was higher than the initial muscle biopsy (10). Soreness may be caused by swelling and pressure within the muscle. Along with swelling and pressure in the muscle, noxious chemicals have been suggested to influence soreness (1). Howell et al. (11) found that pressure was the physical stimulus for the pain associated with soreness. It is also suggested that soreness is caused by direct micro-trauma in myofibers (12). Oosthuyse and Bosch (12) saw a prolonged DOMS response in women compared to men and suggested it may be influenced by menstrual phase, but other studies have found no gender difference or women having less DOMS (13, 14). Both a decreased in range of motion (ROM) and an increase in limb girth are also indicators of inflammation within the muscle and muscle damage (1).

A prior bout of ECC exercise has been shown to produce a protective adaptation reducing the amount of muscle damage caused by the same exercise for the second time. Investigators found that following a second about of the same ECC exercise there was attenuated muscle strength loss, swelling, DOMS, ROM reduction, and less increase in blood CK activity. This phenomenon is known as the repeated bout effect (RBE) (3). It is also known as the ispilateral repeated bout effect (IL-RBE) because the second bout of the ECC exercise is performed on the same limb as the original exercise bout. The exact mechanism for the RBE is unknown, but many theories point to cellular, mechanical, and neural adaptations (4).

More and more research found that the protective adaptation following an initial bout of ECC exercise was also transferred to the unexercised, contralateral limb (3, 4). It was termed as the contralateral repeated bout effect (CL-RBE). After an initial bout of ECC exercise was done on one limb, there was a reduction in muscle strength loss, swelling, DOMS, ROM reduction, and less increase in blood CK activity when the similar ECC exercise was performed by the contralateral limb, which was not involved in the initial exercise (3).

The first study (3) on CL-RBE found that a second bout of exercise on the contralateral limb has reduced changes in the indirect muscle damage markers compared to the first bout. There were large reductions in CK, DOMS, and maximal voluntary contraction (MVC). Howatson and van Someren (3) reported that the CL-RBE was evident when a second bout of three sets of 15 maximal isokinetic (30°/sec) eccentric contractions of the elbow flexors was done 2 weeks after the first bout. Participants in the study were men that were familiar with resistance training but not accustomed to ECC exercise. The study also reported that the CL-RBE had a smaller protective effect than the IL-RBE (3). Chen *et al.* (15) examined the magnitude of the CL-RBE with different time intervals between both exercise bouts. A total of 104 young, untrained men participated in the study, and each of them did two bouts of ECC exercise comprising 5 sets of 6 maximal isokinetic ECC contractions using opposite arms. The participants were divided into groups that performed the second bout at 0.5 h, 6 h, 12 h, 24 h, 7 d, 4 wk, and 8 wk after the first exercise bout. Chen *et al.* (15) found that all of the muscle damage markers were significantly smaller in the second bout of exercise compared to the first bout when the second bout was done 24 h, 7 d, and 4 wk after the initial bout. The magnitude of the CL-RBE was decreased as the intervals increased between 24 h, 7 d, and 4 wk (15). The CL-RBE was not observed in the 0.5 h, 6 h, 12 h, or 8 wk groups. It appears that it takes more than 12 hours for the CL-RBE to be evident and it lasts up to 4 weeks.

Like RBE, the mechanisms for CL-RBE are unknown. There are two theories (16) to explain the CL-RBE. One states that the spinal and cortical motor pathways to the contralateral limb become more efficient after exercising one limb. The other hypothesis proposes that the motor control areas of the brain that were trained during the initial bout of exercise can be used by the untrained contralateral muscles (17). Both hypothesis are only theoretical.

Although the exact mechanisms for CL-RBE are unknown, many studies used electromyography (EMG) to study the motor unit activation in a particular muscle (4, 18) . When the median frequency (MF) of the EMG data is decreased, it is suggestive of a relatively larger number of slow twitch motor units being recruited during the exercise(4). Chen *et al.* (18) found that a lower MF was consistent with a decreased recruitment of fast-twitch muscle fibers. An increased EMG amplitude is thought to demonstrate an increase in motor unit recruitment. By recruiting more motor units during the second bout of ECC exercise, the stress placed on each individual motor unit would be less and would potentially reduce the damage caused compared to the first bout of ECC exercise (5). Although it is hypothesized that the EMG amplitude should increase after one bout of exercise, many studies have not seen a change in the EMG amplitude in the second bout of exercise (4, 5).

Starbuck and Eston (4) expanded on the Howatson and van Someren (3) study by using EMG to measure the electrical activity in the muscles. Specifically, surface EMG was used to measure the median frequency (MF) and amplitude during the two bouts of exercise. A reduction of 31% in the MF was observed in the second bout of exercise. It is believed that a lower MF indicates an increase in the amount of slow twitch muscle fibers being recruited during the exercise. Reductions in the MF were seen in both the ipsilateral and contralateral groups, but there was a larger reduction in the MF in the contralateral group. In the study, there was no significant difference in EMG amplitude, which suggests that the number of recruited motor units was similar for both the IL-RBE and CL-RBE groups in both bouts (4).

Most studies on the CL-RBE only recruited men or a mix of both sexes. Previous research has shown either no difference between the response after ECC exercise in women compared to men or women showed a more pronounced response after ECC exercise (1). Sewright et al. (19) specifically investigated the sex difference in response to maximal ECC exercise. They found that women had a greater immediate loss of strength after ECC exercise when compared to men. Although women had a higher muscle strength loss immediately post exercise, there were no significant differences between men and women after 12 hours post exercise, so the sex differences in muscle strength loss were only evident immediately after exercise. This suggested a sex difference in muscle fatigue rather than in muscle damage. Men had higher blood CK activity four days after exercise. The increase in blood CK activity post-exercise is expected to be attenuated after the RBE, suggesting less muscle damage in women after exercise. The effect of estrogen on post-exercise blood CK activity is unclear. Some studies suggested that circulating estradiol and/or hormonal contraceptives could affect the post-exercise blood CK activity level (20), but other studies found that circulating estradiol has no effect on CK response after maximal ECC exercise (19). Oosthuyse and Bosch (12) had women participate in downhill running and found that women had a quicker CK activity recovery time. They suggested that it might be because estrogen reduces the secondary phase of EIMD that was caused by local inflammation. The same study did not find any difference in the CK response between menstrual phases (12).

To the best of my knowledge, there has not been a study investigating the CL-RBE only in women. Therefore, the purpose of the current study is to see if the CL-RBE exists in women. We hypothesized that we would see a reduction in muscle strength loss, swelling, DOMS, ROM reduction, and less increase in blood CK activity in the contralateral limb when it is exposed to the same ECC exercise that was initially performed by the ipsilateral limb.

# CHAPTER III

# METHODS

## Subjects

Twelve untrained, healthy women volunteered and completed all the visits. Their mean mean ± SD age, height, and body mass were 20.9 ± 2.5 yr, 1.6 ± 0.11 m, and 66.9 ± 9.8 kg, respectively. Subjects were recruited from the University of Mississippi and Oxford Community via recruitment fliers placed in the Student Union and Turner Center, or mass emails to the enrolled female students at University of Mississippi. They had not participated in resistance training of arms for at least six months prior to the study. All of the subjects had no prior history of surgeries or injuries to the arm, neck, or wrist. They completed a Physical Activity Readiness Questionnaire (PAR-Q) and a medical history questionnaire to ensure they were able to perform the exercise and measurements safely. No subjects had been diagnosed with cardiovascular, pulmonary, metabolic, or any other chronic diseases, and had no routine use of any medications except birth control pills. The subjects were asked to refrain from using oral and topical analgesics, heat or cold treatment, physical therapy, massage or any other muscle treatment regimen during the course of the study. They also refrained from consuming alcohol, alcohol containing products, and cough/cold products during the study period of Stage 1 Visits 1-4 duration and Stage 2 Visits 1-4 duration. All the subjects were informed of the study protocol and signed the informed consent documents that had been approved by the Institutional Review Board of the University of Mississippi.

## Study Design

All the subjects competed nine separate visits which are illustrated in Figure 1. The pre-test screening was conducted over phone. The pre-qualified subjects after phone screening were scheduled for an interview visit (V0). During V0, the subject reviewed and signed the informed consent form and completed the brief medical history and Physical Activity Readiness Questionnaire (PAR-Q) forms. In addition, the arm tested (exercised) in the initial bout of eccentric exercise was determined by alternating from dominant to non-dominant arm as the subjects were enrolled. Therefore, there were equal number of subjects who used dominant or non-dominant arm in the first bout of eccentric exercise. The eligible subject then completed eight visits, which were divided into two stages (Stage 1 Visits 1-4 and Stage 2 Visits 1-4). During Stage 1 Visit 1 (S1V1), the women's height and weight were measured, and the dates of the first day of their last menstrual cycle were recorded. S1V1 consisted of measurements of muscle soreness, range of motion (ROM), upper arm circumference, and isokinetic muscle strength. The blood draw was done before the isokinetic muscle strength measurement, but after the muscle soreness, ROM, and limb girth measurements. Twenty-four hours after the completion of S1V1, each participant returned for her Stage 1 Visit 2 (S1V2). At the beginning of S1V2, muscle soreness, ROM, limb girth, and isokinetic muscle strength of the exercise-assigned arm were measured. The participants then completed 45 maximal eccentric actions of the assigned biceps muscles on an isokinetic dynamometer. Immediately after the eccentric exercise, isokinetic muscle strength (60°/sec) of the arm was measured. On each day of the following two days (S1V3 & S1V4) after S1V2, the subjects had blood drawn and completed the upper arm circumference, muscle soreness, ROM, and strength assessment of the exercised arm. Stage 2 Visit 1 (S2V1) will be 13 days after S1V2. Stage 2 Visits 1-4 were the same as Stage 1 Visits 1-4 except that the opposite arm was exercised and tested. On each blood draw less than 1 tablespoon of blood (~ 10 ml) was taken. A total of 6 blood samples were taken over the whole study. Therefore, about 60 ml blood was taken from each subject over the entire study. x

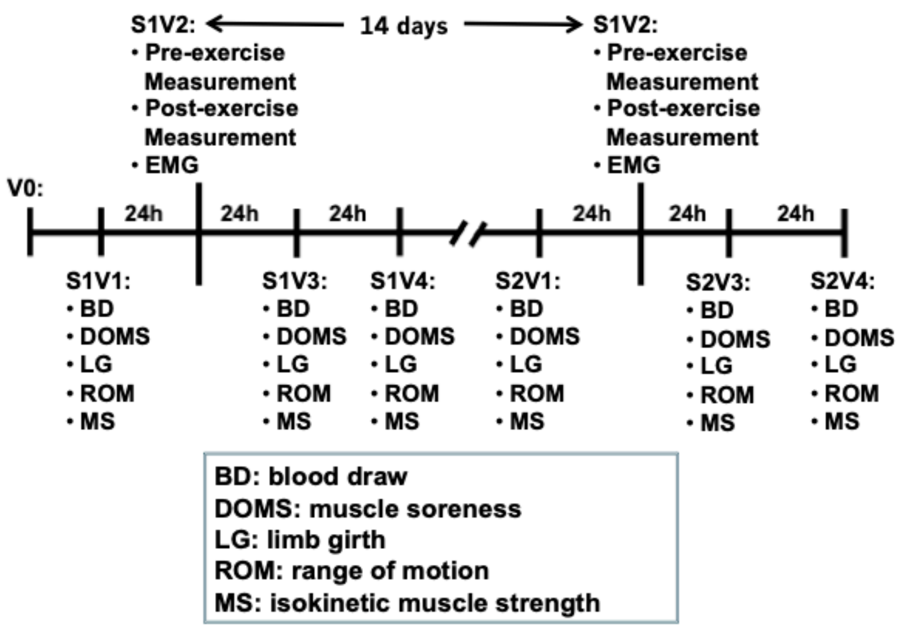


Figure 1: Study layout

*Muscle Soreness Measurement*

Each participant was asked to do two bicep curls with either a one- or two-pound dumbbell. The participant used a one-pound weight if her body mass was under 130 lbs., and a two-pound dumbbell if she has a weight of  over 130 lbs. After the two curls, she was asked to mark her peak soreness level of the tested arm on a 100-mm visual analog scale (VAS), with “no pain” on the left end (0 mm) and “unbearable soreness” on the right end (100 mm). The distance from the left end to the mark was measured and recorded as the soreness level.

*Range of Motion Measurement*

A goniometer was used to measure the ROM of the arm being tested. The participants were asked to keep their arm down by their side and extend it as far as they could comfortably. They were then asked to flex the arm maximally with their palm facing their shoulder. The maximal degree of flexion and extension were recorded. The full range of motion was determined by subtracting the maximal flexed joint angle from the maximal extended joint angle.

*Limb girth Measurement*

Each participant was asked to relax their arms at their side, and the length from the acromion to the lateral epicondyle of the tested arm was measured using a tape measure. The mark was placed at 2/3rds of the length from the acromion to the lateral epicondyle. The circumference of the upper arm was taken surrounding the mark three times. The circumferences were then averaged and recorded.

*Isokinetic Muscle Strength*

Isokinetic muscle strength assessment was conducted on the Biodex System 3 isokinetic dynamometer. The subjects were seated so that their backs were flush against the back of the seat of the dynamometer, and the straps were used to secure their shoulders and waist. The arm rest was raised so that their arm was comfortably resting at around a 45 degree angle. The handle bar was made so that tested/exercised hand was in a supinated position and the subjects could comfortably flex and extend their arm. It was lined up so the subject's lateral humerous epicondyle aligned with the axis of rotation of the dynamometer.

The subjects did 3 maximal biceps curls at the angular velocity of 60º/sec. The peak force of all three trials was recorded. The peak force was the highest amount of force the participant produced during the three trials. The settings of the dynamometer were recorded, therefore every visit had the same identical position as the initial visit.

*Eccentric Exercise*

The same positioning of the subject on the Biodex dynamometer for the muscle strength measurements was used for the eccentric exercise. The subjects performed 3 sets of 15 repetitions of maximal eccentric contractions of the elbow flexor at a speed of 30°/s with 10 seconds rest in between each repetition and 3 minutes of rest between each set. The starting position was 30° from the subject's arm full flexion and ended at full extension (0°). The subject was verbally encouraged to pull maximally during each eccentric contraction. At the end of each eccentric contraction, the arm was moved back to the starting position by the investigator. The work during each set was measured, and the total work accomplished during each bout of eccentric exercise was calculated by adding all of the work done during each of the three sets.

*Blood Creatine Kinase Activity Assay*

*B*lood samples were collected from the antecubital vein and were then spun in the centrifuge for 15 min at 3000 rpm to separate the plasma from the other contents of the blood. The plasma samples were stored in the -80°C freezer until ready to transport. After a subject's blood samples are collected from all visits, the plasma samples were packed up and shipped to North Mississippi Medical Center (NMMC) where the CK activity level was analyzed.

*Electromyography (EMG)*

During all maximal isometric and eccentric contractions, surface EMG signals were recorded through a wireless surface electrode (Trigno™ EMG Sensor, Delsys, Inc., Natick, MA). The sensor was attached over the biceps brachii muscle belly based on the recommendations from SENIAM (21). Prior to any electrode placements, the investigator shaved and cleaned the skin surface with rubbing alcohol. And medical tapes were used to firmly fixate the electrode on the skin. The analog bipolar EMG signals were collected and amplified (gain = 1000) with a Trigno™ Wireless System (Delsys, Inc., Natick, MA) and filtered with high and low pass filters set at 20 and 450 Hz, respectively. The filtered signals were then digitized at a sampling rate of 1926 Hz with a 12-bit analog-to-digital converter (National Instruments, Austin, TX).

For the three maximal isometric contractions, we used a 500 ms-window to select the contraction that yielded the greatest amplitude. The amplitude of each selected window of each EMG signal was calculated as the root mean square (RMS). For the EMG median frequency (MF) analyses, the EMG signal was first filtered with a Hamming window, and the Discrete Fourier Transform (DFT) algorithm was used to derive the EMG signal into the power spectrum. The MF of the spectrum was lastly calculated based on the equation described by Kwatny et al. (22). For each of the eccentric muscle contraction, the mid 1-s portion of the entire 3 seconds was selected for analyses. All raw RMS values from the maximal eccentric contractions were normalized as percentages of the EMG values from the isometric strength testing trial.

*Statistical Analyses*

Muscle strength, ROM, limb girth, and muscle soreness data were analyzed using a two-way repeated measures ANOVA to determine the main effects of time (exercise), bout, and their interaction terms. The MF and amplitude data were analyzed with paired t-tests. A significance level was set at $P < 0.05$. All data analyses were conducted using SPSS statistical software (Version 22, SPSS Inc., Chicago, IL).

# CHAPTER IV

# RESULTS

*Baseline measurements*

Table 1 displays the baseline and pre-exercise values of muscle strength, ROM, and limb girth. No significant differences between the two bouts were observed for any of these variables.

Table 1: Baseline and pre-exercise values of isokinetic strength, ROM, and limb girth of the elbow flexors.

| | Baseline | Pre-Exercise | Difference between baseline and pre-exercise |
|---|---|---|---|
| Isokinetic peak torque (N·m) | | | |
| Bout 1 | 19.5 ± 4.5 | 19.4 ± 4.5 | 3.5 ± 4.1 |
| Bout 2 | 19.5 ± 5.9 | 17.6 ± 4.7 | 2.5 ± 5.9 |
| ROM (˚) | | | |
| Bout 1 | 144.1 ± 5.3 | 145.8 ± 5.9 | 5.0 ± 5.2 |
| Bout 2 | 145.6 ± 4.7 | 145.1 ± 4.5 | 4.8 ± 4.1 |
| Limb girth (cm) | | | |
| Bout 1 | 28.6 ± 3.2 | 28.6 ± 3.1 | 0.3 ± 0.3 |
| Bout 2 | 28.7 ± 3.2 | 28.5 ± 3.2 | 0.3 ±0.2 |

Data are mean ± SD (n = 12). No significant differences between bouts were observed for any variable.

*Eccentric Exercise*

The exercising ROM was107.7± 9.1° and 104.45± 7.3 for Bout 1 and Bout 2, respectively. The exercising ROM difference between Bout 1 and Bout 2 was not statistically significant (p = 0.20). Similarly, no significant difference was observed between the total work completed in the Bout 1(469.4 ± 165.5 J) eccentric exercise and the total work in Bout 2 (453.4 ± 187.9 J) (Figure 2). These results indicate that the subjects provided similar and consistent efforts during both eccentric exercise bouts and thus minimize the potential confounding effects from the difference in both exercise bouts.

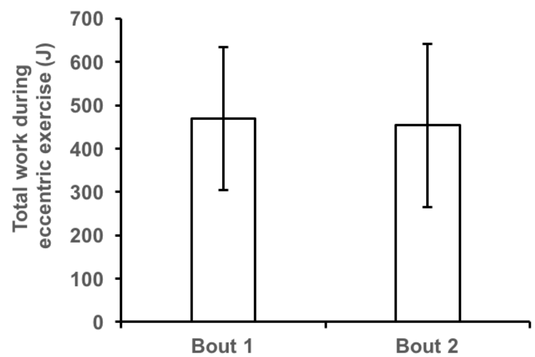


Figure 2: The total work completed during each bout of the eccentric exercise. Values are mean ± SD; n = 12.

*Muscle Soreness*

Muscle soreness was significantly ($p < 0.05$) increased at both 24 h and 48 h post exercise within each bout, indicating that the exercise protocol was successful in inducing muscle damage. However, there were no significant bout ($p > 0.05$) or interaction ($p > 0.05$) effects. (Figure 3).

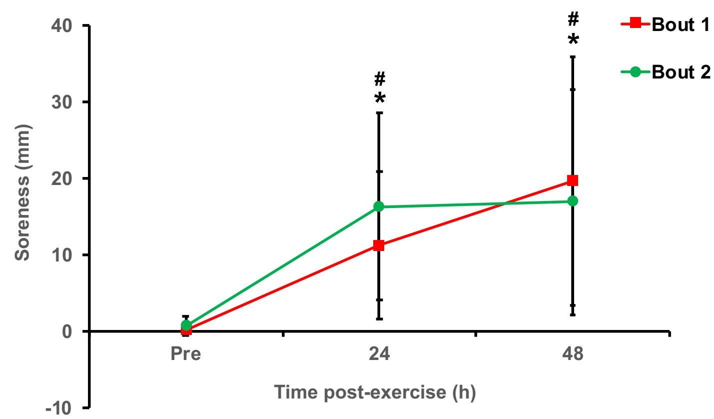


Figure 3: Muscle soreness pre-exercise (Pre), 24 h, and 48 h post-exercise following the two bouts of eccentric exercise. Values are mean ± SD; n = 12 for each time point. * Significant difference compared to pre-exercise in Bout 1. # Significant difference compared to pre-exercise in Bout 2.

*Isokinetic Muscle Strength*

Figure 4 shows the percent changes in isokinetic peak torque at 60°/s at different time points after the two bouts of eccentric exercise. There were significant main effects of time ($p < 0.05$) for both bouts. The maximal isokinetic torque loss was observed at 24 h post-exercise for both exercise bouts, decreasing by 19.3 ± 17.4% and 15.3 ± 15.2% in Bout 1 and Bout 2, respectively. The isokinetic torque returned to pre-exercise levels at 48 h post-exercise in Bout 1 and 24 h post-exercise in Bout 2, respectively which seemed to suggest a faster muscle strength recovery after Bout 2. However, there were no significant bout ($p > 0.05$) or interaction ($p > 0.05$) effects.

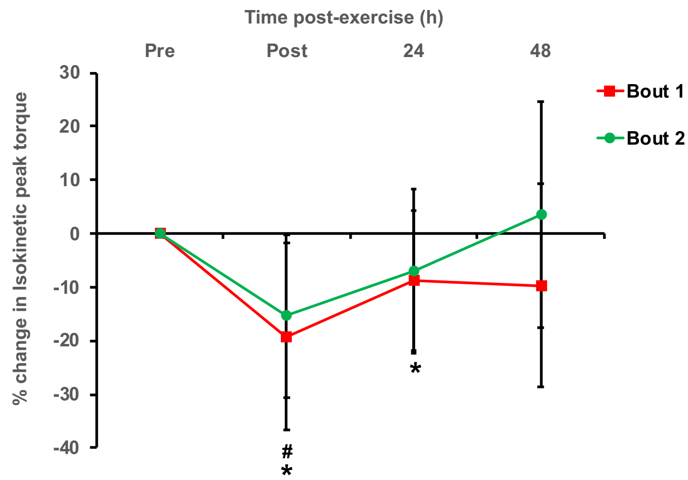


Figure 4: Isokinetic contraction peak torque pre-exercise (Pre), immediately post-exercise (Post), 24 h, and 48 h post-exercise following the two bouts of eccentric exercise. Values are mean ± SD; n = 12 for each time point. * Significant difference compared to pre-exercise in Bout 1. # Significant difference compared to pre-exercise in Bout 2.

*Range of Motion*

Range of motion (ROM) was significantly decreased at 24 h after exercise within each bout, but no significant difference ($p > 0.05$) was observed when comparing the first bout with the second bout (Figure 5).

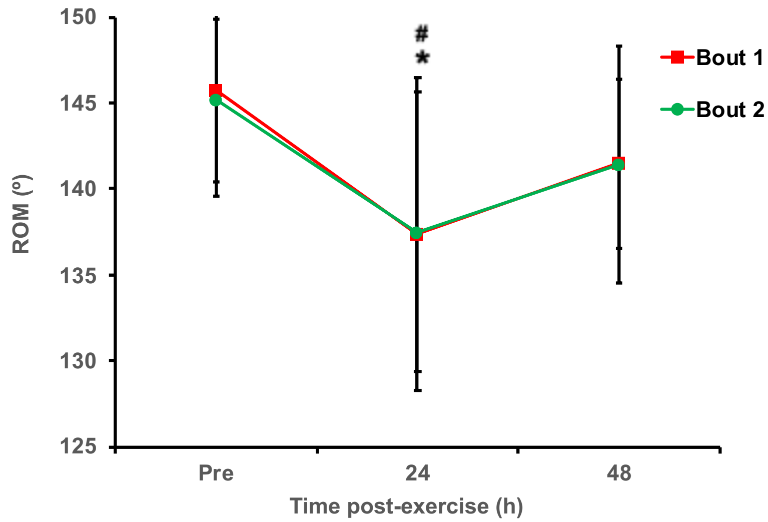


Figure 5: Range of motion (ROM) pre-exercise (Pre), immediately post-exercise (Post), 24 h, and 48 h post-exercise following the two bouts of eccentric exercise. Values are mean ± SD; n = 12 for each time point.  * Significant difference compared to pre-exercise in Bout 1. # Significant difference compared to pre-exercise in Bout 2.

*Blood Creatine Kinase (CK) Activity Assay*

We could not obtain the blood samples form one of the subjects due to her tiny antecubital vein. Therefore, the CK activity data were collected from the blood samples of 11 subjects. There was no significant increase ($p > 0.05$) in the plasma CK activity level over time within each exercise bout (Figure 6). Neither was there significant difference in the CK activity change between the two bouts, and there was no significant interaction ($p > 0.05$).

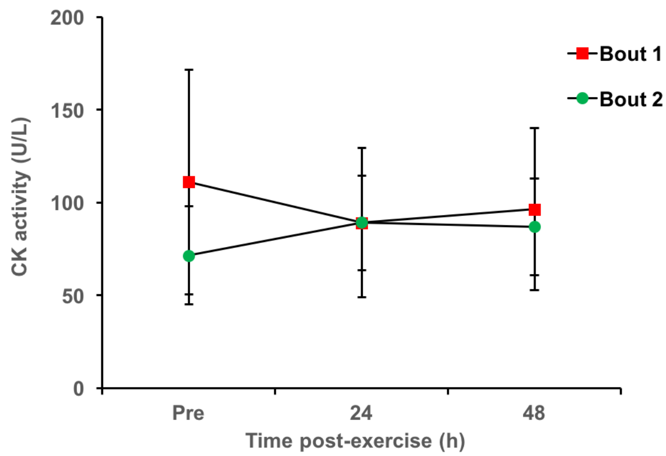


Figure 6: Plasma creatine kinase (CK) activity pre-exercise (Pre), 24 h, and 48 h post-exercise following the two bouts of eccentric exercise. Values are mean ± SD; n = 11 for each time point.

*Limb girth*

The upper limb girth did not differ significantly ($p > 0.05$) over time within each bout or between the two bouts of exercise. No significant difference ($p > 0.05$) was identified in the interaction of time and bout, either.

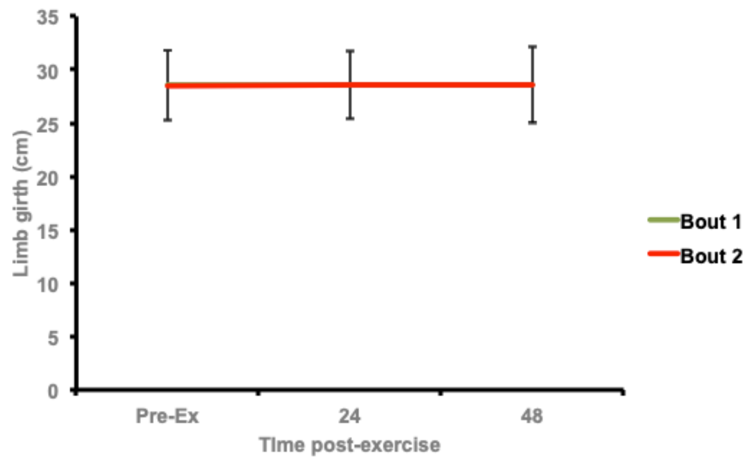


Figure 7: Limb girth pre-exercise (Pre), 24 h, and 48 h post-exercise following the two bouts of eccentric exercise. Values are mean ± SD; n = 12 for each time point.

*Electromyography (EMG)*

Median frequency (MF) and EMG amplitude were the two variables recorded and processed from the raw EMG data. Four participants' EMG data were omitted due to the EMG equipment errors during testing. As a result, only 8 subjects' EMG data were used for the final analyses. Statistical analysis of the MF data revealed no significant difference ($p>0.05$) between sets or between bouts (Figure 8). There were no significant main effects for either set or bout for EMG amplitude (data now shown).

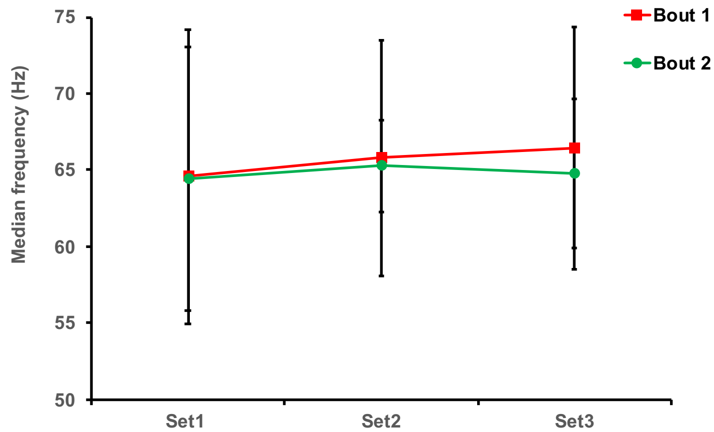


Figure 8: Median frequency (MF) of the 3 sets during both eccentric exercise bouts.

Values are mean ± SD; n = 8 for each time point.

# CHAPTER V

# DISCUSSION

The main objective of this study was to examine whether CL-RBE exists in women. The overall results did not indicate the existence of CL-RBE of the elbow flexors in young women. There were no significant differences in the commonly measured EIMD markers (blood CK activity, muscle strength, muscle soreness, ROM, limb girth, etc.) between the two bouts of eccentric exercise using the opposite arms.

Previous studies (3, 4, 15, 23) have shown evidence of the CL-RBE in men. It has been manifested by attenuated changes in the indirect markers of EIMD following a second bout of the same damaging exercise using the opposite limb. There are two hypotheses (16) to explain the CL-RBE. The first hypothesis states that the spinal and cortical motor pathways to the contralateral limb become more efficient after exercising one limb. The other hypothesis proposes that the untrained contralateral muscles are able to use the motor control areas of the brain that were trained during the initial bout of exercise (17). The exact mechanisms are still unknown, so these hypotheses are only theoretical.

In this study, we measured the changes in most frequently used EIMD indirect markers including muscle strength loss, DOMS, limb girth, ROM, and blood CK activity level (1). We observed significant main effects of time for muscle strength loss, DOMS, and ROM, but not for limb girth or blood CK activity level. Since muscle strength has been considered as the best EIMD indirect marker (7), the significant time effect of muscle strength loss, accompanied by the significant changes in DOMS and ROM over time, provided strong enough evidence to suggest that the eccentric exercise protocol used in this study successfully induced muscle damage.

In contrast to our hypothesis, the data of the current study did not indicate the existence of CL-RBE in untrained, young women. No significant main effect of bout or time-bout interactions were identified for all the measured variables. Although the magnitude of the muscle strength loss seemed to be attenuated in the Bout 2 exercise, the difference between the bouts did not reach statistical significant levels. There are two main possible reasons why the CL-RBE was not evident in this study. First, the eccentric exercise protocol used was not damaging enough to the biceps brachii in the women subjects of this study. Chen *et al. (15)* used a similar exercise protocol in men where the subjects did 30 repetitions of elbow flexor eccentric exercises, and they found a ~ 40% decrease in muscle strength in the initial bout. Howatson and van Someren (3) used the same exercise protocol as in this study during which the male subjects performed two bouts of 45 maximal eccentric contractions using opposite arms and they reported a ~ 25% muscle strength loss in the first bout. In the current study, the loss of muscle strength in the Bout 1 exercise was less than 20%, which was a smaller loss than the aforementioned two studies (~ 40% and ~25% strength loss).This different magnitude of EIMD could be due to the sex difference of the recruited subjects between my study and those two studies. Indeed, female skeletal muscles have displayed less damage following damaging stimuli such as strenuous exercise in both human and animal models (12, 24). Estrogen has been proposed to have a protective role in the inflammatory response following muscle damage (24). Second, the initial bout of eccentric exercise might have generated enough stimulus to provide CL-RBE in women, but the CL-RBE lasted shorter than 2 wk in women so that it was not caught in the current study. I used 2 wk as the time interval between the two exercise bouts based on previous studies (3, 4, 25), but the CL-RBE in women probably already subsided and become undetectable prior to 2 wk. This is possible because Chen et al. (15) reported that the magnitude of CL-RBE in men decreased as the time interval between bouts increased. Therefore, future studies on CL-RBE in women need to adopt shorter time interval between exercise bouts such as 1 wk or even shorter so that the CL-RBE in women might be more profound and become detectable. Regardless the possible mechanisms, the CL-RBE may not have been observed in this study because the initial eccentric exercise did not induce enough damaging stimulus to enact the CL-RBE or we have missed the time interval when the CL-RBE in women was detectable.

There was no significant change in the limb girth throughout either exercise bout in this study. The limb girth may not have significantly changed because there was not enough inflammation to cause a detectable difference in the circumference of the upper arm. Chen *et al.* (15) found that there was a significant increase in limb girth after both exercise bouts, and there was a significant bout main effect. They used a similar protocol, which seemed to induce more muscle damage in men than in women in the current study based on the higher muscle strength loss (~ 40% loss) in their study.

Unaccustomed ECC exercise tends to have a greater reliance on fast twitch motor units, which causes greater disruption to the fast twitch motor units (4). Surface EMG can be used to record motor unit activation. A higher MF has been suggested to indicate that there is a higher recruitment of fast twitch muscle fibers, and a lower MF suggests that there is a higher recruitment of slow twitch muscle fibers. An increased use of slow twitch muscle fibers is known to be associated with the IL-RBE (5). Starbuck and Eston (4) observed an increased recruitment of slow twitch motor units in the contralateral arm during the second bout of eccentric exercise, and they concluded that the CL-RBE they observed was due to neural adaptation, specifically due to higher dependence on slow twitch motor units recruitment in the second bout of exercise. In this study, MF in the second bout appeared to be slightly lower than that in the initial bout, although the difference did not reach the statistical significance. It is possible that there was a non-significant increase in the slow twitch muscle fiber recruitment in Bout 2 compared to Bout 1. As discussed for the muscle strength data, the MF difference was not statistically significant probably due to the insufficient damage stimulus generated by the first bout of eccentric exercise or the CL-RBE already subsided before 2 wk when we did the measurements.

Blood CK activity has been the most frequently used blood biomarker for EIMD (1). The use of any blood biomarker can be problematic because the amount of blood protein in the blood depends on both the rate at which the muscle releases the protein and how quickly it is being cleared from the blood. In addition, blood CK activity has high intersubject variability, which does not seem to be related to sex, muscle mass, or activity level(1). Not surprisingly, there is not a perfect relationship between the blood CK activity level and muscle damage after eccentric exercise (1). It is interesting to note that the blood CK activity level did not change significantly after either bout of the eccentric exercise in the current study, even though some of the other EIMD markers suggested that there was muscle damage. It is unclear as to why the CK results from the current study are inconsistent with those of previous studies (3, 4, 15), probably due to the different CK response to EIMD in either sex.

## Limitations

There are some limitations to the current study, and thus cautions need to be taken while generalizing the findings observed in this study. Firstly, only women were recruited in this study. Ideally, both sexes should be included in the same study to compare the sex difference in CL-RBE. Although we assume men, if recruited in this study, would experience similar EIMD as in previous studies (~ 25%- 40% strength loss) which used the same eccentric exercise protocol with same or fewer number of repetitions (3, 15), it is not 100% certain that men would get higher strength loss than women in our lab. Secondly, we did not control for the menstrual cycle of the subjects in this study. The midluteal (ML) phase has a higher estrogen level than prefollicular and midfollicular (MF) phases. Estrogen has been shown to provide a protective effect on muscle damage following eccentric exercise (20). So, the different menstrual phases under which the two exercise bouts were performed might be a confounding factor in the current study. Lastly, two participants stated that they felt as though the hand grip was putting more strain on their wrist compared to their biceps. This was unexpected because previous studies used the same position of the attachment. To minimize or completely avoid strain on the wrist, an attachment should be found so that the wrist of the participant will be strapped and the participant will be able to relax her hand while doing eccentric contraction of their elbow flexors.

## Conclusion

The CL-RBE of the elbow flexors was not evident in young healthy women in this study. This was probably because the initial bout of the eccentric exercise did not induce enough damage to generate protective adaptation in the contralateral arm during the second bout or the CL-RBE in women lasted shorter than two weeks. Future studies are warranted to further investigate the CL-RBE in women using either more damaging eccentric exercise protocols such as more eccentric contraction repetitions, or with shorter time interval between the two exercise bouts. In addition, more studies are needed to uncover the mechanisms of the CL-RBE.

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