THE EFFECTS OF NEUROMUSCULAR ELECTRICAL STIMULATION ON THE CONTRALATERAL REPEATED BOUT EFFECT OF THE ELBOW FLEXOR MUSCLES

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in partial fulfillment of
the requirements for
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DOCTOR OF PHILOSOPHY
May, 2023

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ACKNOWLEDGMENTS

I would like to expess my deepest gratitude to my advisor, Dr. Michael Trevino and my committee members, Dr. Jason DeFreitas, Dr. Douglas Smith, and Dr. Jerome Hausselle for continued support and encouragement. In addtion, I appreciate my colleagues, Alex Olmos, Stephanie Sontag, and Lyric Richardson, and graduate students for their help. I also want to thank my wife and family who always give me faith.

Name: Sunggun Jeon

Date of Degree: May, 2023

Title of Study: THE EFFECTS OF NEUROMUSCULAR ELECTRICAL STIMULATION ON THE CONTRALATERAL REPEATED BOUT EFFECT OF THE ELBOW FLEXOR MUSCLES

Major Field: Health and Human Performance

Abstract:

PURPOSE: To examine the effects of neuromuscular electrical stimulation (NMES) on the contralateral repeated bout effect (CL-RBE) of the biceps brachii (BB). **METHODS:** Twenty untrained adults were randomly assigned into an ipsilateral (IL) or CL group, and completed 7 visits. Following a familiarization (visit 1), participants completed 3 maximal voluntary isometric contractions (MVICs) and submaximal trapezoidal contractions at 40% and 70% MVIC before and after 45 NMES to the BB muscle (1^{st} bout) at pre (visit 2), post (visit 2), 1 day post (24post [visit 3]), and 2 days post (48post [visit 4]). The same procedures were performed for visits 5 (2^{nd} bout), 6 (24post), and 7 (48post) on the same arm for IL or the contralateral arm for CL. Muscle damage markers (MVIC, elbow range of motion [ROM], visual analog scale [VAS] and pressure pain threshold [PPT] for muscle soreness and pain, and muscle thickness via ultrasonography were measured. Surface electromyography (EMG) and mechanomyography (MMG) were recorded from the BB. The EMG signals were decomposed to calculate y-intercepts and slopes for the motor unit (MU) mean firing rate (MFR) and MU action potential amplitude ($MUAP_{AMP}$) vs. recruitment threshold (RT) relationships. The MMG amplitude (MMG_{RMS})-force relationships were log-transformed to calculate *a* and *b* terms for the linearly varying segments of the trapezoid. EMG amplitude (EMG_{RMS}) and MMG_{RMS} during steady force were normalized (N-EMG_{RMS}, N-MMG_{RMS}) to MVIC. Separate mixed factorial analysis of variance (ANOVAs) were performed. **RESULTS:** MVIC and ROM at post were less than pre, 24post, and 48post ($p<0.05$). Muscle thickness at pre was less than post ($p<0.001$) and 24post ($p=0.019$). PPT and the *b* terms at pre were greater ($p<0.05$) than post, 24post, and 48post. Y-intercepts for the MFR vs. RT relationships during the 70% MVIC were lower at post than pre ($p=0.015$), 24post ($p=0.050$), and 48post ($p=0.016$). VAS were lower ($p=0.003$) during the second bout (0.21±0.27cm) than the first bout (1.06±0.70cm) for the IL group. There were no significant differences between bouts for other dependent variables. **CONCLUSION:** Although there was an IL-RBE for VAS, the other variables did not support the existence of IL- or CL-RBE with NMES.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

1.1. Introduction

It has been reported that unaccustomed exercise generally results in exercise-induced muscle damage (EIMD) [1]. In particular, eccentric exercise can alter indirect muscle damage markers, such as: strength loss, increased muscle protein in the blood, muscle soreness, muscle pain, acute and chronic inflammatory responses [2], muscle thickness [3], decreased range of motion (ROM) [4], M-wave amplitude [5], and maximal rate of force development (RFD) [6]. However, unaccustomed exercise can potentially elicit an acute adaptation that reduces the magnitude of muscle damage if the same or a similar exercise is repeated [7]. This phenomenon, termed as the repeated bout effect (RBE) or protective effect, is characterized by attenuation and a faster recovery of muscle damage in the exercised muscles after the second exercise bout [8]. For example, Muthalib et al. [9] reported multiple indirect muscle damage markers for the elbow flexors were attenuated in a subsequent bout of exercise. In addition, RBE has been reported among different muscle groups [10], training statuses [11], exercise volume [12], types [13], and intensities [14]. It is suggested the potential underlying mechanisms include neural, inflammatory, muscle-tendon complex adaptations, and extracellular matrix structural remodeling [15].

In addition to within-muscle acute adaptations, the RBE has been reported for the contralateral muscle group (contralateral repeated bout effect [CL-RBE]) after an initial

bout of unilateral exercise [16]. For example, Chen et al. [17] reported indirect muscle damage markers were attenuated on the contralateral arm muscles starting 1 day after two bouts of exercise and lasting up to 4 weeks. However, there was no CL-RBE during the same day or following 8 weeks between bouts. Thus, CL-RBE in arm muscles may be time sensitive. In addition, the authors mentioned that 50% of the RBE for the ipsilateral limb transferred to the contralateral limb muscles during a second exercise bouts (ipsilateral RBE). Although numerous studies have examined the CL-RBE among different muscle groups [18], training effects [13], muscle-damaging protocols [19], and exercise intensities [20], some studies have reported mixed results regarding CL-RBE [21,22]. Consequently, more research in necessary to elucidate mechanisms for CL-RBE.

Unlike ipsilateral RBE, the neural and inflammatory adaptations of CL-RBE are likely due to systemic effects [17,18,23,24]. Howatson et al. [25] reported short-interval intracortical inhibition and interhemispheric inhibition to the contralateral motor cortex were diminished, while intracortical facilitation was increased during eccentric contractions. Therefore, eccentric contractions may modulate corticospinal excitability and intracortical and interhemispheric connections in the contralateral limb. In addition, reductions in voluntary activation (VA) were attenuated after a second bout of eccentric exercise performed on the same arm, suggesting modulation in the corticospinal track after the initial bout of eccentric exercise [26]. Moreover, eccentric exercise augments the synchronization of motor units (MU) firings [27] and a greater reliance on lower-threshold MUs to modulate force during the subsequent exercise bouts [28]. Thus, changed descending drive from central nervous system (CNS) after an initial bout of eccentric exercise may possibly modify MU behavior to induce a contralateral protective effect.

Another possible mechanism for the CL-RBE may be inflammatory adaptations. Eccentric muscle damage [29] promotes pro-inflammatory responses [30] and elevates central fatigue [31]. In addition, nuclear factor-kappa B (NF-kB) activation, which mediate the expression of pro-inflammatory responses [30], is enhanced by muscle-damaging exercise [32]. However, Xin et al. [24] revealed NF-kB DNA-binding activity was significantly attenuated in a subsequent exercise bout of the opposite leg, suggesting reduced pro-inflammatory responses in the contralateral limb muscle. Thus, it is plausible that the mitigation of inflammation may partially contribute to the CL-RBE by reducing secondary damage. However, the exact mechanisms for RBE is still unclear and warrants further investigation.

Neuromuscular electrical stimulation (NMES) is the process of inducing a muscle contraction, and can serve as a unique tool for strength and conditioning, rehabilitation, neuromuscular testing, and post-exercise recovery fields [33] to prevent muscle atrophy, increased circulation, and provide a cross-education effect on muscular strength [34] and treatment for neuromuscular disorders and diseases [35]. For example, NMES of the vastus medialis muscles significantly increased strength following 4 weeks of usage [36] and in conjunction with exercise, has improved neuromuscular function in untrained individuals [37], athletes [38], and to contralateral limb muscles [34]. Thus, it is plausible NMES method may improve neuromuscular function as a result of cross-education and may also acutely elicit the CL-RBE.

It is well understood that during voluntary efforts, MUs are recruited by order of increasing size. However, NMES can evoke strong involuntary muscle twitches by recruiting the entire MU pool [33]. Thus, it is plausible that NMES can result in greater

muscle damage and mechanical stress to muscle fibers than voluntary contractions at low target intensities [39] due to a greater activation higher-threshold MUs [40]. In addition, Aldayel et al [41] reported that multiple indirect muscle damage markers were increased after electrical knee extensor muscle stimulation, but were attenuated following a subsequent bout of electrical stimulation, suggesting the existence of an ipsilateral RBE after NMES of lower limb muscles. However, although ipsilateral RBE is evident after two bouts of the NMES [41,42], it remains unknown whether NMES has any potential protective effect on contralateral limb muscles.

1.2. Purpose of the Study

The findings from previous studies examining the CL-RBE have been mixed. In addition, none of these studies utilized NMES when investigating the CL-RBE. Therefore, the purpose of this study was to examine the effects of NMES on the CL-RBE, indirect muscle damage markers, and neuromuscular behavior of the BB muscles.

1.3. Specific Aims

1. The first aim was to examine potential changes of indirect muscle damage markers, including MVIC, muscle thickness, pressure pain threshold, and ROM, on the biceps brachii (BB) muscle following a single bout of NMES.
2. The second aim was to identify the CL-RBE after two bouts of NMES.

3. The third aim was to determine whether neural adaptations occur in the contralateral BB muscles, when compared to ipsilateral BB muscle, by investigating the motor unit firing behavior and mechanical activity of the muscle via EMG signal decomposition and mechanomyography (MMG) techniques, respectively.

1.4. Research Questions

1. Do NMES of both the ipsilateral and contralateral BB muscle alter indirect markers of muscle damage?
2. Does NMES induce the CL-RBE on the BB muscle?
3. Does NMES alter neuromuscular behavior in the contralateral BB muscle?

1.5. Hypotheses

1. NMES will significantly increase indirect muscle damage markers in the stimulated muscle following NMES.
2. The changes in indirect muscle damage markers will be significantly attenuated in the contralateral BB muscle following the second bout of NMES.
3. Motor unit properties and the mechanical activity of the muscle from contralateral BB muscle will be significantly changed following the subsequent bout of NMES when compared to the initial bout of NMES.

1.6. Delimitations

1. Participants were between 18 to 40 years of age.
2. The investigation required the recruitment of approximately 20 males and females to complete this study.
3. All participants must be untrained, healthy, and no history of severe shoulder, elbow, and wrist injuries.
4. All participants must have no any cardiovascular disease or metabolic, renal, hepatic disorder.
5. Seven separate visits were required to complete this study.
6. Participants were asked to refrain from physical activity or exercises involving the upper-extremities.
7. Participants were recruited through email, word of mouth, poster/flyer, and in-person lecture recruitment.

1.7. Limitations

1. Investigator did not control participants' food dietary or life style. Thus, participant's activities from outside of laboratory could have influenced the results.
2. The validation techniques used to assess motor unit firing properties could potentially restrict data analysis

3. NMES could provide pain and discomfort to participants, which can cause stress and demotivation for voluntary participation.

1.8. Assumptions

1. Participants completed a health questionnaire and a physical activity readiness questionnaire (PAR-Q) truthfully.
2. Participants produced voluntary force with their maximal effort during maximal isometric contractions.
3. Participants answered and all questions regarding indirect muscle damage markers honestly and accurately.
4. Participants refrained from any exercise or training in upper body during the entire study period.
5. The EMG and MMG sensors were accurately representing the electrical and mechanical behavior of the entire muscles, respectively.

CHAPTER II

REVIEW OF LITERATURE

The review of literature has four subsections: (1) exercise-induced muscle damage (EIMD), (2) ipsilateral repeated bout effect (IL-RBE), (3) contralateral RBE (CL-RBE), and (4) muscle damage and neuromuscular adaptations induced by neuromuscular electrical stimulation (NMES). It has been structured as a study by study format with the most relevant research study summaries provided chronologically to its respective section are.

2.1. Exercise-Induced Muscle Damage

Friden et al. 1983 [43]

This study was one of the earliest observation to examine myofibrillar damage in knee extensors following eccentric exercise. Twelve males performed eccentric bicycle exercise at 60 rpm for 30 minutes and completed muscle biopsies from the vastus lateralis before, immediately after, 3 days, and 6 days after the bicycle exercise. The results indicated that muscle fibers, especially type II muscle fibers, were disrupted in their size and shape after exercise. Eccentric exercise induced Z-disc streaming and altered straining pattern of structural filaments. Thus, eccentric exercise induced morphological

changes in the structure of the contractile apparatus, and the magnitude of muscle damage was likely muscle fiber dependent.

Shellock et al. 1991 [44]

The purpose of this study was to compare the magnitude of muscle damage after concentric or eccentric exercise using transverse relaxation time (T2) (detection the microstructure and the perfusion of the skeletal muscle) from magnetic resonance imaging (MRI) technique. Five healthy participants (3 men and 2 women), ages 21 – 28 years old, performed both concentric and eccentric exercise until failure with a weight 10 – 20% of their body weight and were measured muscle soreness and pain and T2 relaxation time at before, 1, 3, 5, 10, 25, 40, 50, 60, and 80 days after exercise. The authors reported no significant differences in dependent variables after concentric exercise, but eccentric exercise resulted in increased T2 relaxation time, and muscle soreness and pain from 1 to 5 days post-exercise. The authors suggested greater T2 signal intensity reflected greater muscle damage due to damaged connective tissue in musculotendinous junctions. T2 signal intensity reflect the magnitude and location of muscle damage. In addition, based on indirect muscle damage marker after two different muscle action, eccentric exercise induced greater muscle damage when compared to concentric exercise.

Clarkson et al. 1992 [45]

In this study, the authors provided the time course of changes in indirect muscle damage markers following maximal eccentric elbow flexion exercise. Maximal strength, muscle soreness, muscle swelling, and creatine kinase (CK) levels were examined at pre to 10 days after eccentric exercise. Muscle soreness peaked at 2 to 3 days after exercise and remained at 10 days, and it may be due to the circulating neutrophils activity and increased pro-inflammation. Muscle strength decreased over 50% immediately after exercise and gradually returned to the baseline over time. It is possible that eccentric exercise resulted in muscle fatigue and disruption of the myofibrillar structure, thereby reducing the number of cross-bridges and ability to produce force. Muscle circumference gradually increased after exercise and peaked at 5 days. Blood CK levels was rapidly increased at 2 days and peaked at 4 days after exercise. The possible explanation of this phenomenon is delayed release CK to blood from muscle due to the complex interactions. Thus, each indirect muscle damage marker has different time course and may have different mechanisms.

Kuipers 1994 [46]

This paper examined initial and secondary muscle damage and the factors of exercise-induced muscle damage after muscular overuse. Unaccustomed exercise induced mechanical stress that is a primary factor of muscle damage. One hypothesis is the contractile apparatus is damaged after exercise. Another is that eccentric exercise induces a greater magnitude of muscle damage compared to concentric exercise. It is suggested lower number of motor units (MUs) recruited during eccentric exercise compared to the concentric exercise at the same intensity, implying that the magnitude of the mechanical

stress per fiber is greater during eccentric exercise than concentric exercise. Thus, damaged contractile elements results in strength loss. In addition, the speed of muscle contraction can be a factor of muscle damage. Higher contraction speeds elicit greater muscle damage due to the speed of cross-bridge cycling when compared to the lower speed contraction. Secondary muscle damage includes the cellular inflammatory response post-exercise. Increased sarcoplasmic calcium concentration after eccentric exercise can lead to a decline cellular homeostasis, increased stiffness, and an impairment in ATP generation due to calcium accumulation in the mitochondria. Thus, calcium can be a play an important role in secondary muscle damage.

Dartnall et al. 2008 [47]

The authors examined MU firing rate behavior (motor unit synchronization and coherence) from the BB muscle during low force contractions (1-26% of maximal voluntary contraction [MVC]) after eccentric exercise. Eight health adults performed eccentric exercise for the elbow flexors until they exhibited a 40% reduction in strength. MVC and electromyographic (EMG) signals (surface and intramuscular) were recorded before, immediately after, and 24 hours post-eccentric exercise to examine muscle damage and possible changes in MU firing properties. Force was decreased by 46%, suggesting muscle damage, and MU synchronization and coherence in the low frequency band (0-10 Hz) were significantly greater by 30% and 20% immediately after eccentric exercise, respectively, and remained elevated 24 hours later. Thus, muscle-damaging eccentric exercise leads to adjustments in MU firing rate behavior (synchronization and coherence).

Chen et al. 2011 [48]

In this study, the authors compared the magnitude of muscle damage using indirect muscle damage markers among four limb muscles (elbow flexors [EF] vs. extensors [EE] vs. knee flexors [KF] vs. extensors [KE]) after maximal eccentric exercise. Seventeen sedentary adults completed five sets of six maximal isokinetic eccentric contraction of the EF, EE, KF, and KE with a 4 to 5 weeks interval each limb. All indirect muscle damage markers were measured before to 5 days post-eccentric exercise. All dependent variables significantly deteriorated in EF, EE, and KF, but the optimum angle for maximal force production of the KE, muscle circumference, and muscle echo-intensity were not significantly changed in KE. The magnitude of muscle damage was greatest in the arm muscles (equal EF and EE). Therefore, arm muscles are more susceptible to muscle damage by eccentric exercise than leg muscles. This may be because the leg muscles are used more frequently than the arm muscles in daily life.

Howatson et al. 2011 [25]

The authors investigated the influence of unilateral eccentric and concentric contractions on corticospinal and spinal excitability in contralateral homologous muscles. Forty-one health adults performed eccentric and concentric testing on the left wrist flexors and motor cortical function of the left motor cortex (M1) was measured with transcranial magnetic stimulation (TMS). After the contractions, changes in corticospinal excitability were greater during eccentric than concentric contractions. Short-interval

intracortical inhibition and interhemispheric inhibition to the left M1 (contralateral side) were further diminished during eccentric than concentric contractions. Intracortical facilitation was elevated during the eccentric contractions, but decreased for the concentric contractions. There was also a decrease in H-reflex amplitude for the right wrist flexors during eccentric and concentric contractions. The findings suggested that eccentric contractions may alter corticospinal excitability and inhibition in contralateral limb muscles more than concentric contractions.

Cabral et al. 2021 [49]

Previous research [25] has reported alteration in corticospinal excitability of central mechanisms after eccentric exercise-induced muscle damage. However, the magnitude of muscle damage may have different responses within the muscle depending on location (distal vs. proximal). The purpose of this study was to identify local changes in the M-wave amplitude from BB muscle using 64 monopolar high-density surface EMG during neuromuscular stimulation. Ten healthy young men completed 30 isokinetic eccentric exercise, and MVIC peak torque, muscle soreness, echo intensity, and M-wave peak-to-peak amplitude were recorded before, 1, 2, 3, and 4 days after eccentric exercise. Strength loss, increased muscle soreness and echo intensity were reported across days, indicating that muscle damage was evident after eccentric exercise. The largest supramaximal M-waves amplitude were detected in distal portion of BB muscles from 1 to 4 days, and M-wave amplitudes were decreased at proximal region of BB muscle from 1 to 3 days after eccentric exercise. Disruption of sarcolemma by eccentric exercise can lead to increased permeability due to increased intracellular Na^+ and Ca^+ concentrations.

Increased permeability can induce limited propagation speed of action potentials (AP) within damaged muscle site. Thus, decreased M-wave amplitude at the distal site may due to the impairment of sarcolemma function, and suggested that the distal region of BB muscles may be more susceptible to muscle damage than the proximal region. In addition, at 4 days after eccentric exercise, M-wave amplitudes at proximal region returned to the baseline, suggesting that peripheral alteration to BB muscle excitation may last up to 3 days.

2.2. Ipsilateral Repeated Bout Effect (IL-RBE) and Possible Mechanisms

Byrnes et al. 1987 [50]

Unaccustomed contractions (e.g. eccentric contraction) can lead to disturbances in myofibrillar structure and delayed onset muscle soreness; however, training (e.g., repeated exercise) can reduce the magnitude of muscle damage. Thus, the authors hypothesized that the initial exercise training may provide a protective effect (e.g., decline in muscle damage) in subsequent exercises. Therefore, the purpose of this study was to examine muscle soreness and creatine kinase (CK) activity after repeated downhill running, separated by 3, 6, and 9 weeks. Muscle soreness and CK activity were significantly increased after the initial bout of downhill running, but less soreness and decreased CK activity was reported 3 and 6 weeks later downhill running. This suggested that initial exercise bout can provide protective effect (repeated bout effect: RBE) that attenuated the magnitude of muscle damage and this effect may last up to 6 weeks.

Clarkson and Tremblay 1988 [51]

The authors investigated exercise-induced muscle damage, repair, and RBE after repeated eccentric exercises. Eight healthy women competed two bouts of eccentric elbow flexion exercise. One arm performed two bouts of 70 maximal eccentric contractions 2 weeks apart and the other arm performed a bout of 24 maximal eccentric contractions. Indirect muscle damage markers including strength, muscle soreness, CK concentration, range of motion (ROM) were measured before to 5 days after exercise. The high volume of exercise induced greater muscle damage and slower recovery than low volume of exercise. However, changes in indirect muscle damage markers were reduced after second bout of exercise and the recovery rate was faster after the second bout when compared to after initial bout. Thus, RBE can provide resistance to muscle damage and repair damage at a faster rate.

Warren et al. 2000 [52]

This study examined potential mechanisms for RBE using surface EMG to assess EMG amplitude and median frequency after two bouts of eccentric or concentric dorsiflexion exercise, 1 week apart. Twenty healthy men (eccentric group; n = 10, concentric group n = 10) completed 50 maximal eccentric or concentric contractions, and the results indicated that both groups displayed no difference in EMG amplitude between two bouts. However, EMG median frequency was significantly lower in the subsequent bout of eccentric exercise. It was suggested that no change in EMG amplitude coupled with the decrease in EMG median frequency for the second bout indicates that the same

motor units were recruited but with lower discharging rates, and the number of recruited MUs with slow-twitch properties increased during the second bout. Thus, attenuation in muscle damage after the second bout of eccentric exercise may be partly due to an increase in the activation of MUs with slow-twitch properties in conjunction with a decrease in recruitment of fast twitch MUs.

Nosaka et al. 2001 [53]

The authors investigated the maximal time course of the RBE on indirect muscle damage markers after two bouts of eccentric exercise, 6, 9, and 12 months apart. Untrained men performed two bouts of 24 maximal eccentric elbow flexions, and strength, ROM, arm circumference, muscle soreness, CK activity, and T2 relaxation time of magnetic resonance images (MRI) were recorded from BB muscle before and 5 days after exercise. All indirect muscle damage markers deteriorated after the first bout of exercise, but changes in indirect muscle damage markers were reduced in subsequent bout after 6 and 9 months. However, there was no significant difference between two bouts after 12 months. Therefore, the RBE of BB muscle was evident for the interval of 6 and 9 months but was lost between 9 and 12 months.

Chen et al. 2007 [14]

Earlier studies examined the effects of maximal eccentric exercise to identify IL-RBE; therefore, this study investigated a wide range of intensities (40, 60, 80, or 100% of MVIC) during the initial bout. Fifty-two men completed the initial (40 to 100% of

MVIC) and second (100% of MVIC) bout of eccentric elbow flexion exercise, separated by 2 to 3 weeks. The initial exercise at 80% and 100% of MVIC elicited the RBE on all indirect muscle damage marker at 1 to 5 days post exercise in subsequent bout. Although the initial exercise at 40% and 60% of MVIC did not induce any RBE 1 to 4 days after second bout, RBE was evident at 5 days post-exercise. The magnitude of RBE at 5 days post was greatest for the 100% of MVIC intensity and gradually decreased 80% to 40% of MVIC. The findings of this study supported the existence of IL-RBE on BB muscle after submaximal eccentric exercise.

Falvo et al. 2009 [11]

This study investigated the IL-RBE after two bouts of 100 eccentric barbell bench press repetitions, 2 weeks apart, in resistance-trained men. Eleven trained men were measured for strength, rate of force development (RFD), muscle soreness, CK activity, and surface EMG parameters (amplitude and median frequency) at pre, 15-min, 1, and 2 days post two bouts of eccentric exercise. Despite a main effect for strength and EMG median frequency when collapsed across time, the initial bout of eccentric exercise did not confer any protective effect against muscle damage following a subsequent bout of the same exercise. The findings suggested that there was no IL-RBE in resistance-trained individuals.

Nosaka and Aoki 2011 [8]

The purpose of this review paper was to provide information of RBE and underlying mechanisms. RBE is referred to the protective effect against muscle damage in subsequent bout of same or similar exercise. The RBE against maximal eccentric exercise can be elicited by submaximal intensities (40, 60, and 80% of MVIC), lower volume (6 vs. 24 eccentric contractions), slower velocities ($30°\cdot s^{-1}$ vs. $210°\cdot s^{-1}$), and isometric exercise at a long muscle length (160°). The potential mechanisms for RBE is not fully understand, but it seems to involve neural, mechanical, and cellular adaptations. For neural adaptations, an initial bout of exercise can increase in MU synchronization, usage of synergist muscles, and the number of lower-threshold MUs recruited in a subsequent bout to reduce muscle damage for an ensuing bout. In addition, an increase in the number of sarcomere in series, up-regulation of desmin, talin, vinculin, and dystrophin, remodeling of intermediate filament system, and increased protein synthesis can also be associated with the RBE as mechanical and cellular adaptations.

Gorianovas et al. 2013 [54]

This study compared the magnitude of RBE among children, adults, and older adults after two bouts of 100 intermittent drop jumps, separated by 2 weeks. The results indicated that the magnitude of RBE was greater in young adults than children and older adults. The findings suggested that boys and older adults are more resistant to muscle damage than young adults. The authors assumed that less muscle damage may be due to greater proportion of type I muscle fiber in boys and older adults than young adults, and insufficient muscle damage from initial bout may induce less RBE in subsequent bout.

Chen et al. 2019 [10]

The authors compared the changes in indirect muscle damage markers on EF, EE, KF, KE, pectoralis, plantar flexors, latissimus, abdominis, and erector spinae muscles after two bouts of 50 eccentric contractions, separated by 2 weeks. Changes in indirect muscle damage markers were greater in arm muscles than the other muscle groups after the initial bout, but the magnitudes of RBE were similar among the muscles. The findings suggested that despite the different response in muscle damage, IL-RBE was similar in larger muscle groups.

2.3. Contralateral Repeated Bout Effect (CL-RBE) and Possible Mechanisms

Connolly et al. 2002 [21]

This study was examined the CL-RBE after two bouts of bench step-on and -off exercise, separated by 2 weeks. Twelve participants completed step exercises, and muscle strength, pain, and tenderness were measured pre, and 1 to 4 days post exercise. The initial and second bouts induced muscle damage as indicated by strength loss, increased muscle pain, and decreased muscle tenderness over time. However, there were no differences in strength and tenderness between the initial and second bout, indicating no CL-RBE on leg muscles.

Howatson and van Someren 2007 [16]

This study investigated changes in CK activity, muscle soreness, MVIC, and ROM of the elbow flexors for the IL and contralateral arms following exercise bouts separated by 2 weeks. Sixteen men were assigned into IL group (n = 8) and CL (n = 8) and completed two bouts of 45 maximal eccentric contractions in same arm for IL-RBE group and in different arms for CL group. Changes in indirect muscle damage markers, including CK activity, MVIC and muscle soreness, were significantly attenuated in the subsequent bout when compared to the initial bout for both the IL and CL groups. However, the magnitude of the protective effect was greater for IL than CL. This was the first study to report CL-RBE on elbow flexor muscles.

Starbuck and Eston 2012 [28]

The purpose of this study was to investigate CL-RBE on elbow flexors and examine possible neural adaptations with surface EMG technique. IL group performed two bouts of 60 eccentric contractions in same arm separated by 2 weeks, whereas the CL group completed the initial bout with one arm and second bout with contralateral arm. A reduction in symptoms of muscle damage was observed in both IL and CL groups after the second bout, indicating IL-RBE and CL-RBE for the elbow flexors. Although there was no significant change in EMG amplitude, EMG median frequency decreased by 31% in the subsequent bout when compared to the initial bout with no difference between groups (group collapsed). As indicated in an earlier study [52], it is believed that decreased EMG median frequency indicated an increased reliance on MU expressing slow-twitch characteristics to modulate force during the second bout in both IL and CL arm, suggesting that CL-RBE may be partly mediated by neural adaptation.

Xin et al. 2014 [24]

This study investigated nuclear factor-kappa B (NF-kB: an important regulator of muscle inflammation) DNA-binding activity after two bouts of 100 eccentric knee extension contractions, where one leg completed the initial bout and the other leg conpleted the second bout, 4 weeks apart. Isometric and isokinetic strength, CK activity and muscle soreness were measured as indirect muscle damage markers at pre and 1 to 5 days post exercise, and NF-kB DNA-binding activity was obtained as the inflammatory factor at pre- and post-exercise. Isometric and isokinetic strength and NF-kB DNA-binding activity were significantly attenuated in the contralateral leg after the second bout, but muscle soreness and CK activity did not exhibit any CL-RBE. The findings suggested that inhibition of inflammatory responses after muscle-damaging exercise can be one of the underpinned mechanisms for CL-RBE. In addition, there was a CL-RBE for strength but not for muscle soreness and CK activity. Thus, it is possible that each indirect muscle damage marker has different mechanisms for CL-RBE.

Chen et al. 2016 [17]

The authors investigated the CL-RBE for different time intervals between two bouts of 30 eccentric elbow flexion contractions to identify the maximal duration of CL-RBE on elbow flexors. Untrained young men completed two bouts of eccentric exercise separated by 0.5, 6, 12, and 24 hours and 1, 4, and 8 weeks. CL-RBE was observed for 24

hours and 1 and 4 weeks intervals but not for 0.5, 6, and 12 hours and 8 weeks intervals. The findings suggested that CL-RBE for elbow flexors may last between 4 and 8 weeks.

Chen et al. 2018a [18]

Earlier research investigated the duration of CL-RBE on elbow flexors; therefore, this study examined the maximal duration of CL-RBE for knee flexors after two bouts of 60 maximal eccentric contractions, separated by 1, 7, and 28 days, and compared between the magnitude of CL-RBE for elbow flexors and knee flexors. Young untrained men completed eccentric exercise and were measured for strength, ROM, CK activity, and muscle soreness at pre and 1 to 5 days post exercise. Multiple indirect muscle damage markers were significantly diminished in a subsequent bout at 1 and 7 days intervals when compared to the initial bout, but not at 28 days post. The findings suggested that CL-RBE for knee flexors may last 1 week and disappear between 1 to 4 weeks. In addition, the magnitude of CL-RBE for elbow flexors was greater than CL-RBE for knee flexors. Thus, the magnitude of CL-RBE may be muscle dependent, unlike IL-RBE.

Chen et al. 2018b [20]

Previous study have reported submaximal eccentric exercise induced IL-RBE [14]. Thus, this study investigated the magnitude of CL-RBE during low intensity eccentric exercise of elbow flexors. Young untrained men completed two bouts of 30 eccentric contractions, separated by 1, 2, and 7 days, where the initial bout consisted of eccentric

exercise at 10% of MVIC and the second bout consisted of maximal eccentric exercise. Multiple indirect muscle damage markers were attenuated after the second bout for days 1, 2, and 7. The findings suggested eccentric exercise performed at 10% MVIC can elicit a protective effect to the contralateral arm muscles.

Tseng et al. 2019 [13]

In this study, the authors compared the magnitude of CL-RBE using different muscle actions (concentric vs. eccentric) and training (concentric training vs. eccentric training). Young sedentary men were assigned into IL (IL-RBE), CL (CL-RBE), ET (eccentric training for CL-RBE), or CT (concentric training for CL-RBE) groups. The IL and CL groups completed two bouts of 30 maximal eccentric contractions, separated by 2 weeks for IL group and 1 week for CL group. The ET and CT groups performed 5 weeks of training (once a week 30 eccentric or concentric contractions) for the initial bout and 30 maximal eccentric contractions for the second bout. The magnitude of the protective effect was shown as IL > ET > CL > CT. The findings suggested that cross-education effect was greater after eccentric training than a single eccentric exercise.

Ochi et al. 2021 [55]

The CL-RBE has mostly been investigated in arm and leg muscles. Therefore, this study examined CL-RBE on the flexor pollicis brevis (FPB) muscle. Thirty-two sedentary men completed two bouts of 100 eccentric contractions, separated by 2, 4, and 8 weeks. MVIC, ROM, muscle soreness, and motor and sensory nerve conduction

velocities measured via surface EMG were recorded at pre, post and 1, 2, 3, and 5 days post exercise. Eccentric exercise induced strength loss, increased muscle soreness, and decreased ROM and motor and sensory nerve conduction velocities. However, there was no CL-RBE for dependent the variables. The findings suggested that eccentric contractions of the FPB muscle caused dysfunction of nerve function and muscle damage, but did not induce any contralateral protective effect on hand muscle.

2.4. Neuromuscular Electrical Stimulation (NMES)

Feiereisen et al. 1997 [56]

This study used fine wire EMG to investigate MU recruitment order during voluntary and involuntary contractions (NMES). A total of 302 MUs from the tibialis anterior with recruitment threshold ranges of 1 to 88% of MVIC were recorded from five men. The results showed that MUs were recruited in order of motoneuron size during voluntary contraction, while a recruitment pattern reversal was shown during NMES. Thus, NMES can induce a unique pattern for MU recruitment that is not present during voluntary muscle activation.

Jubeau et al. 2007 [57]

The authors investigated motor unit recruitment patterns during NMES. Sixteen healthy men completed submaximal isometric trapezoidal contraction at 20, 40, and 60% of MVIC. Paired stimuli were delivered during the steady force segment of the

contractions, and the characteristic of the superimposed doublet (i.e., peak torque: PT and time to peak torque: TPT) was recorded. TPT of the superimposed doublet was longer during NMES than during the voluntary contraction. TPT of the superimposed doublet during voluntary contractions was decreased as intensity was increased, whereas TPT of the superimposed doublet during NMES did not change at all intensities. In addition, PT of the superimposed doublet was higher during NMES than during voluntary contractions. The findings suggest that NMES may activate MUs at random, as opposed to the size principal typically observed during voluntary contractions.

Jubeau et al. 2008 [58]

The study investigated muscle damage and hormone response after voluntary contractions and NMES. Nine healthy men completed 40 isometric leg press contractions and 40 NMES of the quadriceps muscles. MVIC, growth hormone (GH), CK activity, and lactate concentration were recorded. Strength decrements, GH, CK activity, and lactate concentration were greater after NMES compared to the isometric contractions. The authors assumed that greater changes in all dependent variables after NEMS may be due to the unique patterns of MU recruitment, such as temporally synchronous and non-selective recruitment. A greater amount of high-threshold MUs recruitment during NMES can lead to more muscle damage and fatigue, resulting in greater GH release. Thus, the findings suggest that NMES can result in a greater GH response and muscle damage than voluntary contractions.

Black and Mccully 2008 [42]

This study was one of earliest observation to examine IL-RBE with NMES in humans. The NMES group (n = 10) completed two bouts of 80 electrically stimulated evoked eccentric contractions and the voluntary group (n = 9) performed two bout of 80 eccentric knee extension contractions, 7 weeks apart. The initial bout decreased strength, increased muscle soreness and T2 signal intensity for both groups, indicating muscle damage. After the repeated bout, changes in all dependent variables were smaller than the initial bout and the magnitude of reduction was similar between two groups. The finding suggested that eccentric contractions via NMES can induce a protective effect on the same muscle group and the mechanism for IL-RBE may be involve mechanical adaptation. T2 signal intensity is reported to reflect the magnitude and location of muscle damage. In this study, T2 signal intensity was increased after the initial bout, but the change of T2 signal intensity was reduced after second bout. Thus, mechanical adaptation can be one of the potential mechanisms for IL-RBE via NMES. In addition, IL-RBE via NMES can last at least 7 weeks.

Toca-Herrera et al. 2008 [59]

This study investigated cross-education after one NMES session for the leg muscles. The NMES group completed NMES for 10 minutes on the non-dominant leg, and the control group relaxed for 10 minutes without any activity. Strength, EMG_{RMS}, and MMG_{RMS} were recorded for dominant leg before and after NMES. Strength and EMG_{RMS} were significantly increased for the dominant leg after NMES on the non-

dominant leg, but there was no change for MMG_{RMS}. The increased EMG activity of the agonist muscle and the decreased EMG activity of the antagonist muscle can partially explain the increase in muscle strength. It was suggested the more efficient MU recruitment and discharge rate between the agonist and antagonist muscles may have increased the muscle-generating capacity due to neural adaptations. In addition, no change in MMG_{RMS} suggested cross-education did not alter the mechanical behavior of the muscles. The findings suggested that one NMES session lead to neural mediated cross-education on strength.

Aldayel et al. 2010 [41]

Previous studies have demonstrated an IL-RBE after two bouts of ES evoked eccentric contractions [42,60]. However, no study has explored the protective effect after NMES contractions. The purpose of this study was to examine whether the initial bout of NMES contractions at a maximal tolerable intensity can induce any protective effect in subsequent bouts of NMES contractions. Nine untrained men completed two bouts of 45 NMES contractions in knee extensors for 15 minutes, separated by 2 weeks. MVIC torque, muscle soreness, pressure pain threshold (PPT), and CK activity were measured before and 1, 24, 48, 72, and 92 hours after NMES. The initial bout of NMES contractions inducted muscle damage, such as strength loss, increased soreness, tenderness, and CK activity, but the magnitude of muscle damage was smaller in subsequent bout, indicating IL-RBE. Thus, NMES without eccentric muscle actions can lead to a protective effect on same muscle.

CHAPTER III

METHODOLOGY

3.1. Experimental Design

The study used a between-group design to identify whether an initial bout of neuromuscular electrical stimulation (NMES) on the biceps brachii (BB) muscles can induce any potential protective repeated bout effect (RBE) on the contralateral muscle in subsequent bout of the NMES. In addition, the mechanical activity and motor unit (MU) control strategies of the BB were also examined for the purpose of exploring the potential mechanisms of the contralateral repeated bout effect (CL-RBE). A total of seven separate visits to the laboratory was required to complete this investigation. After a familiarization visit (Visit 1), 45 NMESs were applied to a randomly-chosen (ipsilateral vs. contralateral) BB muscle (Visit 2). Before (pre), after (post), 1 day (24 post: Visit 3), and 2 day (48 post: Visit 4) after the NMES, indirect muscle damage markers were recorded. Following a rest interval of one week, participants completed the exact same muscle-damaging electrical stimulation and measurements (Visit 5) at the same time points (Visit 6 and 7) using same BB muscle for IL group and contralateral BB muscle for CL group (Fig. 1).

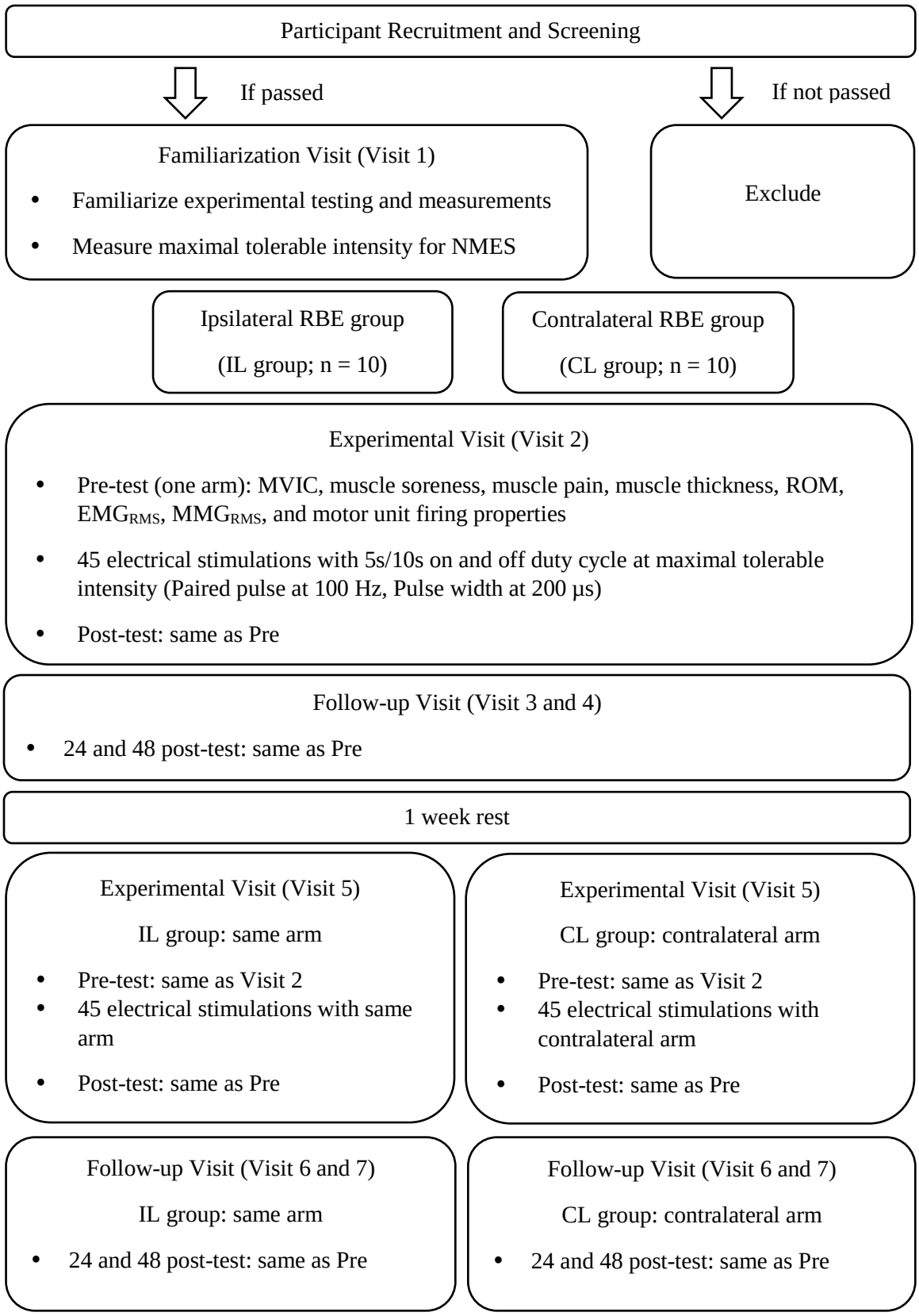

Figure 1. Experimental design

3.2. Participants

The target population was healthy and untrained males and females between the age of 18-40. Since no study has examined the effects of NMES on CL-RBE of the BB muscles, Chen et al. [17] was used to estimate the necessary sample size for the current study as the authors investigated the CL-RBE of elbow flexors. It was estimated that at least 10 participants per group were necessary with the effect size of 0.8, an α level of 0.05, and a power (1 – β) of 0.80 by G*Power (G*power 3.1.9.7, Heinrich-Heine-Universität Düsseldorf, Dusseldorf, Germany). Thus, a sample size of 20 was used for the current study. Inclusion criteria required participant to have no any cardiovascular disease or metabolic, renal, and hepatic disorders. Twenty untrained adults (Mean ± SD; age = 26.1 ± 6.2 years, height = 171.9 ± 8.7 cm, weight = 93.3 ± 29.5 kg) participated in this study. Of these 20 subjects, 11 were male (Mean ± SD; age = 27.9 ± 6.8 years, height = 177.3 ± 5.4 cm, weight = 105.6 ± 32.3 kg) and 9 were female (Mean ± SD; age = 23.8 ± 4.9 years, height = 165.2 ± 7.3 cm, weight = 78.1 ± 17.2 kg). In addition, exclusion criteria was any history of severe shoulder, elbow, and wrist injuries, or other pathological conditions that impair motor control. Before the study, all participants completed a physical activity readiness questionnaire (PAR-Q), a health history questionnaire, and informed consent form approved by the University Institutional Review Board (Protocol number: IRB-21-359).

3.3. Procedures

Seven laboratory visits were required to complete this study. All procedures were thoroughly explained to participants, and participants were familiarized with all the experimental testing and measurements during Visit 1. First, the investigator measured participants' height and weight, and the dominant arm was determined by asking which hand would throw a ball [61]. One arm was randomly selected (right vs. left) during the first visit to avoid dominant muscle effects. The subjects were randomly assigned into the ipsilateral RBE group (IL) or the contralateral RBE group (CL). Lastly, the maximal tolerable intensity of NMES for each participant was determined with a constant-current stimulator (Digitimer model DS7AH; Hertfordshire, England, UK). Two stimulating electrodes (5 × 5 cm square electrodes, model USX2020, Axelgaard Manufacturing Co., Ltd., Fallbrook, CA, USA) connected to a constant-current stimulator were placed over the proximal belly (cathode) and the distal tendon (anode) of BB muscle. Prior to electrode placement, the surface of the skin was shaved with a disposable razor, superficial dead skin was removed with adhesive tape, and the electrode area was sterilized with alcohol. The participants relaxed their arm muscles and the investigator recorded their maximal stimulation levels of with a series of stimuli (paired pulses at 100 Hz, 200 μs pulse-width) by increasing 20 miliamps (mA) until the plateau of involuntary elbow flexion twitch force [62].

At least 24 hours after Visit 1, participants had 45 electrical stimuli to the designated BB muscle (e.g., ipsilateral side during Visit 2 and the same side for the IL group or the contralateral side for the CL group during Visit 5) with 5-sec on and 10-sec off duty at pre-determined maximal tolarable intensity. Participants were seated in chair with an upright position, and the treated arm was comfortably rested on a custom-made

elbow ergometer (Model: MUC1, OT Bioelettronica SRL, Torino, Italy) with an elbow joint angle of 180°. Electrodes were placed over the proximal belly and the distal tendon of the BB muscle after skin preparation, paired pulses of 45 electrical stimuli cycle were delivered total 225 seconds with 450 seconds of resting. Before, after, 1 day, and 2 days after NMES, participants completed all measurements, including MVICs, muscle soreness and pain, ROM, and muscle thickness, and randomly ordered submaximal isometric trapezoidal contractions at 40% and 70% MVIC. After performing a few submaximal elbow flexions at a lower %MVIC as a warm-up, participants were asked to contract isometrically as fast and hard as possible for three, five second maximal voluntary contractions to assess MVIC of the elbow flexors. Three minutes of rest were provided between contractions. Maximal strength was calculated as the greatest 0.25 second epoch among the MVICs. At least 3 minutes after maximal strength testing, participants performed randomly ordered submaximal isometric trapezoidal contractions at 40% and 70% of MVIC for the current visit. The template for the isometric trapezoid contractions contained a linearly increase from baseline at a rate of 10% MVIC per second, a 12 seconds steady force segment at the targeted force (40% or 70% MVIC), and a linearly decrease to baseline at a rate of 10% MVIC per second. Therefore, the contractions for the 40% and 70% isometric trapezoidal contractions lasted 26 and 32 seconds, respectively. During the isometric trapezoidal contractions, a monitor provided the targeted force template and the participants' real time force feedback. Participants were given a second attempt if they were unable to maintain the targeted force during the intial trial.

3.4. Measurements

3.4.1. Muscle Soreness

A visual analog scale (VAS) was used to record muscle soreness before, after, 1 day, and 2 day after NMES. The VAS consisted of a 100 mm line from 0 mm "not sore at all" to 100 mm "worst soreness ever". Participants marked their perceived muscle soreness on 100 mm line of the VAS when they were extended and flexed their BB muscles throughout the ROM.

3.4.2. Pressure Pain Threshold (PPT)

A hand-held digital pressure algometer (1 cm diameter of probe head, Wagner Force Ten^{TM} Model FDX 50, Wangner Instruments, Greenwich, USA) was used to measure the PPT at 3, 9, and 15 cm above the elbow crease [63]. Participants were seated in a chair with their arm relaxed and hand supinated on the table. The probe head of algometer was placed vertically over the targeted surface skin of the BB muscle, and pressure was gradually applied until the participant verbalized they felt slight muscle pain. The investigator then recorded the force level (Unit: kg) displayed digitally on the algometer. Three measurements were taken for each test.

3.4.3. Range of Motion (ROM)

Elbow joint ROM was determined as the difference in the joint angles between voluntary maximal elbow flexion and extension using an 8 inch goniometer (EMI Plastic

Goniometer, Elite Medical Instruments, Inc., Fullerton, CA, USA). The maximal elbow flexion was measured when participants maximally tried to touch their shoulder of the same side by flexing the elbow joint while keeping the elbow joint at the side of the body in a standing position. The extension was measured when participants attempted to extend their elbow joint as much as possible with the elbow held by their side and the palm toward the body. At least three measurements were taken for each angle.

3.4.4. Ultrasound Imaging

Muscle thickness of the BB muscle was taken using a B-mode ultrasound (LogiQ S8, General Electric Company, Milwaukee, WI, USA) with a multifrequency linear-array probe (ML6-15-D, 4-15 MHz, 50 mm field of view, General Electric Company, Milwaukee, WI, USA) using the LogicView function. The setting for the muscle thickness measurements of the BB muscle was set at 60 dB and a frequency of 12 MHz. Participants laid supine on a rehabilitation bed with the testing arm relaxed, supinated, and abducted from the torso. The probe was placed at two-thirds the distance between the medial acromion of the scapula and the fossa cubit [17]. Before the measurement, a generous amount of water-soluble transmission gel was applied to the skin to reduce possible near-field artifacts and enhance acoustic coupling. All images were analyzed with ImageJ software (Version 1.47v, N National Institutes of Health, Bethesda, MD, USA). The average for three muscle thickness measurements was used in subsequent analyses. Muscle thickness was defined as the distance from the border of the fascia of the muscle to the top edge of the humerus.

3.4.5. Electromyographic (EMG) Acquisition and Decomposition

Surface EMG signals were recorded from the BB muscles during the MVIC and submaximal trapezoidal contractions with a 5 pin array sensor consisting of a 0.5 mm diameter, with 4 pins located at the corners of a 5 × 5 mm square and the 5th pin at the center of the square (dEMG sensor, Delsys, Boston, MA, USA). The dEMG sensor was placed over the muscle belly of the BB based on the recommendations from the surface electromyography for the non-invasive assessment of muscles (SENIAM) project [64] with surgical tape. The reference electrode was attacted on the seventh cervical vertebrae (C7). Prior to application of the electrodes, the surface of the skin was shaved, adhesive tape was used to remove superficial dead skin, and the skin was sterilized with alcohol. A permanent marker was used to mark the location of the electrode on the skin and participants were instructed to remark the location when necessary. In addition, the position of the electrodes was measured by using anatomical landmarks as a reference to confirm similar electrode placement between experimental sessions. The signals were sampled at 20 kHz and stored on a computer for off-line analysis.

The surface EMG signals recorded during the isometric trapezoidal contractions were decomposed to examine individual MU properties. For detailed information regarding signal procesessing of the EMG signals, refer to De Luca et al. [65] and Nawab et al. [66]. The Precision Decomposition III (PD III) algorithm (version 4.1.1.0) was used to extract action potentials into single MU firing events from the four separate EMG signals as described by De Luca et al. [65]. The reconstruct-and-test procedure tested the accuracy of the decomposed firing instances [66], and only MUs with > 90% accuracies was included

for further analysis. For each detected MU, three parameters were extracted from the firing rate data: 1) recruitment threshold (RT) (expressed as %MVIC), 2) mean firing rate (MFR) at the target force level (pulses per second [pps]), and 3) MU action potential amplitude ($MUAP_{AMP}$). The RT was calculated as the average force from a 0.10 ms epoch of force that began at the first discharge of the MU. The MFR was calculated as the inverse of the average interspike intervals during the first 8 seconds of the steady force segment from the trapezoidal contraction. The average of the peak-to-peak amplitude (mV) from each of the 4-action potential waveforms was used to calculate action potential amplitude.

3.4.6. EMG Amplitude (EMG_{RMS})

Channel 1 of the 4 bipolar EMG channels for the dEMG sensor was selected for the time-domain (amplitude) analysis. The EMG channels were bandpass filtered (fourth-order Butterworth) at 10-500Hz. Peak EMG amplitude was calculated as the average RMS value from the highest 0.25 s peak force epoch during the MVIC from the respective visit. In addition, the average EMG_{RMS} calculated during the 8 s epoch analyzed during the steady force segments of the isometric trapezoidal contractions to quantify MU MFRs was normalized ($N\text{-}EMG_{RMS}$) to peak EMG_{RMS} for the current visit.

3.4.7. Mechanomyographic (MMG) Acquisition

During the MVICs and submaximal isometric trapezoidal contractions, MMG signals were recorded from BB muscle using an active miniature accelerometer (model

352A24, frequency response of 1-8000 Hz, sensitivity of 10.2 mV m/s^2, PCB Piezotronics, Inc., Depaw, NY, USA). The accelerometer was placed over the BB muscle at one third the distance of the fossa cubit to medial acromion. Double-sided tape was used to affix the accelerometer to the skin. The location of the accelerometer was marked with a permanent marker pen (Sharpie, Atlanta, GA, USA) to confirm similar placement for each visit, and participants were instructed to remark the location when necessary. In addition, the position of the electrodes was measured by using anatomical landmarks as a reference to confirm similar electrode placement between experimental sessions.

3.4.8. MMG Amplitude (MMG_{RMS})

For the MVICs, peak MMG_{RMS} was calculated as the average RMS value the from the highest 0.25 s peak force epoch for the respective visit. For the submaximal muscle actions, the force and MMG signals were analyzed with consecutive, non-overlapping 0.25 s epochs. In addition, the average MMG_{RMS} calculated during the 12 s steady force segment of the isometric trapezoidal contractions was normalized (N-MMG_{RMS}) to peak MMG_{RMS} for the current visit. The amplitude of the MMG signal was calculated with root mean square (RMS).

3.4.9. Surface EMG, MMG, and Force Signal Processing

For the MVICs and isometric trapezoidal contractions, the surface EMG (μV), MMG (m/s^2) and force (N) signals were simultaneously sampled at 2 kHz with a National Instruments compact data acquisition system (NI cDAQ-9174). All subsequent signals

were stored and processed off-line with custom-written software (Labview version 18; National Instruments, Austin, Tx). EMG signals were bandpass filtered (fourth-order Butterworth) at 10-500 Hz, whereas the MMG signals were bandpass filtered (fourth-order Butterworth) at 5-100 Hz. During the isometric trapezoidal muscle actions, consecutive, non-overlapping 0.25-s epochs were analyzed for the force, EMG, and MMG signals. Root mean square (RMS) was used to calculate the amplitude of the EMG and MMG. Zero to 10% was not analyzed during the linearly increasing and decreasing muscle actions to avoid transient phenomena from rest to exertion and vice versa when obtaining the integrated EMG and MMG signals [67].

3.5. Statistical Analysis

3.5.1. Motor Unit Properties

For each contraction, linear regressions were applied to the $MUAP_{AMP}$ and FR vs. RT relationships [68,69]. Slope and y-intercepts were calculated for each subject and used for statistical analysis.

3.5.2. Surface EMG_{RMS}, MMG_{RMS}, and Force

For the linearly increasing (Fig. 2-A) and decreasing (Fig. 2-C) segments of the trapezoidal contractions at 40% and 70% MVIC, simple linear regression models were fit to log-transformed MMG_{RMS}-force relationships [70,71]. The equations were represented as:

$$\ln[Y] = b(\ln[X]) + \ln[a] \quad (1)$$

Where ln[Y] = the natural log of the MMG_{RMS} values, ln[X] = the natural log of the force values, b = slope, and ln[a] = the natural log of the y-intercept. This can also be expressed as an exponential equation after antilog transformation:

$$Y = aX^b \quad (2)$$

Where Y = the predicted MMG_{RMS} values, X = force, b = slope of equation (1), and a = the antilog of the y-intercept from equation (1). Slopes (b) was calculated using Microsoft Excel (Microsoft Excel, version 2010; Microsoft, Inc., Redmond, WA, USA).

For the steady force segment of the isometric trapezoidal contraction (Fig. 2-B), EMG_{RMS} and MMG_{RMS} were calculated by averaging the values for each 0.25 sec epoch from the entire 12 sec targeted %MVIC and normalized to peak EMG_{RMS} and MMG_{RMS} for the MVICs from the current visit, respectively.

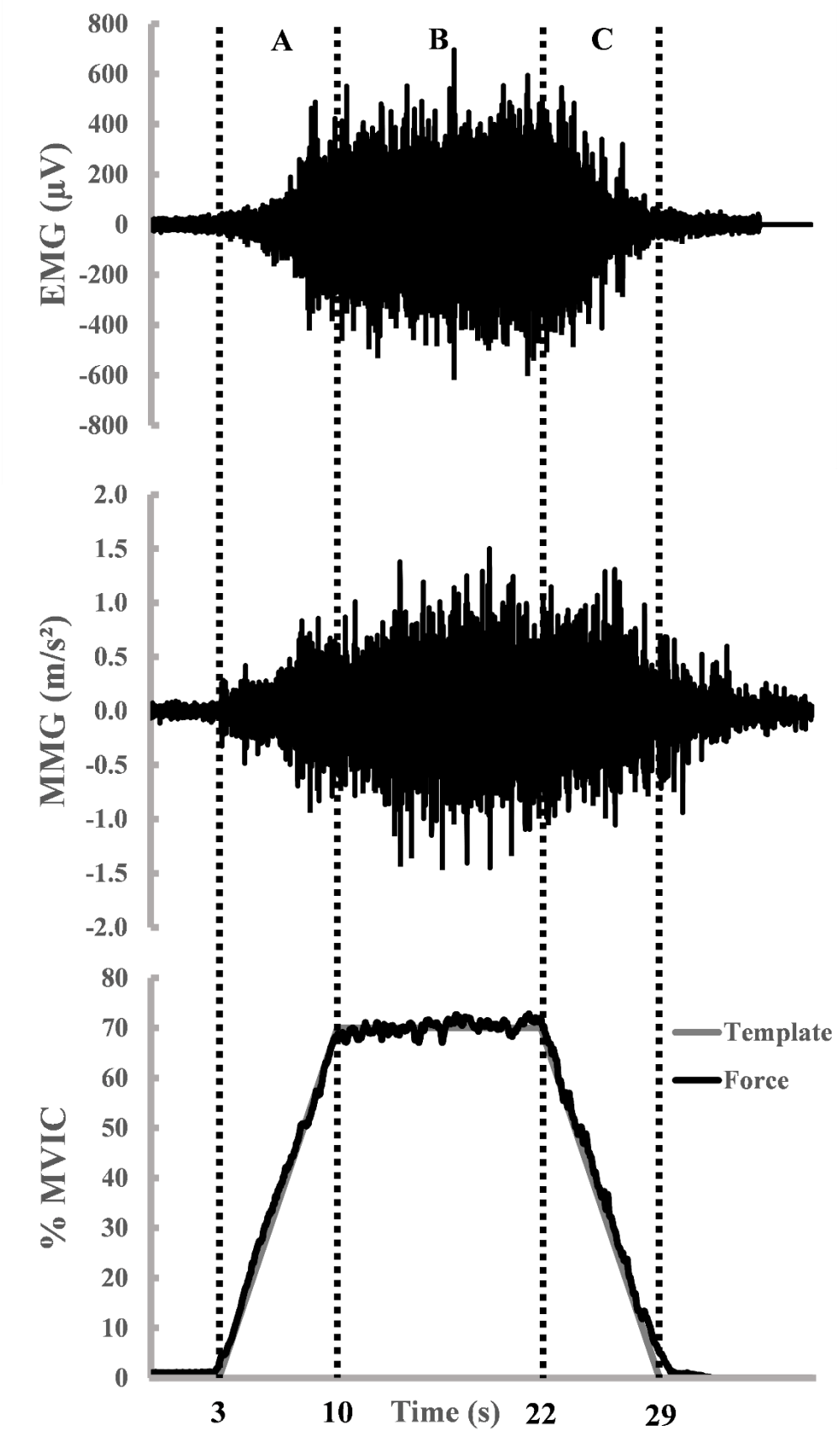
A
B
C
800
600
400
200
0
-200
-400
-600
-800
EMG (μV)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
MMG (m/s²)
80
70
60
50
40
30
20
10
0
% MVIC
Template
Force
3
10
Time (s)
22
29

Figure. 2. The electromyographic (EMG; top) and mechanomyographic (MMG; middle) signals recorded from the biceps brachii (BB) during a 70% isometric trapezoidal contraction based on their maximal isometric voluntary contraction (MVIC) from one participant. The force signal (bottom) is overlaid onto the trapezoidal template as it appeared for the participant during the trial. The vertical dotted lines indicated the (A) linear force increase, (B) the steady force, and (C) the linear decrease segments of the 70% isometric trapezoidal contraction. The EMG and MMG signals that corresponded with the contraction segment (A-C) was selected for analysis.

3.5.3. Analysis of Variance (ANOVA) Models

Separate three-way mixed factorial ANOVAs (time [pre vs. post vs. 24 post vs. 48 post] × bout [1st bout vs. 2nd bout] × group [IL group vs. CL group]) were used to analyze possible differences in MVIC, musle soreness, ROM, and muscle thickness. A four-way mixed factorial ANOVA (time [pre vs. post vs. 24 post vs. 48 post] × bout [1st bout vs. 2nd bout] × group [IL group vs. CL group] × location [proximal vs. mid vs. distal]) was used to examine differences in PPT for the BB muscle. Separate five-way mixed factorial ANOVAs (time [pre vs. post vs. 24 post vs. 48 post] × bout [1st bout vs. 2nd bout] × group [IL group vs. CL group] × contraction [40% vs. 70%] × segment [linear increase vs. linear decrease]) were used to examine differences in the *a* and *b* terms from the log-transformed MMG_{RMS}-force relationships during linear increasing and decreasing segements of submaximal isometric trapzoidal contraction at 40% and 70% MVIC. Separate four way-mixed factorial ANOVAs (time [pre vs. post vs. 24 post vs. 48 post] × bout [1st bout vs. 2nd bout] × group [IL group vs. CL group] × contraction [40% vs. 70%]) was used to examine possible differences in normalized MMG_{RMS} and EMG_{RMS} during the steady force segment, and the slope and y-intercepts from the MU MFR and $MUAP_{AMP}$ vs. RT relationships during the submaximal isometric trapzoidal contraction at 40% and 70% MVIC. When appropriate, follow-up tests included lower level ANOVA models and dependent and independent sample t-tests with Bonferroni corrections. The

level of significance was set at $p \leq 0.05$ and all data were reported as mean ± standard deviation (SD). The partial η^2 statistics were calculated for paired comparisons, with 0.2, 0.5, and 0.8 corresponding to small, medium, and large effect size, respectively. Hedges' g was calculated for paired comparisons, with 0.2, 0.5, and 0.8 corresponding to small, medium, and large effect size, respectively. All statistical analyses were conducted using SPSS 24.0 (IBM SPSS statistics 24.0, IBM, Armonk, NY, USA).

CHAPTER IV

RESULTS

4.1. Indirect Muscle Damage Markers

4.1.1. Maximal Voluntary Isometric Contraction (MVIC)

For MVIC, the analysis indicated no significant three-way interaction (time × bout × group, $F = 1.215$, $p = 0.311$, $\eta^2 = 0.063$) or two-way interactions (time × group, $F = 2.454$, $p = 0.073$, $\eta^2 = 0.120$; bout × group, $F = 0.762$, $p = 0.394$, $\eta^2 = 0.041$; time × bout, $F = 2.626$, $p = 0.060$, $\eta^2 = 0.127$). However, there was a significant main effect for time ($F = 27.854$, $p < 0.001$, $\eta^2 = 0.607$), but not for group ($F = 0.490$, $p = 0.493$, $\eta^2 = 0.026$) or bout ($F = 0.029$, $p = 0.868$, $\eta^2 = 0.002$). MVIC was significantly less at post (202.22 ± 71.29 N, $p < 0.001$) when compared to pre (238.91 ± 89.85 N, $g = 0.452$), 24 post (226.88 ± 80.78 N, $g = 0.324$), and 48 post (234.68 ± 84.80 N, $g = 0.414$) (Fig. 3).

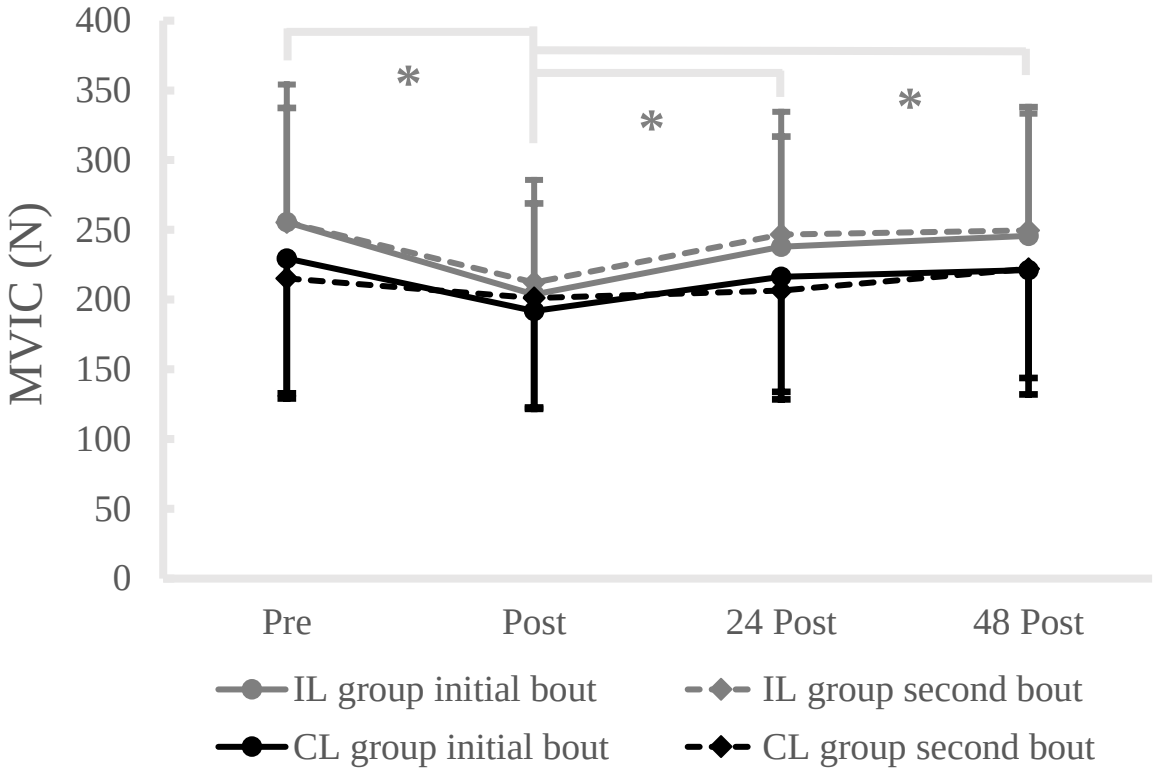

Figure 3. Plotting means for maximal voluntary isometric contraction (MVIC) before (pre), immediately after (post), 1 day post (24 post), and 2 days post (48 post) neuromuscular electrical stimulation for the ipsilateral- (IL) and contralateral-group (CL). Horizontal bars represent the standard deviation at each time point for the respective groups. * indicates MVIC at post was less than pre, 24 post, and 48 post, when collapsed across bout and group ($p < 0.001$).

4.1.2. Visual Analog Scale (VAS) for Muscle Soreness

For VAS scores, the analysis indicated no significant three-way interaction (time × bout × group, $F = 2.379$, $p = 0.080$, $\eta^2 = 0.117$). There was a significant two-way interaction (bout × group, $F = 5.900$, $p = 0.026$, $\eta^2 = 0.247$), but no significant time × group interaction ($F = 2.087$, $p = 0.113$, $\eta^2 = 0.104$) or time × bout interaction ($F = 1.410$, $p = 0.250$, $\eta^2 = 0.073$). For the IL group, muscle soreness was significantly lower ($p = 0.003$, $g = 1.602$) during the second bout (0.21 ± 0.27 cm) than the first bout (1.06 ± 0.70

cm). In addition, muscle soreness for the CL group was greater (p = 0.004, g = 1.335) during second bout (1.51 ± 1.35 cm) compared to the IL group (0.21 ± 0.27 cm) (Fig. 4).

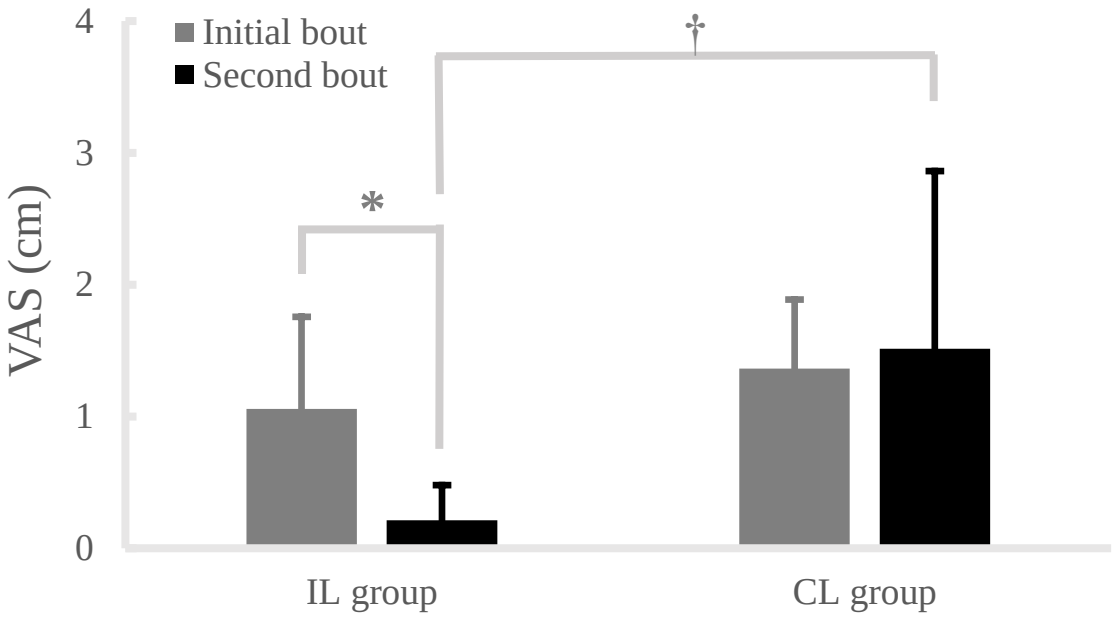

Figure 4. Plotted mean visual analogue scale (VAS) scores representing muscle soreness during the initial and second bouts for the ipsilateral (IL) group and contralateral (CL) group. Horizontal bars represent the standard deviation at each bout. * indicates VAS scores was lower during second bout than the first bout (p = 0.003) for the IL group. † indicates greater VAS scores was higher for the CL group than the IL group during the second bout (p = 0.004).

4.1.3. Range of Motion (ROM)

For ROM, the analysis indicated no three-way interaction (time × bout × group, F = 0.149, p = 0.930, η^2 = 0.008) or two-way interactions (time × group, F = 0.182, p = 0.908, η^2 = 0.010; bout × group, F = 0.182, p = 0.675, η^2 = 0.010; time × bout, F = 0.751, p = 0.526, η^2 = 0.040). There was a significant main effect for time (F = 11.759, p < 0.001, η^2 = 0.395), but not for bout (F = 2.121, p = 0.162, η^2 = 0.105) or group (F = 0.056, p = 0.816, η^2 = 0.003). ROM at post (117.29 ± 8.03 °) was significantly lower than

at pre (121.19 ± 8.57 °, $p < 0.001$, $g = 0.470$), 24 post (120.14 ± 7.68 °, $p = 0.019$, $g = 0.363$), and 48 post (120.56 ± 6.56 °, $p = 0.003$, $g = 0.446$), when collapsed across bout and group (Fig. 5).

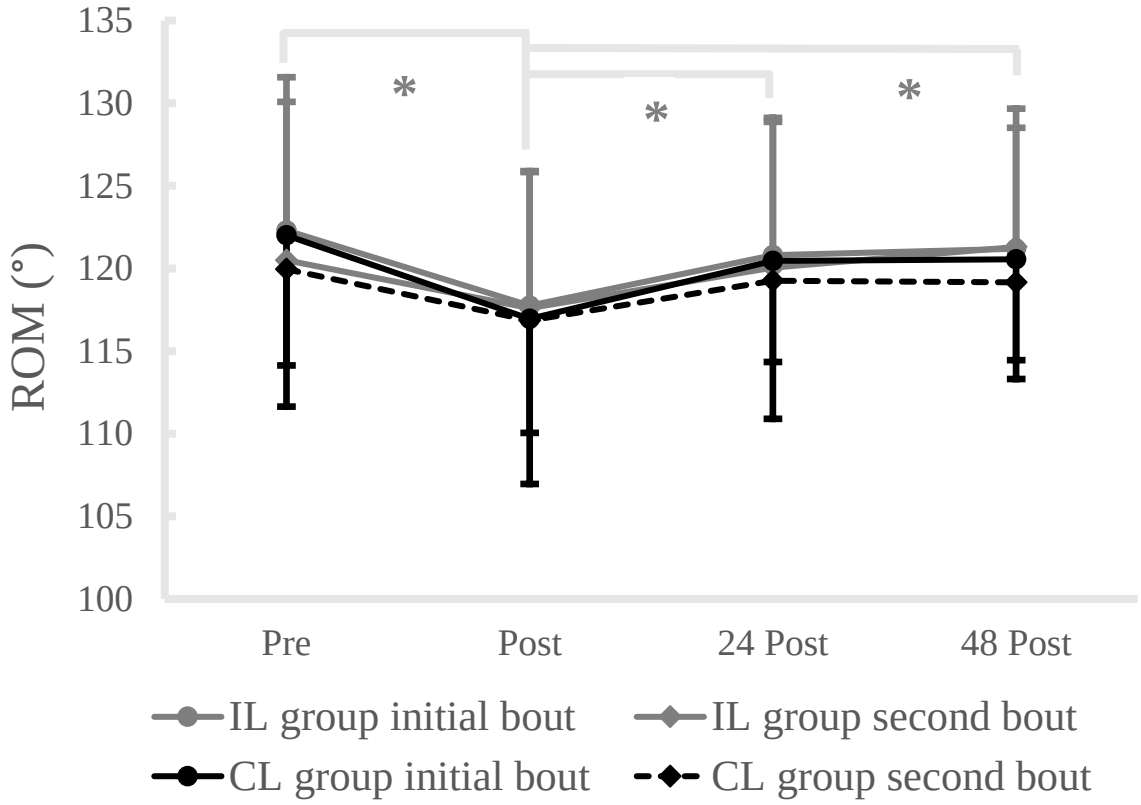

Figure 5. Plotted means for range of motion (ROM) before (pre), immediately after (post), 1 day post (24 post), and 2 days post (48 post) neuromuscular electrical stimulation for the ipsilateral (IL) group and contralateral (CL) group. Horizontal bars represent the standard deviation at each time point for the respective groups. * indicates ROM at post was less than pre, 24 post, and 48 post, when collapsed across bout and group ($p < 0.001$).

4.1.4. Muscle Thickness

For the muscle thickness, the analysis indicated no significant three-way interaction (time × bout × group, $F = 0.983$, $p = 0.408$, $\eta^2 = 0.052$) or two-way interactions (time × group, $F = 0.893$, $p = 0.451$, $\eta^2 = 0.047$; bout × group, $F = 1.463$, $p =$

0.242, $\eta^2 = 0.075$; time × bout, $F = 1.648$, $p = 0.189$, $\eta^2 = 0.084$). However, there was a significant main effect for time ($F = 11.464$, $p < 0.001$, $\eta^2 = 0.389$), but not for bout ($F = 1.381$, $p = 0.255$, $\eta^2 = 0.071$) and group ($F = 0.350$, $p = 0.561$, $\eta^2 = 0.019$). Muscle thickness at pre (2.91 ± 0.69 cm) was less than at post (3.10 ± 0.77 cm, $p < 0.001$, $g = 0.250$) and 24 post (3.03 ± 0.75 cm, $p = 0.019$, $g = 0160$). In addition, muscle thickness at post was greater than at 48 post (2.97 ± 0.76 cm, $p = 0.006$, $g = 0.170$) (Fig. 6).

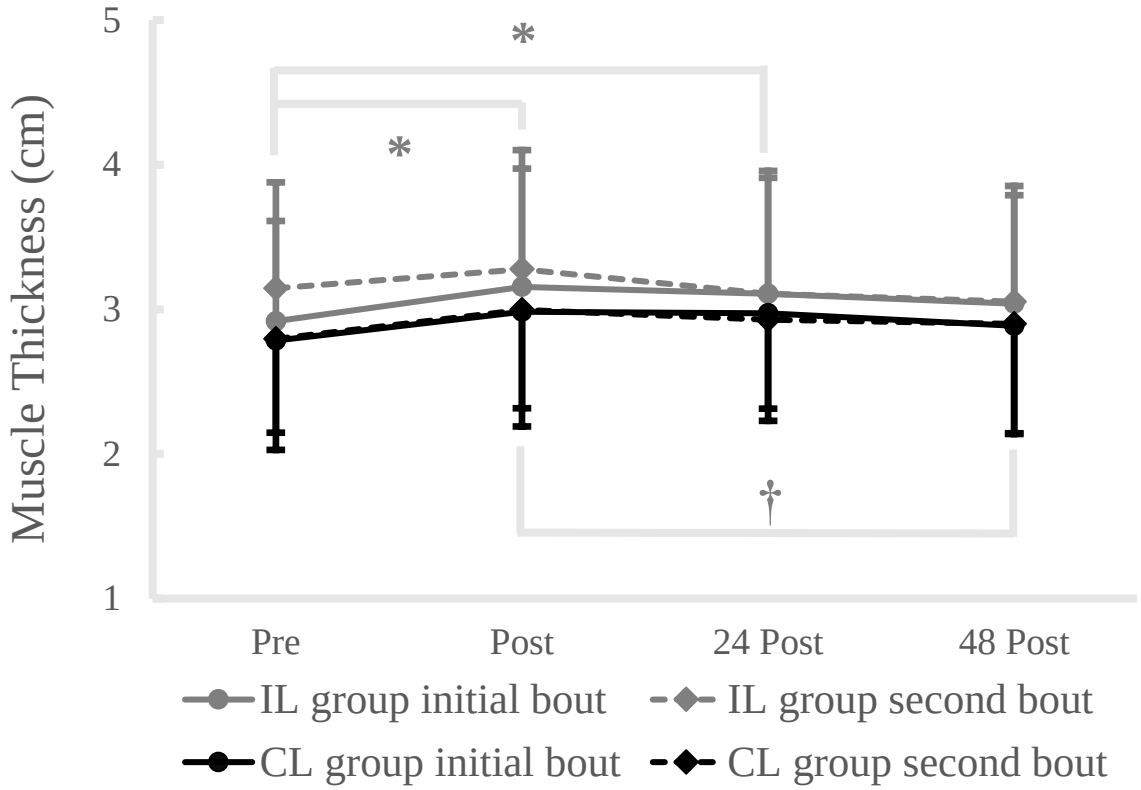

Figure 6. Plotted means for thickness at before (pre), immediately after (post), 1 day post (24 post), and 2 days post (48 post) neuromuscular electrical stimulation for the ipsilateral (IL) group and contralateral (CL) group. Horizontal bars represent the standard deviation at each time point for the respective groups. * indicates muscle thickness at pre was less than post ($p < 0.001$) and 24 post ($p = 0.019$), when collapsed across bout and group. † indicates muscle thickness at post was greater than 48 post ($p = 0.006$), when collapsed across bout and group.

4.1.5. Pressure Pain Threshold (PPT)

For PPT, the analysis indicated no significant four-way interaction (time × bout × group × location, $F = 1.041$, $p = 0.403$, $\eta^2 = 0.055$) or three-way interactions (time × bout × group, $F = 1.045$, $p = 0.380$, $\eta^2 = 0.055$; time × location × group, $F = 1.338$, $p = 0.247$, $\eta^2 = 0.069$; bout × location × group ($F = 1.336$, $p = 0.275$, $\eta^2 = 0.069$; time × bout × location, $F = 0.466$, $p = 0.832$, $\eta^2 = 0.025$). However, there was a significant two-way interactions (time × group, $F = 5.378$, $p = 0.003$, $\eta^2 = 0.230$). The follow-up one-way ANOVA for time was significant for the IL group ($p = 0.001$, $\eta^2 =0.449$) and the CL group ($p < 0.001$, $\eta^2 =0.568$). For the IL group, PPT at pre (1.26 ± 0.57 kg) was greater ($p = 0.004$, $g = 0.339$) than post (1.07 ± 0.55 kg). For the CL group, PPT at 24 post (0.89 ± 0.42 kg) was lower than pre (1.17 ± 0.51 kg, $p = 0.001$, $g = 0.599$) and post (1.04 ± 0.42 kg, $p = 0.007$, $g = 0.357$). In addition, there was significant two-way interaction for bout × group ($F = 5.059$, $p = 0.037$, $\eta^2 = 0.219$); however, there were no significant differences between bouts ($p = 0.134 – 0.143$, $g = 0.195 – 0.381$) and groups ($p = 0.146 – 0.798$, $g = 0.133 – 0.683$), when collapsed across time and location with Bonferroni corrections applied. Furthermore, there was significant two-way interaction for time × bout ($F = 4.593$, $p = 0.016$, $\eta^2 = 0.203$). The follow-up one-way ANOVA for bout was significant for the initial bout ($p < 0.001$, $\eta^2 = 0.448$), but not the second bout ($p = 0.079$, $\eta^2 = 0.128$). For the initial bout, PPT at pre (1.29 ± 0.42 kg) was greater than post (1.08 ± 0.35 kg, $p = 0.001$, $g = 0.543$), 24 post (0.99 ± 0.36 kg, $p < 0.001$, $g = 0.767$), and 48 post (1.02 ± 0.39 kg, $p = 0.003$, $g = 0.666$) (Fig. 7).

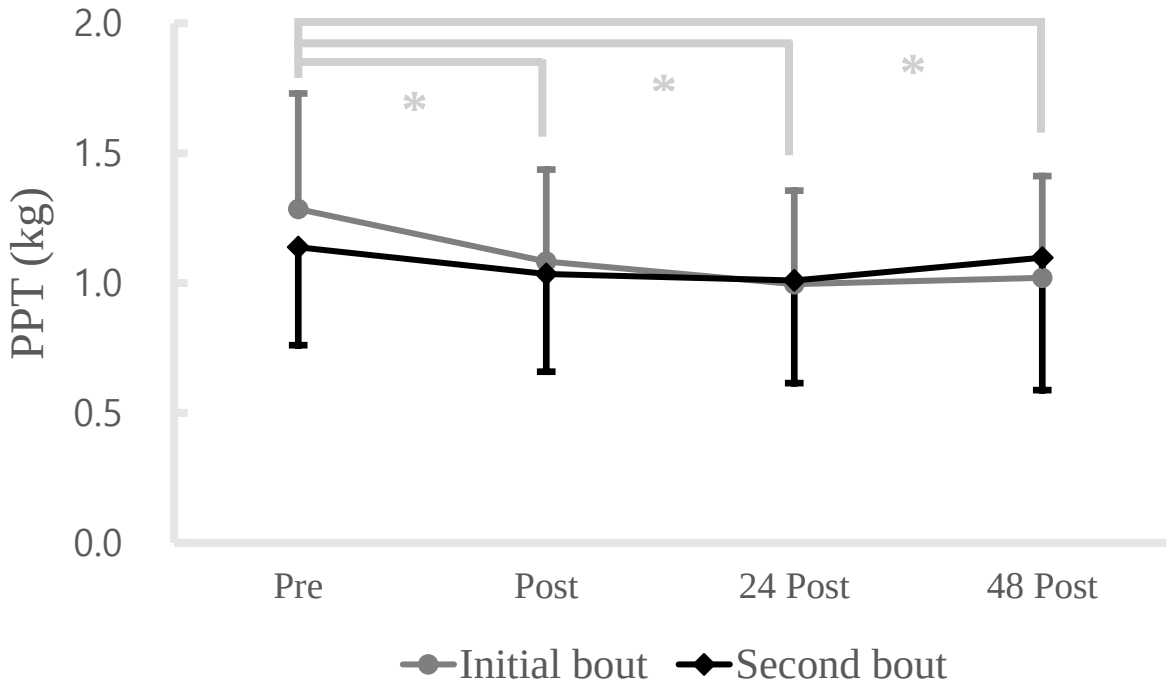

Figure 7. Plotted means for pressure pain threshold (PPT) for the initial and second bouts at before (pre), immediately after (post), 1 day post (24 post), and 2 days post (48 post) when collapsed across group and location. Horizontal bars represent the standard deviation at each time point for the respective bouts. * indicates higher PPT at pre than post ($p = 0.001$), 24 post ($p < 0.001$), and 48 post ($p = 0.003$) during the initial bout.

4.2. Mechanomyographic Amplitude (MMG_{RMS})

4.2.1. Linearly Increasing and Decreasing MMG_{RMS}-Force Relationships

For the *a* terms, the analyses indicated no significant five-way interaction (time × bout × group × contraction × segment, $F = 0.847$, $p = 0.474$, $\eta^2 = 0.045$), four-way interactions (time × segment × contraction × group, $F = 2.000$, $p = 0.896$, $\eta^2 = 0.011$; time × segment × bout × group, $F = 0.914$, $p = 0.441$, $\eta^2 = 0.048$; time × contraction × bout × group, $F = 0.462$, $p = 0.710$, $\eta^2 = 0.025$; segment × contraction × bout × group, $F = 0.556$, $p = 0.465$, $\eta^2 = 0.030$; time × segment × contraction × bout, $F = 0.214$, $p = 0.886$, $\eta^2 =$

0.012), three-way interactions (time × segment × group, $F = 0.303$, $p = 0.823$, $\eta^2 = 0.017$; time × contraction × group, $F = 0.863$, $p = 0.466$, $\eta^2 = 0.046$; segment × contraction × group, $F = 1.482$, $p = 0.239$, $\eta^2 = 0.076$; time × segment × contraction, $F = 0.611$, $p = 0.611$, $\eta^2 = 0.033$; time × bout × group, $F = 0.426$, $p = 0.735$, $\eta^2 = 0.023$; segment × bout × group, $F = 0.285$, $p = 0.600$, $\eta^2 = 0.016$; time × segment × bout, $F = 0.129$, $p = 0.943$, $\eta^2 = 0.007$; contraction × bout × group, $F = 1.739$, $p = 0.204$, $\eta^2 = 0.088$; time × contraction × bout, $F = 1.586$, $p = 0.203$, $\eta^2 = 0.081$; segment × contraction × bout, $F = 2.379$, $p = 0.140$, $\eta^2 = 0.117$), two-way interactions (time × group, $F = 0.967$, $p = 0.415$, $\eta^2 = 0.051$; segment × group, $F = 1.025$, $p = 0.325$, $\eta^2 = 0.054$; contraction × group, $F = 3.229$, $p = 0.089$, $\eta^2 = 0.152$; bout × group, $F = 3.318$, $p = 0.085$, $\eta^2 = 0.156$; time × segment, $F = 0.775$, $p = 0.513$, $\eta^2 = 0.041$; time × contraction, $F = 0.345$, $p = 0.793$, $\eta^2 = 0.019$; segment × contraction, $F = 0.265$, $p = 0.613$, $\eta^2 = 0.014$; time × bout, $F = 1.528$, $p = 0.218$, $\eta^2 = 0.078$; segment × bout, $F = 1.206$, $p = 0.287$, $\eta^2 = 0.063$; contraction × bout, $F = 0.518$, $p = 0.481$, $\eta^2 = 0.028$), or main effects for time ($F = 0.842$, $p = 0.477$, $\eta^2 = 0.045$), segment ($F = 0.094$, $p = 0.763$, $\eta^2 = 0.005$), bout ($F = 1.181$, $p = 0.291$, $\eta^2 = 0.062$), or group ($F = 3.996$, $p = 0.061$, $\eta^2 = 0.182$). However, there was a significant main effect for contraction ($F = 17.220$, $p = 0.001$, $\eta^2 = 0.489$). The *a* terms were lower for the 70% MVIC (0.015 ± 0.013) compared to the 40% MVIC (0.069 ± 0.067, $p = 0.001$, $g = 1.119$) (Table 1).

Table 1. Mean (SD) values for the *a* terms calculated from the log-transformed mechanomyographic amplitude (MMG_{RMS})-force relationship during linearly increasing and decreasing segment of the 40% and 70% maximal voluntary isometric contractions for the ipsilateral (IL) and contralateral (CL) groups before (pre), immediately after (post), 1 day (24 post), and 2 days after (48 post) two bouts of neuromuscular electrical stimulation.

Group	Intensity	Bout	Pre		Post		24 Post		48 Post	
			Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease

IL	40%	1st	0.033 (0.034)	0.063 (0.091)	0.102 (0.191)	0.086 (0.125)	0.072 (0.120)	0.091 (0.110)	0.060 (0.062)	0.072 (0.070)
		2nd	0.050 (0.063)	0.153 (0.312)	0.048 (0.052)	0.158 (0.214)	0.098 (0.110)	0.068 (0.096)	0.115 (0.197)	0.313 (0.850)
	70%	1st	0.015 (0.026)	0.008 (0.009)	0.015 (0.013)	0.026 (0.053)	0.020 (0.022)	0.019 (0.024)	0.016 (0.020)	0.025 (0.034)
		2nd	0.010 (0.007)	0.018 (0.022)	0.035 (0.032)	0.026 (0.017)	0.040 (0.072)	0.012 (0.012)	0.039 (0.067)	0.025 (0.034)
CL	40%	1st	0.028 (0.042)	0.018 (0.017)	0.071 (0.071)	0.035 (0.029)	0.132 (0.319)	0.040 (0.066)	0.033 (0.058)	0.014 (0.012)
		2nd	0.027 (0.032)	0.035 (0.057)	0.053 (0.087)	0.030 (0.035)	0.023 (0.019)	0.022 (0.018)	0.046 (0.051)	0.022 (0.029)
	70%	1st	0.004 (0.004)	0.007 (0.008)	0.014 (0.011)	0.019 (0.022)	0.009 (0.009)	0.006 (0.007)	0.009 (0.010)	0.007 (0.005)
		2nd	0.007 (0.009)	0.004 (0.004)	0.019 (0.030)	0.007 (0.004)	0.006 (0.004)	0.007 (0.007)	0.010 (0.010)	0.007 (0.007)

Main effect for contraction ($p = 0.001$).

For the *b* terms, the analyses indicated no significant five-way interaction (time × bout × group × contraction × segment, $F = 0.461$, $p = 0.711$, $\eta^2 = 0.025$), four-way interactions (time × bout × segment × group, $F = 1.966$, $p = 0.130$, $\eta^2 = 0.098$; time × bout × contraction × group, $F = 0.442$, $p = 0.724$, $\eta^2 = 0.024$; time × segment × contraction × group, $F = 0.298$, $p = 0.827$, $\eta^2 = 0.016$; bout × segment × contraction × group, $F = 0.018$, $p = 0.896$, $\eta^2 = 0.001$; time × bout × segment × contraction, $F = 0.380$, $p = 0.768$, $\eta^2 = 0.021$), three-way interactions (time × bout × group, $F = 1.885$, $p = 0.143$, $\eta^2 = 0.095$; time × segment × group, $F = 0.412$, $p = 0.745$, $\eta^2 = 0.022$; bout × segment × group, $F = 0.080$, $p = 0.780$, $\eta^2 = 0.004$; time × bout × segment, $F = 0.522$, $p = 0.668$, $\eta^2 = 0.028$; time × contraction × group, $F = 0.482$, $p = 0.696$, $\eta^2 = 0.026$; bout × contraction × group, $F < 0.001$, $p = 0.993$, $\eta^2 < 0.001$; time × bout × contraction, $F = 0.412$, $p = 0.745$, $\eta^2 = 0.022$; segment × contraction × group, $F = 2.502$, $p = 0.131$, $\eta^2 = 0.122$; time × segment × contraction, $F = 0.188$, $p = 0.904$, $\eta^2 = 0.010$; bout × segment × contraction, $F = 0.515$, $p = 0.482$, $\eta^2 = 0.028$), two-way interactions (time × group, $F = 0.238$, $p = 0.870$, $\eta^2 = 0.013$; bout × group, $F = 3.605$, $p = 0.074$, $\eta^2 = 0.167$; segment × group, $F = 0.993$, p

= 0.332, η^2 = 0.052; contraction × group, F < 0.001, p = 0.994, η^2 < 0.001; time × bout, F = 0.702, p = 0.555, η^2 = 0.038; time × segment, F = 0.743, p = 0.531, η^2 = 0.040; bout × segment, F = 0.026, p = 0.874, η^2 = 0.001; time × contraction, F = 1.524, p = 0.219, η^2 = 0.078; bout × contraction, F = 0.591, p = 0.452, η^2 = 0.032; segment × contraction, F = 0.728, p = 0.405, η^2 = 0.039), or main effects for bout (F = 0.004, p = 0.949, η^2 < 0.001) or segment (F = 0.246, p = 0.626, η^2 = 0.013). However, there were significant main effects for time (F = 5.191, p = 0.003, η^2 = 0.224), contraction (F = 64.605, p < 0.001, η^2 = 0.782), and group (F = 4.942, p = 0.039, η^2 = 0.215). The *b* terms were higher at pre (0.59 ± 0.25) than at post (0.46 ± 0.19, p = 0.007, g = 0.586), 24 post (0.50 ± 0.18, p = 0.035, g = 0.413), and 48 post (0.50 ± 0.27, p = 0.032, g = 0.346). In addition, the *b* terms were lower for the 40% MVIC (0.36 ± 0.22) compared to the 70% MVIC (0.67 ± 0.22, p < 0.001, g = 1.409), and the *b* terms were higher for the CL group (0.61 ± 0.29) than the IL group (0.41 ± 0.29, p = 0.039, g = 0.690) (Table 2).

Table 2. Mean (SD) values for the *b* terms calculated from the log-transformed mechanomyographic amplitude (MMG_{RMS})-force relationship during linearly increasing and decreasing segment of the 40% and 70% maximal voluntary isometric contractions for the ipsilateral (IL) and contralateral (CL) groups before (pre), immediately after (post), 1 day (24 post), and 2 days after (48 post) two bouts of neuromuscular electrical stimulation.

Group	Intensity	Bout	Pre		Post		24 Post		48 Post	
			Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease
IL	40%	1st	0.391 (0.276)	0.367 (0.445)	0.328 (0.499)	0.214 (0.271)	0.333 (0.342)	0.180 (0.354)	0.262 (0.378)	0.188 (0.386)
		2nd	0.358 (0.408)	0.192 (0.513)	0.353 (0.343)	0.097 (0.329)	0.182 (0.389)	0.193 (0.276)	0.246 (0.460)	0.170 (0.417)
	70%	1st	0.665 (0.261)	0.757 (0.250)	0.569 (0.207)	0.583 (0.238)	0.566 (0.269)	0.612 (0.275)	0.572 (0.216)	0.578 (0.354)
		2nd	0.637 (0.232)	0.560 (0.233)	0.370 (0.274)	0.443 (0.201)	0.443 (0.309)	0.640 (0.212)	0.451 (0.316)	0.573 (0.275)
CL	40%	1st	0.458 (0.370)	0.539 (0.307)	0.209 (0.348)	0.377 (0.323)	0.257 (0.384)	0.411 (0.358)	0.483 (0.390)	0.543 (0.277)
		2nd	0.547 (0.548)	0.575 (0.594)	0.479 (0.473)	0.578 (0.559)	0.461 (0.297)	0.473 (0.421)	0.375 (0.513)	0.538 (0.518)
	70%	1st	0.866 (0.245)	0.797 (0.276)	0.615 (0.305)	0.620 (0.229)	0.713 (0.239)	0.848 (0.270)	0.684 (0.232)	0.782 (0.279)
		2nd	0.841 (0.404)	0.874 (0.349)	0.726 (0.429)	0.790 (0.275)	0.798 (0.281)	0.802 (0.384)	0.712 (0.376)	0.810 (0.412)

Main effect for time (p = 0.003), contraction (p < 0.001), and group (p = 0.039).

4.2.2. N-MMG_{RMS} at Steady Force

For the MMG_{RMS}, the analysis indicated no significant four-way interaction (time × bout × group × contraction, $F = 0.695$, $p = 0.559$, $\eta^2 = 0.037$), three-way interactions (time × bout × group, $F = 0.380$, $p = 0.768$, $\eta^2 = 0.021$; time × contraction × group, $F = 2.121$, $p = 0.108$, $\eta^2 = 0.105$; bout × contraction × group, $F = 0.712$, $p = 0.410$, $\eta^2 = 0.038$; time × bout × contraction, $F = 2.093$, $p = 0.112$, $\eta^2 = 0.104$), two-way interactions (time × group, $F = 1.714$, $p = 0.175$, $\eta^2 = 0.087$; bout × group, $F = 0.156$, $p = 0.697$, $\eta^2 = 0.009$; contraction × group, $F = 2.619$, $p = 0.123$, $\eta^2 = 0.127$; time × bout, $F = 1.054$, $p = 0.376$, $\eta^2 = 0.055$; time × contraction, $F = 0.863$, $p = 0.466$, $\eta^2 = 0.046$; bout × contraction, $F = 0.063$, $p = 0.805$, $\eta^2 = 0.003$), or main effects for time ($F = 0.188$, $p = 0.904$, $\eta^2 = 0.010$), bout ($F = 0.010$, $p = 0.921$, $\eta^2 = 0.001$), group ($F = 0.056$, $p = 0.816$, $\eta^2 = 0.003$). However, there was a significant main effect for contraction ($F = 75.624$, $p < 0.001$, $\eta^2 = 0.808$). N-MMG_{RMS} was higher at 70% MVIC (96.79 ± 39.93 %) than at 40% MVIC (52.67 ± 27.70 %, $p < 0.001$, $g = 1.284$) (Table 3).

Table 3. Mean (SD) normalized mechanomyographic amplitude (N-MMG_{RMS}) during steady force at 40% and 70% maximal voluntary isometric contraction for the ipsilateral (IL) and contralateral (CL) groups before (pre), immediately after (post), 1 day (24 post), and 2 days (48 post) after two bouts of neuromuscular electrical stimulation.

			N-MMG_{RMS} (%)			
Group	Intensity	Bout	Pre	Post	24 Post	48 Post
IL	40%	1st	58.19 (43.63)	49.89 (29.87)	40.10 (18.37)	51.20 (20.82)
		2nd	56.79 (56.50)	49.76 (38.81)	45.52 (26.08)	50.76 (27.58)
	70%	1st	99.48 (45.75)	115.66 (77.52)	90.67 (42.40)	111.75 (38.86)
		2nd	108.44 (75.56)	97.22 (65.27)	92.95 (44.19)	104.68 (54.49)
CL	40%	1st	51.76 (28.62)	60.28 (40.74)	54.07 (31.45)	51.99 (55.04)
		2nd	55.99 (37.77)	58.17 (43.73)	55.58 (30.14)	52.67 (29.33)
	70%	1st	79.52 (32.59)	104.10 (45.53)	103.63 (42.99)	69.68 (29.61)
		2nd	93.12 (54.54)	80.88 (34.21)	102.67 (45.47)	94.17 (47.58)

Main effect for contraction ($p < 0.001$)

4.3. Motor Unit (MU) Data

For the examination of MU properties, a total of 5 (n = 2 for IL group, n = 3 for CL group) participants were excluded from the analyses due to a lack of similarly recorded recruitment threshold ranges among contractions. Such strict recruitment threshold criterion ensures that different segments of the MU pool are not being compared to each other across days by better guarding against calculating relationships on MUs with intrinsically different firing rates, such as, lower-threshold MUs that achieve higher firing rates in comparison to higher-threshold MUs [72-74]. Therefore, a total of fifteen participants (n = 8 for the IL group, n = 7 for the CL group) were included for the MU analysis.

4.3.1. MU Mean Firing Rate (MFR) vs. Recruitment Threshold (RT) Relationship

For the slopes, the analysis indicated no significant four-way interaction (time × bout × group × contraction, $F = 1.409$, $p = 0.255$, $\eta^2 = 0.098$), three-way interactions (time × bout × group, $F = 0.739$, $p = 0.537$, $\eta^2 = 0.054$; time × contraction × group, $F = 0.030$, $p = 0.993$, $\eta^2 = 0.002$; bout × contraction × group, $F = 0.002$, $p = 0.967$, $\eta^2 < 0.001$; time × bout × contraction, $F = 1.409$, $p = 0.255$, $\eta^2 = 0.014$), two-way interactions (time × group, $F = 0.311$, $p = 0.817$, $\eta^2 = 0.023$; bout × group, $F = 3.717$, $p = 0.076$, $\eta^2 = 0.222$; contraction × group, $F = 0.057$, $p = 0.816$, $\eta^2 = 0.004$; time × bout, $F = 1.667$, $p = 0.190$, $\eta^2 = 0.114$; time × contraction, $F = 1.190$, $p = 0.326$, $\eta^2 = 0.084$; bout × contraction, $F = 0.002$, $p = 0.965$, $\eta^2 < 0.001$), or main effects for bout ($F = 0.631$, $p = 0.441$, $\eta^2 = 0.046$), contraction ($F = 0.428$, $p = 0.525$, $\eta^2 = 0.032$), or group ($F = 0.950$, $p = 0.348$, $\eta^2 =$

0.068). There was a significant main effect for time (F = 3.977, p = 0.014, η^2 = 0.234); however, there were no significant differences among pre, post, 24 post, and 48 post when collapsed across bout, group, and contraction with Bonferroni corrections applied (p = 0.058 to > 0.999, g = 0.040 to 0.574) (Table 4).

For the y-intercepts, the analysis indicated no significant four-way interaction (time × bout × group × contraction, F = 1.380, p = 0.263, η^2 = 0.096), three-way interactions (time × bout × group, F = 0.940, p = 0.431, η^2 = 0.067; time × contraction × group, F = 0.354, p = 0.787, η^2 = 0.026; bout × contraction × group, F = 2.987, p = 0.108, η^2 = 0.187; time × bout × contraction, F = 0.259, p = 0.854, η^2 = 0.020), or two-way interaction (time × group, F = 0.587, p = 0.627, η^2 = 0.043; bout × group, F = 4.095, p = 0.064, η^2 = 0.240; contraction × group, F = 0.379, p = 0.549, η^2 = 0.028; time × bout, F = 1.030, p = 0.390, η^2 = 0.073; bout × contraction, F = 0.130, p = 0.724, η^2 = 0.010). However, there was two-way interaction (time × contraction, F = 4.084, p = 0.013, η^2 = 0.239). The follow-up one-way ANOVA was significant for the 70% MVIC (p = 0.001, η^2 = 0.314), but not the 40% MVIC (p = 0.064, η^2 = 0.157). For the 70% MVIC, the y-intercepts at post (34.13 ± 6.67 pps) were lower than at pre (41.50 ± 8.22 pps, p = 0.015, g = 0.985), 24 post (41.02 ± 8.85 pps, p = 0.050, g = 0.879), 48 post (40.82 ± 7.79 pps, p = 0.012, g = 0.923). In addition, follow-up paired sample t-tests indicated the y-intercepts for the 40% MVIC at pre (29.42 ± 7.63 pps), post (29.30 ± 8.37 pps), 24 post (33.16 ± 11.40 pps), and 48 post (31.54 ± 9.76 pps) were less than the y-intercepts for the 70% MVIC at pre (41.50 ± 8.22 pps, p < 0.001, g = 1.523), post (34.13 ± 6.67 pps, p = 0.008, g = 0.638), 24 post (41.02 ± 8.86 pps, p = 0.016, g = 0.770), and 48 post (40.82 ± 7.79 pps, p = 0.004, g = 1.051), respectively (Table 4).

4.3.2. MU Action Potential Amplitude ($MUAP_{AMP}$) vs. RT Relationships

For the slopes, the analysis indicated no significant four-way interaction (time × bout × group × contraction, $F = 0.953$, $p = 0.425$, $\eta^2 = 0.068$), three-way interactions (time × bout × group, $F = 1.094$, $p = 0.363$, $\eta^2 = 0.078$; time × contraction × group, $F = 0.367$, $p = 0.777$, $\eta^2 = 0.027$; bout × contraction × group, $F = 1.894$, $p = 0.192$, $\eta^2 = 0.127$; time × bout × contraction, $F = 0.045$, $p = 0.987$, $\eta^2 = 0.003$), two-way interactions (time × group, $F = 0.754$, $p = 0.526$, $\eta^2 = 0.055$; bout × group, $F = 2.386$, $p = 0.146$, $\eta^2 = 0.155$; contraction × group, $F = 0.243$, $p = 0.631$, $\eta^2 = 0.018$; time × bout, $F = 1.354$, $p = 0.271$, $\eta^2 = 0.094$; time × contraction, $F = 1.440$, $p = 0.246$, $\eta^2 > 0.999$; bout × contraction, $F = 0.890$, $p = 0.363$, $\eta^2 = 0.064$), or main effects for time ($F = 3.431$, $p = 0.053$, $\eta^2 = 0.209$), bout ($F = 1.498$, $p = 0.243$, $\eta^2 = 0.103$), or group ($F = 0.312$, $p = 0.586$, $\eta^2 = 0.023$). However, there was a significant main effect for contraction ($F = 7.802$, $p = 0.015$, $\eta^2 = 0.375$). The slope coefficients were greater for 70% MVIC (0.014 ± 0.002 mV/%MVIC) compared to the 40% MVIC (0.007 ± 0.001 mV/%MVIC, $p = 0.015$, $g = 4.427$) when collapsed across time, bout, and group (Table 4).

For the y-intercept, the analysis indicated no significant four-way interaction (time × bout × group × contraction, $F = 0.972$, $p = 0.416$, $\eta^2 = 0.070$), three-way interactions (time × bout × group, $F = 0.537$, $p = 0.660$, $\eta^2 = 0.040$; time × contraction × group, $F = 0.363$, $p = 0.780$, $\eta^2 = 0.027$; bout × contraction × group, $F = 1.836$, $p = 0.198$, $\eta^2 = 0.124$; time × bout × contraction, $F = 0.087$, $p = 0.967$, $\eta^2 = 0.007$), two-way interactions (time × group, $F = 0.401$, $p = 0.753$, $\eta^2 = 0.030$; bout × group, $F = 1.740$, $p =$

0.210, $\eta^2 = 0.118$; contraction × group, $F = 0.134$, $p = 0.720$, $\eta^2 = 0.010$; time × bout, $F = 0.797$, $p = 0.503$, $\eta^2 = 0.058$; time × contraction, $F = 2.247$, $p = 0.098$, $\eta^2 = 0.147$; bout × contraction, $F = 1.274$, $p = 0.279$, $\eta^2 = 0.089$), or main effects for time ($F = 2.875$, $p = 0.084$, $\eta^2 = 0.181$), bout ($F = 0.935$, $p = 0.351$, $\eta^2 = 0.067$), or group ($F = 0.346$, $p = 0.567$, $\eta^2 = 0.026$). However, there was a significant main effect for contraction ($F = 8.881$, $p = 0.011$, $\eta^2 = 0.406$). The y-intercepts were greater for 40% MVIC (-0.044 ± 0.132 mV) compared to the 70% MVIC (-0.314 ± 0.418 mV, $p = 0.011$, $g = 0.871$) when collapsed across time, bout, and group (Table 4).

Table 4. Mean (SD) for the slopes and y-intercepts of the motor unit (MU) mean firing rate (MFR) (pulses per second [pps]) and motor unit action potential amplitude ($MUAP_{AMP}$) (mV) vs. recruitment threshold (RT) (Maximal voluntary isometric contraction [%MVIC]) relationships for the ipsilateral (IL) and contralateral (CL) groups during the 40% and 70% MVICs before (pre), immediately after (post), 1 day (24 post), and 2 days (48 post) after two bouts of neuromuscular electrical stimulation.

	Group	Initial bout				Second bout			
		Pre	Post	24 Post	48 Post	Pre	Post	24 Post	48 Post
40% MVIC MFR vs. RT Slopes (pps/%MVIC)	IL	-0.479 (0.192)	-0.490 (0.267)	-0.584 (0.331)	-0.538 (0.366)	-0.566 (0.293)	-0.479 (0.357)	-0.588 (0.329)	-0.606 (0.349)
	CL	-0.428 (0.234)	-0.462 (0.177)	-0.575 (0.446)	-0.643 (0.386)	-0.420 (0.196)	-0.453 (0.191)	-0.484 (0.259)	-0.410 (0.133)
40% MVIC MFR vs. RT y-intercepts (pps)	IL	28.29 (8.10)	29.56 (9.12)	34.19 (12.78)	32.21 (12.44)	31.47 (9.93)	29.37 (12.03)	34.68 (12.78)	33.48 (13.54)
	CL	29.34 (7.69)	29.36 (8.11)	33.53 (18.18)	33.18 (9.98)	28.33 (6.70)	28.87 (7.26)	29.89 (8.97)	26.93 (3.87)
70% MVIC MFR vs. RT Slopes (pps/%MVIC)	IL	-0.470 (0.155)	-0.417 (0.106)	-0.557 (0.212)	-0.561 (0.111)	-0.647 (0.196)	-0.437 (0.120)	-0.534 (0.181)	-0.523 (0.161)
	CL	-0.490 (0.089)	-0.378 (0.122)	-0.544 (0.227)	-0.497 (0.134)	-0.414 (0.073)	-0.361 (0.107)	-0.382 (0.087)	-0.411 (0.226)
70% MVIC MFR vs. RT y-intercepts (pps)	IL	39.61 (7.23)	33.66 (5.56)	43.17 (8.26)	42.96 (8.36)	48.94 (12.59)	35.54 (8.36)	43.54 (13.50)	44.07 (11.32)
	CL	42.08 (9,61)	34.85 (10.76)	42.47 (15.15)	40.43 (8.98)	34.56 (3.86)	32.34 (4.61)	34.23 (4.55)	35.06 (8.81)
40% MVIC $MUAP_{AMP}$ vs. RT Slope (mV/%MVIC)	IL	0.0080 (0.0092)	0.0049 (0.0049)	0.0091 (0.0123)	0.0115 (0.0186)	0.0098 (0.0137)	0.0068 (0.0099)	0.0085 (0.0112)	0.0088 (0.0104)
	CL	0.0041 (0.0037)	0.0041 (0.0018)	0.0087 (0.0099)	0.0097 (0.0081)	0.0051 (0.0070)	0.0033 (0.0016)	0.0076 (0.0101)	0.0053 (0.0066)
40% MVIC $MUAP_{AMP}$ vs. RT y-intercepts (mV)	IL	-0.0356 (0.1364)	0.0154 (0.0345)	-0.0915 (0.1921)	-0.1393 (0.3519)	-0.0740 (0.1938)	-0.0523 (0.2109)	-0.0963 (0.2478)	-0.0595 (0.1786)
	CL	0.0233 (0.0417)	0.0184 (0.0811)	-0.0954 (0.1856)	-0.0770 (0.1260)	-0.0154 (0.0863)	0.0479 (0.1026)	-0.0510 (0.1217)	-0.0291 (0.0864)
70% MVIC	IL	0.0201 (0.0326)	0.0118 (0.0147)	0.0155 (0.0181)	0.0156 (0.0145)	0.0226 (0.0265)	0.0080 (0.0077)	0.0168 (0.0204)	0.0179 (0.0212)

$MUAP_{AMP}$ vs. RT Slope (mV/%MVIC)	CL	0.0139 (0.0119)	0.0080 (0.0062)	0.0129 (0.0193)	0.0187 (0.0283)	0.0110 (0.0191)	0.0094 (0.0161)	0.0104 (0.0134)	0.0064 (0.0063)
70% MVIC $MUAP_{AMP}$ vs. RT y-intercepts (mV)	IL	-0.5391 (1.1671)	-0.2275 (0.3633)	-0.3208 (0.4038)	-0.3468 (0.3002)	-0.6820 (0.9260)	-0.0864 (0.1418)	-0.3550 (0.5816)	-0.3926 (0.4913)
	CL	-0.4056 (0.3932)	-0.1337 (0.2161)	-0.3071 (0.6191)	-0.5190 (0.9279)	-0.2854 (0.6722)	-0.1511 (0.2873)	-0.1761 (0.1921)	-0.0896 (0.1269)

Main effect for time ($p = 0.014$) for slopes of MU MFR vs. RT relationship.
Two-way interaction (time × contraction, $p = 0.013$) for y-intercepts of MU MFR vs. RT relationship.
Main effect for contraction ($p = 0.015$) for slopes of $MUAP_{AMP}$ vs. RT relationship.
Main effect for contraction (p = 0.011) for y-intercept of $MUAP_{AMP}$ vs. RT relationship

4.3.3. N-EMG_{RMS} at Steady Force

For N-EMG_{RMS} at steady force during 40% and 70% MVIC, the analysis indicated no significant four-way interaction (time × bout × group × contraction, $F = 0.379$, $p = 0.768$, $\eta^2 = 0.022$), three-way interactions (time × bout × group, $F = 0.428$, $p = 0.734$, $\eta^2 = 0.025$; time × contraction × group, $F = 1.441$, $p = 0.242$, $\eta^2 = 0.078$; bout × contraction × group, $F = 0.002$, $p = 0.963$, $\eta^2 < 0.001$; time × bout × contraction, $F = 1.121$, $p = 0.349$, $\eta^2 = 0.062$), two-way interactions (time × group, $F = 0.296$, $p = 0.828$, $\eta^2 = 0.017$; bout × group, $F = 0.186$, $p = 0.672$, $\eta^2 = 0.011$; contraction × group, $F = 2.531$, $p = 0.130$, $\eta^2 = 0.130$; time × bout, $F = 1.344$, $p = 0.271$, $\eta^2 = 0.073$; time × contraction, $F = 0.133$, $p = 0.940$, $\eta^2 = 0.008$; bout × contraction, $F = 1.554$, $p = 0.229$, $\eta^2 = 0.084$), or main effect for time ($F = 2.587$, $p = 0.063$, $\eta^2 = 0.132$) or bout ($F = 0.007$, $p = 0.934$, $\eta^2 < 0.001$). However, there were significant main effects for contraction ($F = 575.048$, $p < 0.001$, $\eta^2 = 0.971$) and group ($F = 4.773$, $p = 0.043$, $\eta^2 = 0.219$). N-EMG_{RMS} was higher at 70% MVIC (82.68 ± 14.65 %) than at 40% MVIC (36.40 ± 7.20 %, $p < 0.001$, $g = 4.010$) when collapsed acrossed time, bout, and group. In addition, N-EMG_{RMS} was lower for the IL group (54.15 ± 15.60 %) than for the CL group (64.93 ± 14.79 %, $p = 0.043$, $g = 0.709$) when collapsed across time, bout, and contraction (Table 5).

Table 5. Mean (SD) values for normalized electromyographic amplitudes (N-EMG_{RMS}) for the ipsilateral (IL) and contralateral (CL) groups during the 40% and 70% maximal voluntary isometric contraction before (pre), immediately after (post), 1 day (24 post), and 2 day (48 post) after two bouts of neuromuscular electrical stimulation.

Group	Intensity	Bout	N-EMG_{RMS} (%)			
			Pre	Post	24 Post	48 Post
IL	40%	1st	28.70 (10.05)	35.54 (18.15)	29.37 (8.07)	34.84 (8.35)
		2nd	30.19 (6.41)	41.85 (19.19)	28.11 (8.39)	31.76 (7.99)
	70%	1st	81.31 (20.76)	74.57 (37.81)	75.66 (19.69)	79.57 (24.43)
		2nd	68.61 (18.39)	79.85 (24.49)	68.91 (21.94)	77.60 (32.61
CL	40%	1st	38.70 (9.78)	42.33 (13.72)	39.94 (19.75)	35.35 (14.74)
		2nd	38.13 (7.41)	48.82 (10.30)	37.17 (9.16)	43.55 (19.46)
	70%	1st	86.88 (20.46)	94.79 (26.83)	94.22 (34.30)	84.94 (31.12)
		2nd	82.52 (15.03)	101.85 (30.19)	78.55 (14.63)	93.09 (32.04)

Main effect for contraction ($p < 0.001$) and group ($p = 0.043$)

CHAPTER V

DISCUSSION

The aims of this study were to examine whether neuromuscular electrical stimulation (NMES) performed on the elbow flexors could induce a protective repeated bout effect (RBE) on the administered muscles (ipsilateral) and related homologous muscles (contralateral) in untrained men and women. In addition, the mechanical activity of muscle and motor unit behavior were examined via mechanomyography (MMG) and electromyography (EMG) signal decomposition to explore the potential mechanisms of RBE induced by NMES. The main findings of this study were: (1) NMES caused muscle damage, as evidenced by the changes in indirect muscle damage markers (i.e., maximal strength, muscle soreness, pain pressure threshold [PPT], range of motion [ROM], and muscle thickness), (2) muscle soreness assessed on the visual analog scale (VAS) was significantly different between the first and second bouts for the ipsilateral (IL) (when collapsed across time), but not the contralateral (CL) group, and (3) the trend in the mechanical activity and motor unit (MU) behavior for the biceps brachii (BB) was not significantly altered between bouts and groups. Therefore, although NMES induced muscle damage, neither the IL- nor CL-RBE was elicited on the BB muscle via NMES.

It is well documented that repeated high intensity voluntary or involuntary muscle contractions causes exercise-induced muscle damage (EIMD), including changes in the indirect muscle damage markers [41,75]. For example, the unaccustomed eccentric

exercise can result in strength loss, limited ROM, increased muscle soreness and pain, and increased inflammatory responses [48] on upper and lower body muscle groups [10], and the negative changes in indirect muscle damage markers may last at least 7 days post-exercise [2]. In addition to dynamic exercise-induced muscle damage, involuntary contractions induced by NMES result in impairment in skeletal muscle [42]. The findings from the current study are in line with previous examinations using NMES that reported strength loss, increased muscle pain and muscle thickness, and limited ROM in the BB muscle after NMES, regardless of bout and muscle groups (ipsilateral or contralateral).

The present study reported a 15% reduction in MVIC after NMES; however, the magnitude of strength reduction was relatively lower than previous NMES studies that reported a ~30% reduction in strength after a single bout of NMES to leg muscles [76,77]. This may be due to the difference of stimulus delivery method and intensity. Previous studies used NMES utilized a frequency of 75 to 100 Hz, pulse-width 400 to 450 μs, and stimulation current amplitude 75 mV, which resulted in an evoked isometric force during NMES that was 25 % to 40 % of pre-MVIC [42,58,76-78]. Due to equipment limitations, our frequency (100 Hz) and pulse-width (200 μs) were less that the aforementioned studies, and may be responsible for our smaller evoked force (7.06 ± 3.84 %) relative to pre-MVIC with NMES. Thus, our lower intensity of NMES caused less muscle damage compared to other NMES studies. Our findings may suggest that a higher intensity of NMES is necessary with effective intensity producing evoked contraction force ranging from 40 % to 60 % of pre-MVIC (Maffiuletti 2010).

NMES-induced muscle damage also affects morphological, and neuromuscular alterations [39,79]. Histological assessment and blood analysis has showed macrophage

infiltration, z-lines disruption, and modified desmin-negative staining and increased creatine kinase activity after a single bout of NMES [39]. In addition, decreases in M-wave amplitude and duration [80] and peak evoked force during double stimulations at 10 Hz (Db_{10}) and 100 Hz (Db_{100}) (10:100 ratio) [77] have been reported after NMES. These results suggested that NMES negatively affect excitation-contraction coupling and morphological structures at the myofiber and sarcomere levels, and is also associated with peripheral alterations. Furthermore, Laurin et al. [76] reported a decrease in volitional (V) wave following NMES, suggesting changes in α-motoneurons excitability and/or alteration in presynaptic inhibition of Ia afferents. Foure et al. [77] reported that voluntary activation significantly decreased 2 days after NMES, but there were no changes immediately after- and 1 day after-NMES. Thus, neural alterations may not occur immediately after NMES, but central factors could be involved in muscle damage. Therefore, NMES can induce muscle damage, and it may be accompanied by peripheral (e.g., morphological changes) and/or central (e.g., neural changes) alterations.

In the current study, there were changes in MU firing rate behavior at post when collapsed across bout and group. For example, the y-intercepts of MU mean firing rate (MFR) vs. recruitment threshold (RT) relationship at 70% MVIC significantly decreased after NMES, while there were no changes in the slopes. A decrease in y-intercepts indicates an overall reduction in the firing rate of recruited MUs, whereas no change in the slope indicated the decrease in the mean firing rates with increments in recruitment thresholds at the targeted force was unaffected by NMES [81]. In addition, there were no changes in N-EMG_{RMS} or the slopes and y-intercepts for the motor unit action potential

amplitude ($MUAP_{AMP}$) vs. RT relationships. Therefore, NMES altered the MU firing rate-excitation relationship, but not MU recruitment patterns.

NMES is suggested to nonselectively activate both slow and fast fibers [82], which may result in greater muscle damage, and also induce relatively more fatigue in the less fatigue-resistance type II muscle fibers compared to low- and moderate intensity contractions [83]. Muscle damage has been associated with reduced MU conduction velocity [84], which would lead to a greater overlap of MU action potentials and increase action potential summation [85]. Subsequently, this would allow MU tetanus at lower firing frequencies, and may explain how our participants were able to maintain the same relative force with lower overall MU firing rate-excitation relationship at post. Another possibility for the decreased MU firing rate-excitation relationship at post is the influence of pain. In the current study, NMES significantly increased muscle pain as assessed by PPT. Indeed, Farina et al. [86] reported decreases in MU firing rates were negatively correlated with muscle pain intensity. Although the exact mechanism for the reduction in MU firing rates with increasing muscle pain is not fully understood, it may be due to reflex-mediated inhibition involved in small diameter group III and IV afferent activity [87]. Thus, it is speculated that the reflex-mediated inhibition induced by the muscle pain may negatively affect MU behavior [88]. Therefore, the reduction in overall MU firing rates after NMES likely indicates a greater percentage of fused MUs when producing a relative force, which be result of slowing conduction velocity and/or increased muscle pain and/or reflex-mediated inhibitions.

There were also changes in mechanical behavior of the BB following NMES. For example, the *b* terms from the log-transformed MMG amplitude (MMG_{RMS})-force

relationships significantly decreased at all time points following NMES (when collapsed across bout, contraction, segment, and group). MMG records the low-frequency lateral oscillations of muscle fibers [89], and the amplitude of the signal is influenced by active stiffness of fibers modulated by the number of MU recruited and firing rate of the recruited MU [90]. MMG_{RMS}-force relationships may provide information regarding the distinction between MU firing rates and recruitment as the primary mechanism to modulate force [91]. Although MMG_{RMS} is thought to primarily reflect recruitment, the amplitude can be blunted by MU tetanus. For example, Cooper and Herda [91] reported lower *b* terms for a muscle that primarily relies on firing rates to modulate force at higher targeted intensities (first dorsal interosseous muscle) compared to muscles that relies on MU recruitment (vastus laterails and rectus femoris). In addition, individuals that express greater amount of type I % myosin heavy chain isoform content [70,92,93], or have solely engaged in chronic endurance training [94], have also exhibited lower *b* terms. Therefore, MMG_{RMS}-force relationships appear to be sensitive to differences in motor control strategies.

At post, the MFR vs. RT relationships suggested a decrease in overall firing rates due to MU tetanus, and likely explain the lower *b* terms at the respective time point. However, the *b* terms remained lower at 24 and 48 hours compared to pre after NMES, despite the MFR vs. RT relationships indicating firing rate behavior had returned to baseline values. This may be due increase muscle stiffness and intramuscular fluid pressure. The amplitude of the MMG signal is negatively affected to muscle stiffness and intramuscular fluid pressure [95,96]. Muscle stiffness at high intensities can elevate due to intramuscular tension and decreased muscle compliance, which restricts muscle

oscillations and reduces MMG amplitude [97]. Although the current study did not measure muscle stiffness, Vanderthommen et al. [98] reported NMES increased muscle stiffness for 2 days following treatment. In addition, Sejersted et al. [99] demonstrated the positive relationship between muscle thickness and intramuscular fluid pressure. The current study reported muscle thickness increased at post and 24 post after NMES, which was likely due to edema-induced muscle swelling [100]. Thus, it is speculated that the decrease in *b* terms at post was due to a combination of increased active stiffness and intramuscular fluid pressure, and a greater percentage of the MU pool tetanizing, whereas MU tetanus was no longer responsible for the decrease in *b* terms at 24 and 48 hours post NMES as firing rates had increased back to baseline values while muscle thickness remained elevated.

The IL- and CL-RBE has been broadly investigated [10,13,18,101]. The protective RBE can be elicited by voluntary muscle movements [102] as well as NMES [41]. For example, Vanderthommen et al. [103] revealed that there was an IL-RBE for muscle soreness, as measured by VAS scores, and creatine kinases activity on quadriceps muscles after two bouts of 100 electrical stimuli separated by 5 weeks. These results were partially in agreement with the current study as only VAS was significantly lower after the second bout than after the initial bout in the IL group (when collapsed across time).

The potential mechanisms for RBE include neural, inflammatory, muscle-tendon complex, and extracellular matrix remodeling adaptation [15]. NMES can result in morphological (e.g., disruption of z-line and desmin) and mechanical changes (e.g., tranverse strain and shear stress in intramuscular structures) [39,104] as well as an increased inflammatory response (e.g., cytokines) [105]. These changes in the peripheral factors may

be involved with IL-RBE. Thus, due to no differences in MU behavior between the two bouts, inflammatory, muscle-tendon complex, and/or extracellular matrix remodeling could be associated with IL-RBE for the changes in VAS scores induced by NMES on BB muscle. However, since the present study did not collect any inflammatory responses, blood sample, or muscle biopsies to identify other mechanisms, we cannot confirm whether other mechanisms were involved in the IL-RBE. Thus, the examination of other muscle damage markers related other mechanisms should be explored in future studies. However, VAS scores are a subjective assessment and weak or no correlations have been reported with other muscle damage markers [106]. In addition, other indirect muscle damage markers (i.e., strength, ROM, MT, and PPT) did not indicate any protective RBE in the IL and CL groups for the current study. Therefore, although protective effects on muscle soreness existed in the same muscle group, it is difficult to suggest that NMES generally provides a RBE.

Ultimately, the findings of the current study (i.e., maximal strength, ROM, PPT, and muscle thickness) did not provide evidence for the IL- or CL-RBE on the BB muscle after NMES despite a protective RBE for muscle soreness. These results contrast previous examinations that reported changes in indirect muscle damage markers were attenuated in the second bout of NMES [41,103]. This may be due to insufficient muscle damage by the lower intensity of NMES we applied during the initial bout. For example, Lavender and Nosaka [107] reported a smaller magnitude of IL-RBE in older- compared to younger-adults for the elbow flexors. The authors suggested this may be a result of physiological changes that occur with age, such as loss of type II muscle fibers and a reduction in joint ROM, which may have attenuated muscle damage for the older

individuals during the initial bout. McHugh and Pasiakos [108] also reported a lack of RBE, and suggested is was likely due to the minimal symptoms of muscle damage from the initial bout. Due to equipment limitations, the current study administered relatively lower intensity NMES (stimulation current amplitude: 46.5 ± 15.7 mA, evoked isometric force: 7.06 ± 3.84 % relative of pre-MVIC) compared to the previous studies (stimulation current amplitude: 75 mA, evoked isometric force during NMES: 25 % to 40 % of pre-MVIC) [42,58,76-78]. In addition, our magnitude of strength loss (15 %) was also lower than another study that reported ~26% reduction in strength after initial NMES [41]. Furthermore, the magnitude of CL-RBE is smaller (approximately 50%) than the magnitude of IL-RBE [18]. Therefore, the intensity of the initial bout may not have been enough to utilize the mechanism for each indirect muscle damage maker for inducing the IL- or CL-RBE.

Another possible explanation for the absence of IL- or CL-RBE could be due to different mechanisms for inducing RBE. Xin et al. [24] found there were CL-RBE for strength and nuclear factor-kappa B (NF-kB) activation after two bouts of 100 eccentric contraction, but no CL-RBE for creatine kinase activity and muscle soreness. Similarly, IL-RBE for VAS and creatine kinase activity was revealed after two bouts of NMES, but no RBE existed for strength [78,103]. Thus, it is possible that each indirect muscle damage markers have different mechanisms for RBE. In addition, the current study reported IL-RBE for VAS despite no difference in MU behavior or mechanical activity between bouts. This suggested that MU activity may not be related to the RBE after NMES, but muscle damage markers associated with muscle soreness and inflammatory responses

may have IL-RBE after NMES based on the findings from present study and previous NMES studies [41,42,103].

Although the current study had some interesting findings, it is necessary to mention that there are some limitations. First, this study measured several indirect markers including MU behavior and the mechanical activity of the BB to examine IL- or CL-RBE after NMES, but no inflammatory response markers or muscle biopsies were collected. Since previous studies found the evidence for RBE for inflammatory and structural factors [41,42], examining other muscle damage markers may provide further insight into the mechanisms for inducing RBE with NMES. Second, due to limitations in the capabilities of our equipment, this study used a relatively lower current stimulation intensity than previous studies [42,58,76-78]. Our findings suggest the magnitude of muscle damage during the initial bout could be one of the important factors for inducing RBE [108]. Thus, future studies should utilized higher current stimulation intensity than we did to investigate RBE. Lastly, the current study performed two NMESs one week apart between bouts. Chen et al. [17] revealed that magnitude of protective effect can vary depending on the duration between bouts. Because inflammatory activities can last at least 7 days after NMES [39], one week may not be sufficient to washout muscle damage by NMES. Therefore, different durations between bouts for RBE could be explored in future research.

In conclusion, the current study reported no evidence of the IL- or CL-RBE in the BB muscle for indirect muscle damage markers including strength, ROM, muscle thickness, muscle pain, MU behavior, and mechanical activity of muscle following NMES, despite IL-RBE for VAS. These findings may further emphasize the importance

regarding the magnitude of muscle damage from the initial bout with NMES. Future studies should investigate different factors and/or different mechanisms. However, our results did indicate that NMES can induce muscle damage with even lower current stimulation. In addition, NMES elicited unique MU firing rate-excitation relationships (e.g., overall reduction in the firing rates of recruited MUs) and altered the mechanical behavior (e.g., reduction in the *b* terms) of the BB muscle. However, future work is still needed in regard to RBE to examine the inflammation response and other mechanism with different NMES intensities, durations between bouts, or different muscle groups.

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VITA

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