INTERNATIONAL JOURNAL OF
-EXERCISE SCIENCE-

Original Research

Examining The Contralateral Repeated Bout Effect in Females While Accounting for Menstrual Cycle-Related Estrogen Fluctuations

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Abstract

International Journal of Exercise Science 19(7): 7006, 2026. The Contralateral Repeated Bout Effect (CL-RBE) occurs when an initial exercise protects the opposite, unexercised muscle from muscle damage during subsequent exercises. CL-RBE has been widely demonstrated in male populations, but research involving females remains limited. Recent research, including exclusively female subjects, reported an absence of CL-RBE, suggesting a possible sex-specific difference in the phenomenon. This null result may have been due to estrogen changes during the menstrual cycle, since estrogen has been suggested to protect from muscle damage. Therefore, this study controlled for estrogen fluctuations in fourteen females while testing for CL-RBE. Each participant completed the same eccentric exercise consisting of 60 repetitions on opposite arms, two weeks apart, with muscle damage markers assessed pre-exercise, immediately (muscle strength only), 24 hours, and 48 hours post-exercise. Significant main effects of time were observed in strength ($p = .006$) and soreness ($p < .001$), confirming muscle damage after the eccentric exercises. A significant bout effect was observed for strength ($p < .028$). However, no bout × time interaction emerged for any measured marker ($p > .05$). These findings suggest that estrogen fluctuation alone may not explain the absence of CL-RBE in females, and support emerging evidence of sex-specific differences in CL-RBE. While this study incorporated increased exercise volume and controlled for estrogen fluctuation, limitations include the indirect estimation of estrogen levels, which may have allowed subtle CL-RBE to go undetected. This highlights the need for future investigation into female-specific mechanisms governing muscle adaptation to develop evidence-based rehabilitation and training protocols that accurately reflect female physiology.

Keywords: Eccentric exercise, exercise-induced muscle damage, hormones, female physiology, elbow flexors

Introduction

The Contralateral Repeated Bout Effect (CL-RBE) describes a phenomenon in which exercise performed by one limb reduces muscle damage in the opposite, unexercised limb when a similar exercise is repeated.[1] It is widely known that repeated bouts of exercise typically result in reduced exercise-induced muscle damage (EIMD) in the same limb due to muscle and /or neural adaptation, a phenomenon called the Ipsilateral Repeated Bout Effect (IL-RBE).[2] While IL-RBE is well established, less is known about the presence and underlying mechanisms of when it crosses over for CL-RBE.[1,3] A better understanding of CL-RBE has important applications, including shaping rehabilitation strategies for injured athletes by protecting strength on an injured limb, refining resistance training protocols for contralateral strength gains, and informing maintenance or injury prevention strategies in older adults or clinical populations.[4]

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Eccentric exercise, in which muscle lengthens under a load, is commonly used in exercise science research because it reliably induces measurable EIMD, allowing observation of patterns in muscle adaptation and recovery.[2-3,5] Muscle damage during eccentric exercise results from mechanical microtears in muscle fibers, causing structural disruptions, impaired motor unit recruitment, and the subsequent inflammatory and oxidative responses.[6-7] These responses manifest as hallmark signs and symptoms of EIMD, such as increased muscle soreness, decreased range of motion (ROM), loss of muscle strength, and increased limb girth (LG).[1] Measuring these over a period of time post-exercise allows researchers to detect muscle adaptation patterns after repeated bouts of exercise.

Notable work by Starbuck et al,[1] used electromyography (EMG) to find a lower median frequency (MF) in Bout 2 than Bout 1, indicating a higher proportion of recruited type I muscle fibers in Bout 2. Type I fibers are generally more fatigue-resistant, so this suggests more efficient motor unit recruitment during Bout 2, and a possible neural adaptation mechanism underlying CL-RBE.[8-10] Supporting a neural adaptation mechanism, recent work on male rats by Tokuda et al,[11] suggested CL-RBE is mediated through central nervous system changes rather than peripherally. Despite recent advances, research on CL-RBE has largely focused on males, leaving a substantial gap in understanding this protective effect in females. To address this gap, Brown et al,[12] examined exclusively female participants who performed two bouts of eccentric exercise on opposite arms separated by two weeks. Unlike previous studies on males, CL-RBE was not observed, and the authors speculated that an insufficient exercise intensity may have contributed to this null finding.[12] However, the lack of control for estrogen fluctuation may also have influenced the outcome, pointing to an area of further investigation regarding CL-RBE in females.

The underrepresentation of females in CL-RBE research is particularly notable given documented sex-based differences in muscle response to eccentric exercise.[13-14] Females may experience protective mechanisms differently due to biological distinctions such as hormonal composition.[15] Estrogen, typically higher in females than males, plays a key role in female physiology and is linked to maintaining muscle mass and strength, with antioxidant properties that may help protect muscle membranes and reduce exercise-induced damage.[16-18] Relevant to this, Sipilä et al,[14] found a positive relationship between muscle strength and estradiol concentrations in post-menopausal females, suggesting a protective role of estrogen. In the context of CL-RBE, estrogen changes across the menstrual cycle may therefore contribute to variability in muscle damage and recovery and help explain previously observed null CL-RBE findings in females. A typical menstrual cycle lasts approximately 28 days and consists of four main phases: menstrual, follicular, ovulation, and luteal.[19] Estrogen levels fluctuate predictably during these phases, peaking twice (pre-ovulation and mid-luteal) and dropping to their lowest toward the end of the cycle and during menstruation.[19-20] Given that estrogen levels have been associated with muscular functioning, changes in estrogen quantity throughout the menstrual cycle may affect the extent of muscle damage and adaptation in females.[20]

The purpose of this study was to examine CL-RBE in healthy, young, moderately active females after completing an intense eccentric exercise protocol while accounting for estrogen fluctuations during a menstrual cycle. Participants were evenly distributed to perform the first exercise in the late luteal/menstrual phase (low estrogen level), or the pre-ovulation/mid-luteal phase (high estrogen level). The eccentric exercise consisted of 60 eccentric contractions rather than the 45 used by Brown et al,.[12] This study hypothesized that controlling for estrogen fluctuations and increasing the exercise volume would allow for the detection of CL-RBE in females. Ultimately, this work seeks to contribute a more comprehensive understanding of muscle adaptation and recovery in females, with implications for exercise prescription and rehabilitation across diverse populations.

Methods

Participants

A priori power analysis was conducted using G*Power (version 3.1.9.6, University of Düsseldorf, Düsseldorf, Germany) to determine the minimum required sample size. The analysis was performed with a desired power level of 0.80, an alpha level of 0.05, and an effect size of 0.8 calculated from previous studies.[3,12] Based on this analysis, 14 female participants were recruited (mean ± SD, age 21 ± 1.6 yr; height 165.2 ± 5.2 cm; body mass 67.5 ± 13.5 kg). Participants were healthy, non-smoking, recreationally active, and were recruited from Simmons University through recruitment fliers, a mass email sent to enrolled students, or social media announcements. Recreationally active status was defined according to Bolger et al,[21] as participating in moderate aerobic activity for less than 80 minutes per week. This criterion was used to minimize the influence of prior resistance training in highly trained individuals while maintaining applicability to a generally active population. Exclusion criteria included smoking, having an irregular menstrual cycle, pregnancy or plans to become pregnant, musculoskeletal injury, and the presence of a serious medical condition such as diabetes, hypertension, kidney, cardiovascular, or pulmonary disease. A regular menstrual cycle was defined as having a consistent cycle between 21 and 35 days over the previous year, with cycles outside of this range being regarded as irregular.[22] Participants were also required to refrain from upper-body exercise, use of cold medicine, any oral or topical analgesics, heat or cold treatment, physical therapy, massage, or any other muscle treatment regimen during the study. The use of oral or other hormonal contraceptives was not used as an exclusion criterion in this study. All individuals signed an informed consent document, completed a Physical Activity Readiness Questionnaire for Everyone (PAR-Q+), and a medical history questionnaire.[12,23] This study was approved by the Institutional Review Board (IRB) at Simmons University and was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science.[24] All data were collected respectfully, allowing participants to self-identify. Personal information regarding gender identity or sexual orientation was only collected if relevant to the study.

Protocol

Each participant completed eight visits over 17 days (Figure 1). Baseline measurements of maximal isometric muscle strength (ISO), ROM, perceived soreness, and LG of both biceps brachii were obtained during V1, and participants were familiarized with the exercise equipment and procedures for the following day.

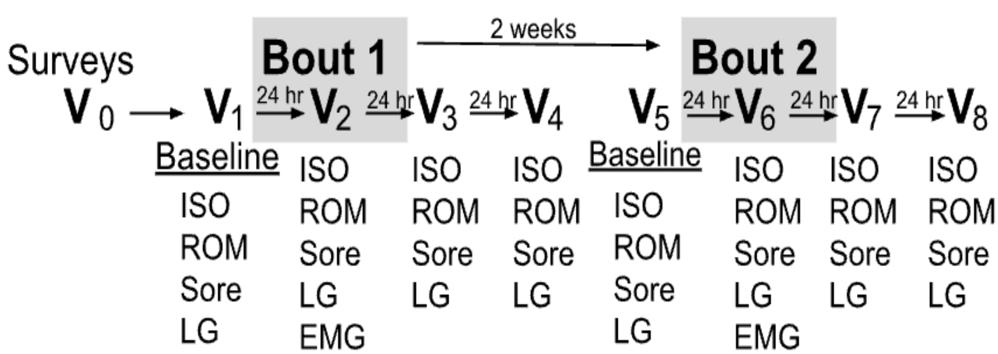

Figure 1. Experimental overview. Isometric strength (ISO), range of motion (ROM), perceived soreness (Sore), limb girth (LG), and electromyography (EMG).

ISO of the biceps brachii was measured using a dynamometer (Nextech Sales, Bangkok, Thailand) attached to a cable machine (Merax, City of Industry, CA, USA) (Figure 2). The cable attachment was positioned at the height of the participant's hand while seated, with the tested arm placed on the platform of a biceps curl machine (Titan Fitness, Memphis, TN, USA) (Figure 2). A 100lb load was attached to the cable machine to simulate an immovable resistance while participants performed three maximal isometric contractions at 75° from full extension (0°). The highest value of the three trials was recorded as ISO. ROM was assessed using a goniometer measuring the elbow angle at full flexion.[12] Perceived muscle soreness was measured following two bicep curls using a light dumbbell (1 lb for participants with body mass <59 kg; 2 lb for participants ≥ 59 kg). Participants then indicated their peak soreness of their 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 participant's mark was measured and recorded as the soreness level.[12] LG was measured using a tape measure at a point located two-thirds of the distance from the acromion to the lateral epicondyle on the biceps brachii.[12]

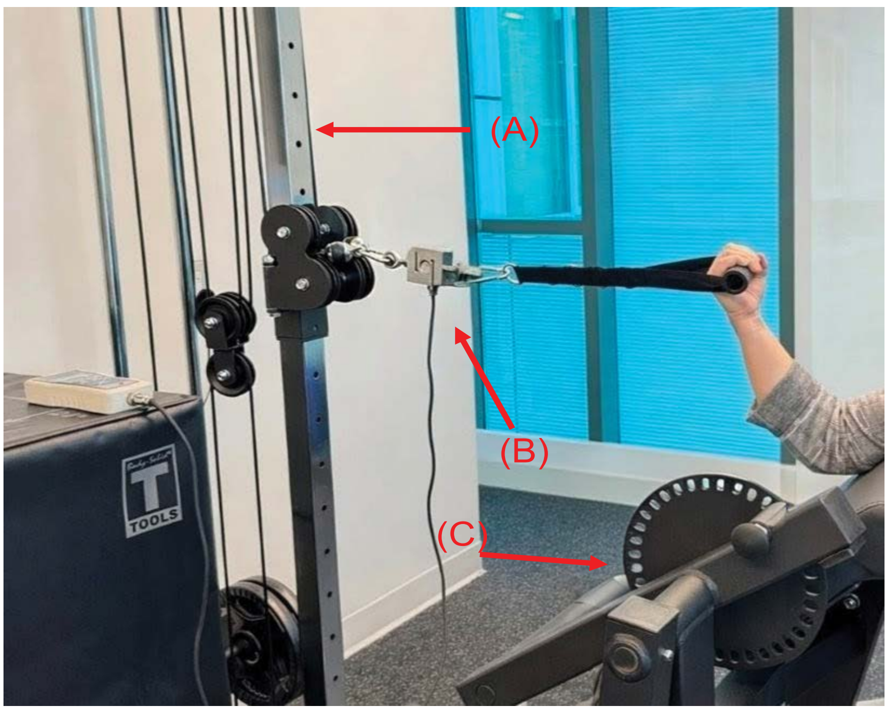

Figure 2. Measurement of Isometric muscle strength using the cable machine (A), the dynamometer (B), and the bicep curl machine (C).

Twenty-four hours after V1, participants returned for V2, which began with reassessing ISO, ROM, soreness, and LG of the exercising arm that would complete Bout 1. Participants were randomly and evenly assigned to begin the exercise with either their dominant or non-dominant arm, and complete the first bout (V2) during a period of either high (pre-ovulation or mid-luteal) or low (late-luteal or menstruation) estrogen level based on their self-reported menstrual cycle information.[19] Participants reported the first day of their most recent menstrual period, based on which the menstrual cycle day for each exercise bout was determined using a standard estrogen curve.[20] During each eccentric exercise, EMG was recorded on the exercising arm by a wireless EMG system (Trigno Lite System, Delsys, Natick, MA, USA). Before the exercise, the skin over the belly of the biceps brachii was prepared with an alcohol wipe, and the EMG electrode was placed parallel to the biceps muscle fibers using an adhesive electrode sticker on the exercising arm.[12]

Following the completion of the exercise protocol, EMG data were exported for MF analysis using Delsys EMGworks Analysis (EMGworks. Version 4.8. Natick, MA, USA). The average MF for each bout was calculated for further analysis.

Eccentric exercise was carried out using a biceps curl machine and dumbbells (Yaheetech.shop, Ontario, CA, USA). The dumbbell load was set to 50% of each participant's previously measured ISO. Seat height was adjusted to the participant's comfort, and participants were instructed to sit upright with their back straight and their exercising arm resting on the platform. Each repetition began at an elbow angle of 130°, when the researcher handed the dumbbell to the participant, and ended at 10°, when the researcher collected the dumbbell (Figure 3). The participant lowered the dumbbell at a rate of 30°/sec over 4 seconds, for a total range of motion of 120°. Participants performed six sets of ten repetitions, with 11 seconds of rest between repetitions and 2 minutes of rest between sets. The researcher monitored each repetition to ensure smooth, controlled eccentric contractions. Participants were periodically reminded to maintain proper posture and to avoid using the non-exercising arm for support. Immediately after the exercise, ISO was reassessed on the exercised arm.

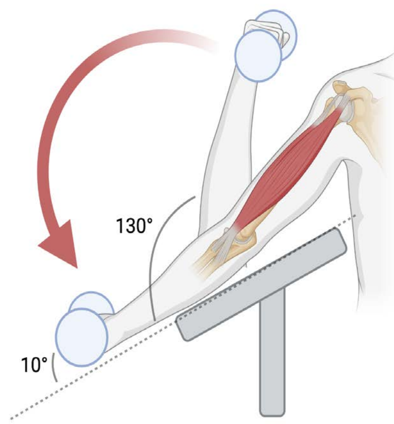

Figure 3. Eccentric exercise (Created in BioRender. Elko, E. (2026) https://BioRender.com/d753ehi).

At 24 hours (V3) and 48 hours (V4) post-exercise Bout 1, ISO, ROM, soreness, and LG of the exercised biceps brachii were measured. Visit 5 (V5) took place 11 days after V4, resulting in a two-week interval between exercise Bouts 1 and 2. Visits 5 through 8 (V5 - V8) replicated procedures of V1 - V4, with testing and exercise performed on the contralateral arm.[12]

Statistical Analysis

All statistical analyses were performed using JASP (version 0.95.4, Amsterdam, The Netherlands). Paired-samples *t*-tests were conducted to compare baseline values and MF between Bout 1 and Bout 2. Two-way repeated measures analysis of variance (ANOVA) was performed to determine the main effects of time (exercise), bout, and their interaction terms for each measure: ISO, ROM, LG, and soreness. Percent change for ISO was calculated before statistical analyses were carried out. Significant main effects were followed by Bonferroni's post hoc comparisons to identify specific differences between time points. Partial eta-squared values (ηp^2) were calculated as measures of effect size. Mauchly's Test of Sphericity indicated that the assumption of sphericity was violated for ISO analysis ($X^2(2) = 9.24$, $P = .010$), so Greenhouse-Geisser corrections were applied ($\varepsilon = 0.65$). Mauchly's Test of Sphericity indicated that the assumption of sphericity was violated for soreness ($X^2(2) = 5.97$, $P < .05$), so Greenhouse-Geisser corrections were applied (ε = 0.72).

Results

To ensure that the two exercise bouts began under comparable conditions, baseline measures of ISO, ROM, LG, and perceived soreness were assessed before each bout (Table 1). There were no significant baseline differences between Bout 1 and Bout 2 for ISO ($t(13) = -0.18$, $P = .859$), ROM ($t(13) = 1.63$, $P = .127$), LG ($t(13) = 1.54$, $P = .148$), or soreness ($t(13) = -0.56$, $P = .583$), confirming that participants entered both exercise bouts with comparable initial muscle status.

Table 1. Baseline Values for ISO, ROM, LG, and Soreness (mean ± SD).

Marker	Bout 1	Bout 2
ISO (lb)	31.14 ± 7.89	27.93 ± 8.00
ROM (°)	132.64 ± 3.37	131.85 ± 3.32
LG (cm)	27.71 ± 3.78	27.44 ± 3.78
Soreness (mm)	0.71 ± 2.67	1.43 ± 3.63

No interaction between bout and time was observed for ISO ($F(1.59, 20.64) = 0.96$, $P = .379$), though there was a significant main effect of time ($F(1.30, 16.92) = 8.66$, $\eta p^2 = 0.441$, $P = .006$) and bout ($F(1,13) = 6.16$, $np^2 = 0.754$, $P = .028$). Specifically, ISO significantly improved regardless of bout at 48 hours relative to immediately post-exercise (-9.61 ± 3.10%; $t(13) = -3.10$, $P = .017$), and relative to 24 hours post-exercise (-7.13 ± 1.55%; $t(13) = -4.61$, $P = .001$). ISO also significantly improved from Bout 1 to Bout 2 regardless of time (-16.06 ± 6.47%; $t(13) = -2.48$, $P = .028$) (Figure 4A). ISO muscle loss was 38.5% immediately post-exercise, 38.9% 24 hours post-exercise, and 29.4% 48 hours post-exercise during Bout 1. During Bout 2, ISO loss was 24.7% immediately post-exercise, then dropped to 19.3% 24 hours post-exercise and 14.6% 48 hours post-exercise.

No main effect of bout ($F(1, 13) = 1.49$, $P = .243$) or bout × time interaction ($F(1.19, 15.42) = 1.55$, $P = .236$) was observed for soreness, though there was a significant effect of time ($F(1.44, 18.68) = 31.54$, $\eta p^2 = 0.708$, $P < .001$). Soreness increased significantly at 24 hours (34.3 ± 4.7mm; $t(13) = -7.35$, $P < .001$) and 48 hours (33.8 ± 6.2mm; $t(13) = -5.42$, $P < .001$) post-exercise compared to pre-exercise values, regardless of bout (Figure 4B). Soreness did not differ significantly between the 24- and 48-hour post-exercise timepoints ($t(13) = 0.14$, $P = .891$).

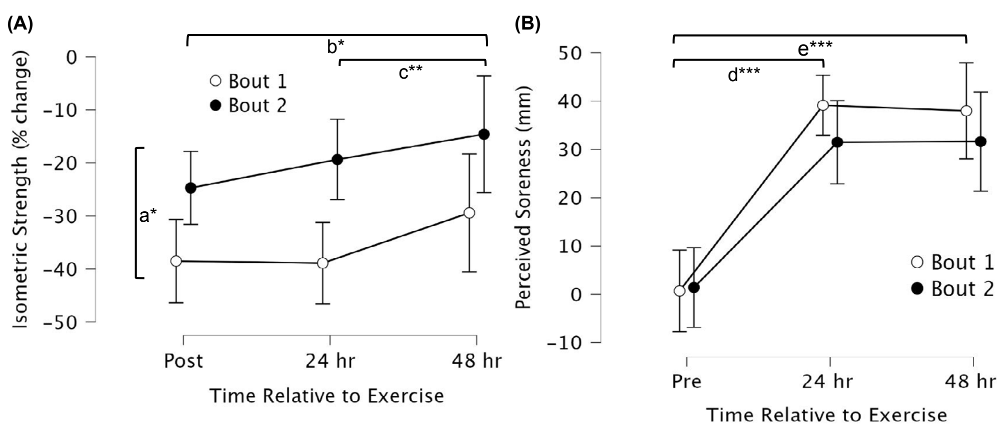

Figure 4. (A) Maximal isometric strength changes following both eccentric exercise bouts. (B) Perceived muscle soreness following both eccentric exercise bouts. (a) main effect of bout (b,c,d,e) main effect of time. * $P < .05$, ** $P < .01$, *** $P < .001$.

No significant main effects of time, bout, or time × bout interaction were observed for ROM or LG (all $P > .05$, data not shown). MF also showed no significant difference between bouts ($t(13) = -0.59$, $P > .05$). These indicators of EIMD remained stable across the testing period and did not differ between exercise bouts.

Discussion

Previous research on CL-RBE has been focused on male populations, with limited and inconsistent findings on exclusively female populations.[1,3] This study hypothesized that accounting for estrogen fluctuations in female participants and increasing exercise volume relative to previous studies (e.g., Brown et al[12]) would allow for the observation of CL-RBE. The current work showed ISO and muscle soreness changes that suggest a successful induction of EIMD by eccentric exercise. ISO changes between bout further illustrate a lower muscle strength loss during Bout 2, and given that isometric muscle force is the most reliable marker for EIMD, it implies a smaller magnitude of muscle damage in the repeated bout.[25] However, no significant changes were observed for all other variables, including ROM, LG, or EMG. Furthermore, although there was less muscle damage in Bout 2, the pattern of muscle recovery over time did not differ between bouts; therefore, CL-RBE cannot be conclusively identified in this study, even with the utilization of a more intense eccentric exercise protocol and accounting for estrogen fluctuations.

The significant time-dependent reductions in ISO and increases in muscle soreness confirmed that EIMD was successfully induced in the current study. Based on the established classifications of EIMD severity, maximal voluntary contraction (MVC) losses are categorized as mild (<20%), moderate (20-50%), or severe (>50%).[26] Accordingly, muscle damage observed in this study is considered moderate throughout the first bout (-38.9% to -29.4%) and immediately after Bout 2 (-24.7%). A resolution to only mild was seen 24 and 48 hours after Bout 2 (-19.3% to -14.6%). This, along with the significant main effect of bout found in this study indicates that EIMD was lower during Bout 2 than Bout 1, suggesting some degree of repeated bout adaptation. However, because the time course of recovery did not differ significantly between bouts, this adaptation cannot be conclusively interpreted as CL-RBE. It is also possible that a contralateral adaptation was emerging, but not sufficiently to produce a significant interaction effect. Contextualized in the literature, the magnitude of strength loss observed in this study was lower than that reported in male-only studies. For example, Chen et al,[3] found approximately 40% MVC reduction following only 30 eccentric contractions during the initial bout. In contrast, Brown et al,[12], who examined females performing 45 eccentric contractions, observed only mild (< 20%) post-exercise strength loss that resolved quickly and failed to demonstrate CL-RBE. The present findings extended this work by employing a more intense exercise protocol of 60 eccentric contractions and inducing sustained moderate MVC loss during Bout 1. Despite this greater stimulus and a present main effect of bout, a significant time × bout effect was not observed. Given that CL-RBE has been identified in males using less demanding protocols, these findings support growing evidence that CL-RBE may not manifest the same in females.

Protective mechanisms likely differ between sexes, as hormonal profiles vary substantially between males and females.[18] Estrogen has been implicated in promoting muscle repair and reducing EIMD.[16,18] Since estrogen levels fluctuate across the menstrual cycle, the confounding effect of estrogen fluctuation may explain the absence of CL-RBE in females.[12] However, controlling for estrogen fluctuation in the current study did not lead to the observation of CL-RBE, suggesting estrogen fluctuations alone are not sufficient to explain the absence of CL-RBE in females. Additional physiological or methodological factors may need to be considered before

CL-RBE can be identified in females.[9] Directly tracking estrogen or other hormones that fluctuate during the menstrual cycle, such as progesterone, may help clarify whether endocrine factors interact with the mechanisms governing CL-RBE or allow for its detection. Progesterone is another sex hormone that exists in higher levels in females than in males. Like estrogen, it fluctuates throughout the female menstrual cycle, but has been correlated with a decline in muscle strength in users of progesterone-based hormonal contraceptives by Sabatini et al,[27].[28] This suggests that factors beyond estrogen may contribute to differences in CL-RBE. Furthermore, the use of oral or other hormonal contraceptives was not controlled for in this study, which may have influenced hormonal profiles. Future studies should account for contraceptive use and directly assess hormonal status to better elucidate the mechanisms underlying these responses.

Additionally, future studies directly comparing male and female participants using identical exercise protocols and hormonal controls may help to clarify sex-based differences regarding CL-RBE expression. It should also be noted that other EIMD markers of LG and ROM showed no changes over time in this study, and therefore, no evidence of muscle damage or CL-RBE. While a moderate level of muscle damage was observed for ISO, our sample size and exercise modality, using 50% each participant's ISO for the weight, may have limited the detection of subtle CL-RBE in females. Though a moderate ISO loss was successfully induced, future studies employing higher exercise intensity and larger sample sizes may further generate conditions necessary to observe EIMD across all muscle damage markers, and ultimately recovery patterns indicating CL-RBE. Additionally, participants starting their exercise during their menstrual cycle (low estrogen) could have experienced performance or maximal strength output complications due to menstrual cramps or general weakness. In the future, the lowest estrogen phase could be specified to be 1 day after menstruation to avoid this.

It is increasingly clear that findings derived from males cannot be directly applied to females. Recent findings emphasize the need to recenter female physiology in research to better inform rehabilitation and training development for this historically underserved group. Similar to the present findings, Brown et al.[12] reported no evidence of CL-RBE in females. By controlling for estrogen fluctuations and increasing eccentric exercise volume from 45 by Brown et al.[12] to 60 contractions, the present study replicated these null findings under improved experimental conditions. Replication of null CL-RBE outcomes despite refined methodology strengthens the argument that this absence reflects a true sex-based physiological difference rather than limits in stimulus or study design. Although it is clear that overall strength was higher during Bout 2, regardless of time, without a significant muscle recovery pattern, CL-RBE may be emerging, but cannot be conclusively determined. These findings reinforce the presence of sex-based physiological differences in contralateral muscle adaptation and are essential for informing evidence-based exercise protocols and advancing a more inclusive understanding of muscle adaptation.

Acknowledgements

This research was supported by the Undergraduate Research Seed-Starter Fund from the Office of Research and Fellowships (ORF) at Simmons University. The funding source had no involvement in study design, data collection, analysis, interpretation, or manuscript preparation. The authors thank the Biology Department at Simmons University for access to equipment and laboratories. The authors thank Dr. Reeder for statistical consultation. Thank you to the participants for their time and effort during this study.

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