Specificity of Sparing Effects with Cross-Education after Eccentric Training

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ABSTRACT

Cross-education (CE) is the phenomenon that occurs after unilateral strength training whereby strength of the untrained contralateral limb is enhanced. A handful of studies have shown that CE can spare the loss of strength and size of an opposite immobilized limb, but specificity of these "sparing" effects is unknown. The purpose was to investigate specificity of CE sparing effects with immobilization. Sixteen participants were randomly assigned to a training (M=1, F=7; ht: 170.3±10.1 cm; wt: 77.2±19.2 kg) and control (M=2, F=6; ht: 169.3±8.5 cm; wt: 85.7±22.7 kg) group. Both groups wore a non-dominant forearm cast for four weeks. Two pre- and one post-testing session involved wrist flexors and extensors muscle thickness (ultrasound), eccentric (ECC), concentric (CON) and isometric (ISO) maximal voluntary contractions (dynamometer), electromyography (EMG) normalized to M_{max}, and forearm muscle cross-sectional area (MCSA; peripheral quantitative computed tomography). Strength training was ECC wrist flexion 3 times per week. Group × time interactions for the immobilized and non-immobilized limbs revealed that only the training group showed strength preservation across all contractions in the wrist flexors of the immobilized limb (Training: pre=12.3±5.4 Nm, post=12.0±4.6 Nm vs. Control: pre=14.8±5.4 Nm, post=11.6±4.6 Nm; p=.04, η_p^2=.25), and increased wrist flexors strength of the non-immobilized limb (Training: pre=12.9±5.5 Nm, post=16.9±7.3 Nm vs Control: pre=14.9±5.5 Nm, post=13.8±7.3 Nm; p=.04, η_p^2=.27). For MCSA there was a significant arm × time interaction for the control group only, p =.02, η_p^2 =.57, where the change in the left arm (pre: 35.2 ± 7.2 cm^2; post: 34.4 ± 8.1 cm^2; -2.3%) was different from the right arm (pre: 34.3 ± 7.7 cm^2; post: 34.7 ± 8.0 cm^2; 1.2%). Muscle thickness change differed between groups (Training: pre=3.3±0.5 cm, post=3.4±0.6 cm; control: pre=3.7±0.7 cm, post=3.7±0.6 cm) for the immobilized wrist flexors only (p=.01, η_p^2=.40). Analyses of normalized EMG data failed to reveal significant between group or co-activation differences regardless of muscle (flexors, extensors), task (flexion, extension) or contraction type (ECC, CON, ISO). Strength preservation was not specific to contraction type (p=.69, η_p^2=.03), yet sparing effects were specific to the trained muscle. The mechanisms of muscle size preservation remain unknown, but these data draw an important link between strength and muscle size sparing with CE and suggest that ECC

training of the non-immobilized limb can preserve size of the immobilized contralateral homologous muscle and strength across multiple contraction types.

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GLOSSARY OF TERMS

Terms	Definition
Concentric	Shortening muscle action
Cross-education	Phenomenon that occurs when unilateral training has a transfer of the trained task to the untrained contralateral limb
Eccentric	Lengthening muscle action
Isometric	Muscle length and joint angle remains constant during a contraction
Isokinetic	Muscle action performed with a constant velocity
Isoinertial	Muscle action performed with a constant resistance
Sparing effect	An attenuation of the loss in muscle size or strength

ABBREVIATIONS

Abbreviation	Definition
1 RM	One repetition maximum
BOLD	blood oxygen level-dependent
CE	Cross-education
CON	Concentric muscle action
ECC	Eccentric muscle action
ISO	Isometric muscle action
M1	Primary motor cortex
MCSA	Muscle cross-sectional area
MT	Muscle thickness
MVC	Maximal voluntary contraction
Mwave	Tracing of an EMG response to the stimulation of a motor nerve
Mmax	Tracing of an EMG response of a maximum compound action potential from the stimulation of a motor nerve.
pQCT	Peripheral Quantitative Computed Tomography
S1	Primary somatosensory cortex
SENIAM	Surface Electromyography for the Non-Invasive Assessment of Muscles

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

INTRODUCTION

Cross-education (CE) of strength is the phenomenon that occurs when unilateral strength training has a transfer of strength to the untrained contralateral limb (Lee & Carroll, 2007). CE effects have also been documented as a transfer of functional skill after performing unilateral tasks (Criscimagna-Hemminger, Donchin, Gazzaniga, & Shadmehr, 2003; Latash, 1999; Teixeira, 2000) and has been referred to as a cross-transfer, cross-training, contralateral strength training effect and bilateral transfer effect. A meta-analysis by Carroll, Hebert, Munn, Lee, and Gandevia (2006) reported that the cross-education effect after unilateral strength training averages ~8% increase in strength in the contralateral limb or ~50% of that achieved in the trained limb. An important more recent finding is that CE can preserve strength and muscle size in the opposite immobilized limb (Farthing, Krentz, & Magnus, 2009). These "sparing effects" have rejuvenated interest in CE as a potential rehabilitation strategy (Farthing & Zehr, 2014). To date, there are only four studies that have investigated the effects of cross-education in healthy participants with an opposite immobilized limb (Farthing et al., 2009; Farthing et al., 2011; Magnus, Barss, Lanovaz, & Farthing, 2010; Pearce, Hendy, Bowen, & Kidgell, 2013). The novelty of an immobilization model is that a disused limb has an accelerated decrease in neural drive, strength and muscle size (Clark, Issac, Lane, Damron, & Hoffman, 2008; Duchateau & Hainaut, 1990; Eastlack et al., 1999; Suetta et al., 2004). All four studies found that CE attenuated the strength loss in the immobilized limb; however, three of these studies also found a sparing effect for muscle size (Farthing et al., 2009; Magnus et al., 2010; Pearce et al., 2013). The link between size and strength sparing is currently unclear. An intriguing way to explore this link is to investigate specificity. CE of strength is widely thought to be specific to the homologous muscle in the untrained limb (Hortobágyi et al., 2011; Lee, Hinder, Gandevia, & Carroll, 2010; Zhou, 2000); however, specificity of CE has never been tested for an immobilized limb. Specificity effects may present differently for an immobilized limb due to alterations in the excitability of the nervous system (Opie, Evans, Ridding, & Semmler, 2016).

The mechanisms of muscle size preservation are currently unclear because cross-education is believed to be driven by neural mechanisms (Carroll et al., 2006; Lee & Carroll, 2007) and not morphological. The discrepancy in findings for muscle size preservation between Farthing et al. (2011) and the other three studies (Farthing et al., 2009; Magnus et al., 2010; Pearce et al., 2013) is curious and warrants further investigation into the sparing of muscle size in an immobilization model. The three studies that observed muscle size sparing effects of CE used ultrasound as a measure of muscle thickness; and although valid and reliable (Cartwright et al., 2013), recent literature has shown that early changes in muscle size observed with ultrasound can be associated with edema and may not represent true morphological changes (Damas et al., 2016). Therefore, since fluid changes can easily influence ultrasound measurements, it is important to revisit the observed sparing effects with a more precise and comprehensive method of muscle imaging such as peripheral quantitative computed tomography (pQCT) or magnetic resonance imaging (MRI) in order to confirm the previous results.

The observed sparing effect of CE has implications for improving rehabilitation of an injured limb (Magnus et al., 2013; Papandreou, Billis, Papathanasiou, Spyropoulos, & Papaioannou, 2013), with the goal of restoring symmetry after unilateral injury (Farthing & Zehr, 2014). The concept of using CE for its sparing effects during rehabilitation from injury has the potential to reduce the total time of recovery, particularly if therapy is started before atrophy and strength deprivation begin to occur (Farthing & Zehr, 2014). Quicker and more complete recovery could reduce the costs and burden of unilateral injury or impairment incurred by national health care systems.

In line with the previous healthy limb immobilization studies, the current study used immobilization of a healthy arm to investigate specificity of CE sparing effects. The novel purpose of this study was to investigate muscle (size; wrist flexors, extensors), task (strength; flexion, extension) and type (strength; ECC, CON, ISO) specificity of CE sparing effects after four weeks of ECC unilateral training with the dominant right arm, while the left arm remained in a forearm cast. The secondary purpose was to investigate the muscle size sparing effects of CE using a measure of MCSA.

1.0 REVIEW OF LITERATURE

1.1 Neural Mechanisms of Cross-Education

1.1.1 Cross-Activation and Bilateral Access Hypotheses

The mechanisms of CE are thought to be neural in nature (Ruddy & Carson, 2013) with adaptations leading to contralateral improvements of strength and performance observed in cortical and subcortical regions (Anguera, Russell, Noll, & Seidler, 2007; Farthing et al., 2011; Farthing, Borowsky, Chilibeck, Binsted, & Sarty, 2007; Hortobágyi et al., 2011; Lee et al., 2010; Pearce et al., 2013). Specifically, the Cross-Activation hypothesis and the Bilateral-Access hypothesis are the two dominant theories of CE, both of which propose that transfer effects are mediated by an interaction between the two hemispheres of the brain (Ruddy & Carson, 2013). Although they are conceptually different, these hypotheses are not necessarily mutually exclusive. There is currently no direct evidence to identify if these hypotheses are in fact responsible for CE transfer effects either working alone or together (Barss, Pearcey, & Zehr, 2016).

The cross-activation hypothesis is based on the notion that unilateral activation (via skill or strength training) not only causes neural plasticity in the contralateral hemisphere that directly innervates the active limb, but also in the ipsilateral hemisphere responsible for activating the opposite limb. On the other hand, the bilateral access hypothesis suggests that cortical adaptations associated with training are only stored in the contralateral hemisphere controlling the trained limb but are accessible by the ipsilateral, untrained hemisphere. Due to the brain's ability to share information through interhemispheric pathways, the ipsilateral hemisphere is able to access the stored motor engram and apply it to movement for the untrained limb. The current body of literature has focused on the primary motor cortices (M1) in each hemisphere when investigating the CE mechanisms. The specific location in each hemisphere where the neural plasticity and adaptation occurs is currently unclear. Regions upstream of the M1 such as the primary somatosensory cortex, supplementary motor area, pre-supplementary motor area, premotor cortex, parietal lobe, cerebellum and the cingulate cortex could be involved in CE (Ruddy & Carson, 2013). For the purposes of this review, the primary focus remains on neural

adaptations within the M1 through changes in surround inhibition, inter-hemispheric inhibition, and intra-hemispheric inhibition and facilitation.

1.1.2 Surround Inhibition

Surround inhibition (SI) is a mechanism in the central nervous system (CNS) that occurs when an excited neuron inhibits the activity of its surrounding neurons (Beck & Hallett, 2011). SI is mediated by GABAergic transmission and contributes to voluntary movement by selective motor output (Mink, 1996; Ziemann, Rothwell, & Ridding, 1996). The inhibition of neural activity in the surrounding neurons allows the nervous system to selectively activate desired motor units without antagonist or other unrelated motor unit activity that would cause undesirable motor output. SI likely occurs in the primary motor cortex (M1) with the inhibition of representative zones (i.e., dedicated regions for different body parts or muscle groups) in the M1 rather than inhibiting descending pathways, which would prevent downstream motor activity (Sohn & Hallett, 2004).

In the context of cross-education, SI is thought to impact cross-activation of the opposite hemisphere. SI occurring in one hemisphere focuses the neural activity through the transcallosal pathway that facilitates specific neural activity in the opposite hemisphere (Ruddy & Carson, 2013).

1.1.3 Inter-Hemispheric Inhibition

Inter-hemispheric inhibition (IHI) is a cortical mechanism that controls the interaction between the two hemispheres of the brain. IHI down-regulates activity between interneurons and is mediated primarily by the transcallosal pathways (Daskalakis, Christensen, Fitzgerald, Roshan, & Chen, 2002). IHI can be demonstrated by transcranial magnetic stimulation (TMS) with the application of a conditioning stimulus to the M1 in one hemisphere which then inhibits the size of the motor evoked potential (MEP) produced by a test stimulus in the M1 of the contralateral hemisphere (Ferbert et al., 1992; Hanajima et al., 2001). With regards to CE of strength, Hortobágyi et al. (2011) conducted the only study to date that has investigated the effects of chronic unilateral strength training on IHI and CE. Participants engaged in 20 training sessions of abduction in the first dorsal interosseus (FDI) muscle of the right index finger over an eight-week period. The trained FDI had a 49.9% increase in strength and the untrained FDI gained

28.1% in strength by the end of the intervention. The chronic training effect decreased IHI by 30.9% over the course of the study and acutely by 8.9% during each training session. Of interest, the rate of strength increase in the untrained FDI was found to increase over the course of the study, which was strongly correlated to the changes (decrease) in IHI by the 20th training session. Additionally, the change in facilitation of MEPs when the right FDI strongly contracted (80% MVC) and the ipsilateral M1 was stimulated with high intensity TMS (160% resting motor threshold) correlated with the change in CE at session five, 10, 15, and 20. Ruddy and Carson (2013) suggest that the decrease in IHI observed by Hortobágyi et al. (2011) is a result of adaptations in the hemisphere ipsilateral to the trained limb with alterations to the excitatory-inhibitory balance within interneuron circuits rather than changes in the way electrical impulses are delivered between hemispheres. The work by Hortobágyi et al. (2011) provides the first evidence of IHI as a possible mechanism driving CE of strength; however, interpreting adaptations as measured by IHI warrants caution. IHI can increase or decrease depending on the intensity of the control stimuli administered via TMS (Ruddy & Carson, 2013) and therefore, can produce different responses in IHI (increased, unchanged or decreased).

Farthing et al. (2011) took a different approach to investigate the supraspinal mechanisms of CE by using functional MRI (fMRI) to analyze active brain regions during an isometric grip task, before and after unilateral strength training of the right arm during three weeks of left forearm cast immobilization. Farthing et al. (2011) observed unique increased neural activation patterns in the motor cortex of the ipsilateral hemisphere responsible for neural drive to the untrained, immobilized limb after unilateral isometric strength training. These unique neural activation patterns were only observed in the training group and were not present in a non-training control group. Unilateral strength training in the Farthing et al. (2011) study may have suppressed inhibitory mechanisms to the immobilized limb in the training group only. The unique neural activity in the ipsilateral hemisphere associated with the immobilized limb provides an indication that increases in ipsilateral motor cortex activation are associated with the sparing of strength in the untrained immobilized limb. A review by Hendy, Spittle and Kidgell (2012) suggested that in the presence of immobilization, changes to interhemispheric connections largely contribute to the increases in ipsilateral motor cortex activation after unilateral strength training. Changes to these interhemispheric connections are possible contributors to the sparing of muscle strength in the immobilized limb. Motor irradiation in the

contralateral hemisphere from unilateral strength training could reduce IHI and cause a 'spill over' effect contributing to the excitation of the ipsilateral motor cortex (Hendy et al., 2012).

1.1.4 Intracortical Inhibition and Facilitation

Intracortical inhibition (ICI) and intracortical facilitation (ICF) represent phenomena that occur in the M1 and impact corticospinal excitability (Wagle-Shukla, Ni, Gunraj, Bahl, & Chen, 2009). Modulation of ICI and ICF in the ipsilateral M1 has been observed with unilateral resistance training; however, the modulatory response presents differently (inhibition vs. disinhibition) depending on the type of muscle actions used in training (ECC, CON, ISO). Acute or chronic CON focused training does not systematically modulate short interval intarcortical inhibition (SICI) or ICF (Tibor Hortobágyi et al., 2011; McCombe Waller, Forrester, Villagra, & Whitall, 2008). Based on the CON focused literature, Ruddy and Carson (2013) concluded that ICI and ICF adaptations are incidental and are not likely mechanisms of CE. However, studies that used eccentric (ECC) muscle actions in training protocols have found that ECC training modulated ICI and ICF differently than CON training. Kidgell et al. (2015) investigated effects of ECC training on ICI (measured by SICI and the duration of the silent period – an interruption in the electromyography (EMG) response after a test pulse) and found that ECC training uniquely reduced ICI confined to the ipsilateral M1 responsible for innervating the untrained limb; a neural adaptation that was not found with CON training. With a three second contraction at 5% isometric MVC torque the ECC training group reduced the duration of the silent period for the left untrained wrist flexors by 27% compared to 4% in the control and CON groups (Kidgell et al., 2015). Kidgell et al. (2015) also observed a 32% reduction in SICI at 40% isometric MVC torque after ECC training compared to 2% after CON training and 1% in the control group. Howatson et al. (2011) also observed that SICI decreased by 92% with ECC muscle actions while SICI only decreased by 69% with CON muscle actions. ICF also diminished during CON muscle actions by 116% while ICF increased with ECC muscle actions by 158% (Howatson et al., 2011). The fact that CE occurs regardless of contraction type indicates that the modulation of intracortical pathways via inhibition or facilitation is not the primary mechanism of CE, which supports the suggestions of Ruddy and Carson (2013). However, it is evident that diminished SICI and increased ICF mediated by ECC training can directly influence the amount of strength obtained in the untrained limb via CE (Howatson et al., 2011; Kidgell et al., 2015). These

findings support previous literature that has demonstrated superior CE effects with ECC training protocols (Farthing & Chilibeck, 2003; Hortobágyi, Lambert, & Hill, 1997; Seger, Arvidsson, & Thorstensson, 1998).

1.2 Immobilization

1.2.1 Muscular Atrophy and Strength Loss

Immobilization of a limb results in a decrease in the size and strength of the muscles responsible for the movement of the immobilized limb. A review by Appell (1990) noted that immobilization reduces muscle weight, induces changes in the ratio of muscle fibre types and changes the size of the individual muscle fibres. Phillips and McGlory (2014) suggest that a number of factors in previous immobilization research urge caution with the interpretation of proposed mechanisms responsible for disuse muscle atrophy. A review by Phillips, Glover and Rennie (2009) outlined the limitations in much of the current literature with the study of disuse atrophy with participants in a diseased state, through analysis of static protein and gene abundances, or through inferences from disuse models in other species such as rodents. Alternatively, Phillips and McGlory (2014) proposed that studying these mechanisms should be done in healthy humans by investigating in vivo measures of skeletal muscle protein turnover. Phillips and McGlory (2014) suggested that in a healthy state there is equilibrium between muscle protein synthesis and muscle protein breakdown, and it is likely that muscle atrophy occurs because of an imbalance in this process.

The primary mechanism of muscular atrophy from disuse or immobilization appears to be due to a decreased rate in protein synthesis and not caused by increased protein breakdown (Phillips & McGlory, 2014). As reviewed by Phillips and McGlory (2014), evidence supporting this notion dates back to a study by Gibson et al. (1987) which found that after unilateral leg immobilization, the immobilized limb had ~30% lower rates of muscle protein synthesis compared to the contralateral non-immobilized limb. This study was further supported in additional unilateral limb immobilization models with De Boer et al. (2007) finding that muscle protein synthesis rates decreased more than 50% over the initial 10 days of lower limb immobilization. However, no further declines in the rate of protein synthesis occurred between days 10 and 21, which resulted in 0.5% muscular atrophy per day over the course of the study. Glover, Yasuda, Tarnopolsky, Abadi and Phillips (2010) also found that markers of muscle

protein breakdown and oxidative stress were not different from baseline after 14 days of knee immobilization, which resulted in a 5.7% decline in muscle cross-sectional area of the quadriceps. Taken together this literature demonstrates that a decline in muscle protein synthesis and not changes in muscle protein breakdown are likely responsible for causing immobilization induced muscle atrophy (Phillips & McGlory, 2014). The findings from De Boer et al. (2007) support the noted time-course of atrophy from immobilization by Appell (1990) with observed losses in muscle weight shown to decrease in early phases of immobilization, yet with prolonged immobilization there appears to be minimal added loss in muscle weight following the initial early decline.

1.2.2 Cortical Adaptation

Although muscular atrophy and strength loss are well understood consequences of immobilization, the cortical adaptations associated with immobilization are less well known. Currently, the dominant belief is that immobilization leads to a decrease in cortical activity (Burianová et al., 2016; Lissek et al., 2009; Opie et al., 2016), which is commonly exhibited by a decrease in amplitude of MEPs from TMS. However, enhanced cortical activity, exhibited by an increase in MEP amplitude has also been reported (Jensen, Christensen, Petersen, Geertsen, & Nielsen, 2006). Clark, et al. (2008) suggested that the discrepant findings are likely a result of the differences in study paradigms, such as the method and duration of immobilization and the health/injury status of the participants. Clark et al. (2008) hypothesized a decrease in corticospinal excitability, yet found mixed results. Resting MEP amplitude increased more than twofold after one week of immobilization, suggesting an increase in corticospinal excitability, yet this finding was accompanied with a 20% increase in the duration of cortical silent period, no change in the active (during contraction) MEP amplitude, and a decrease in estimated voluntary activation assessed by twitch interpolation (electrically stimulating a muscle during an MVC). These findings suggest inhibitory mechanisms are active in the representative area of the immobilized limb in the contralateral hemisphere during submaximal muscle actions (Clark et al., 2008). Additionally, Lissek et al. (2009) reported that short-term hand immobilization led to a significant reduction in hand use and tactile acuity, accompanied by an observed decrease in the representative area of the primary somatosensory cortex (S1) measured with functional magnetic resonance imaging (fMRI). Along with the reduction in S1 activity in the contralateral

hemisphere, a compensatory response was observed in the ipsilateral hemisphere involved with innervating the non-immobilized limb, demonstrating the intercommunication and adaptive nature of the brain under altered conditions (i.e., immobilization). Additionally, Burianová et al. (2016) and Huber et al. (2006) found similar decreases in sensorimotor areas in the contralateral hemisphere to the immobilized hand with fMRI. In addition to the sensorimotor decline observed with fMRI, these two studies also measured corticospinal excitability with TMS. Huber et al. (2006) showed that arm immobilization led to a decreased motor performance in addition to a decline in MEP amplitude. Burianová et al. (2016) found a significant increase in the resting motor threshold of the contralateral M1, which was an indication of decreased corticospinal excitability to the immobilized hand.

1.2.3 Cross-Education and Immobilization

To date there have been four studies to investigate sparing effects of CE with a healthy immobilized limb. Each of the four studies investigated the impact of unilateral training on the muscular strength and size in the untrained, immobilized limb, while two of the studies also investigated cortical contributions to the sparing effects of CE with the immobilized limb. Farthing et al. (2011) used fMRI imaging to investigate the blood oxygen level-dependent (BOLD) signal in each hemisphere to assess the level of activation and contributions of each cortical area. Pearce et al. (2013) used TMS to assess changes in corticospinal excitability of the ipsilateral motor cortex of the trained limb, responsible for activating the immobilized limb. All four studies used an arm immobilization model; however, the type of strength training varied between studies. Unilateral strength training was performed with the dominant right arm in each of the four studies, with heavy load (80% 1RM) isoinertial CON elbow flexion (Pearce et al., 2013), maximal isometric ulnar deviation (Farthing et al., 2009), maximal isometric handgrip training (Farthing et al., 2011), and isometric elbow flexion and extension (Magnus et al., 2010). All four studies observed a ‘sparing’ of muscular strength in the contralateral homologous muscle group to that being trained, and three of the studies also observed a preservation of muscle size in in the contralateral homologous muscle group (Farthing et al., 2009; Magnus et al., 2010; Pearce et al., 2013). Although muscle size preservation is apparently reproducible, these studies have yet to identify a mechanism responsible for the size sparing effects.

Farthing et al. (2011) observed unique activation patterns in the motor cortex of the ipsilateral, untrained, hemisphere for the training group only, while the training group from Pearce et al. (2013) observed a maintenance in corticospinal excitability within the ipsilateral, untrained, motor cortex and the corticospinal tract responsible for innervating the untrained, immobilized limb. The findings from Farthing et al. (2011) and Pearce et al. (2013) provide evidence that unilateral strength training of the non-immobilized limb increases or maintains cortical activity (Farthing et al., 2011) and corticospinal excitability (Pearce et al., 2013) in the ipsilateral motor cortex responsible for activating and driving movement of the immobilized limb. These mechanisms are key indicators that the 'sparing' of muscular strength is largely driven by either the disinhibition or facilitation of intracortical neural pathways in the untrained cortex.

1.3 Eccentric Resistance Training

1.3.1 Muscle Hypertrophy

Muscle hypertrophy is defined as the increase in muscle mass and cross-sectional area due to an increase in size of the individual muscle fibres (Baechle & Earle, 2008). The way a muscle increases in size is by creating new sarcomeres through a process called sarcomerogenesis. The creation of new sarcomeres occurs in two distinct ways. Sarcomeres can be added in series, increasing fascicle length, and they can be added in parallel, increasing cross-sectional area. The way sarcomeres are added depends largely on the type of resistance training the muscle is exposed to. Although the methods of sarcomerogenesis are not mutually exclusive from each other, ECC training has been shown to add more sarcomeres in series than CON training, while CON training has been shown to increase the number of sarcomeres in parallel to a greater extent than ECC training (Franchi et al., 2014). ECC training is generally thought to produce greater overall hypertrophy compared to CON training (Review, Roig et al., 2009). ECC training is more efficient than CON training, producing equal amounts of work with substantially less training volume (i.e. sets × repetitions) (Moore, Young, & Phillips, 2012). A work-matched intervention between ECC and CON training found similar increases in muscle size between ECC (~6.5%) and CON (~4.6%) even though CON performed ~40% more repetitions to match the work of the ECC group. As a result, ECC training resulted in ~60% less work per repetition compared to CON training (Moore et al., 2012). The reason for CON muscle actions requiring

more repetitions was a consequence of ~30% greater peak torque per repetition with ECC muscle actions. Farthing and Chilibeck (2003b) examined the effects of ECC and CON isokinetic resistance training at high velocity (3.14 radians/second) and slow velocity (0.52 radians/second) on muscular hypertrophy and found that high velocity ECC training improved muscle thickness more than all other conditions. The advantages of training with ECC muscle actions are clear, with the ability to produce greater muscle hypertrophy with a more efficient work to volume relationship. These benefits make training with ECC muscle actions an attractive training mode for inducing effective adaptations in all populations from high performance athletes, to clinical populations that may not have the capacity to perform high volume. Given the greater potential for ECC actions to induce hypertrophy with training, this mode of training was used in the current thesis to assess whether muscle size could be preserved in an immobilized limb with cross-education.

1.3.2 Strength

The advantages of using ECC muscle actions to induce muscular strength adaptations is best exemplified by the force-velocity relationship which reveals distinct differences between CON, ISO and ECC muscle actions and how the muscle performs under these different loading conditions. In a CON muscle action, as the force is increased the velocity drastically decreases. ISO muscle actions produce a constant level of force with zero velocity. However, ECC muscle actions can maintain high velocities with high force external loads. This unique performance trait with ECC muscle actions is intriguing from a strength building perspective, as literature suggests the combination of high force and high velocity during resistance training may be an optimal condition for increasing muscle mass and strength (Farthing & Chilibeck, 2003b).

Based on the morphological differences after ECC and CON training (Hypertrophy; section 1.3.1), the two training types also produce differences in muscular performance. Cross-sectional area of a muscle increases by adding sarcomeres in parallel, increasing the capability of the muscle to produce greater amounts of force. However, by increasing the fascicle length in the muscle by adding sarcomeres in series, the muscle can contract and shorten with higher velocities (Franchi et al., 2014). Further, by adding sarcomeres in series through ECC training the length-tension relationship shifts to the right, meaning that optimal actin-myosin cross bridging is achieved at longer muscle lengths, giving the muscle the ability to produce higher

forces at a longer muscle length (Proske & Morgan, 2001; Vogt & Hoppeler, 2014). The physiological advantage of ECC actions to improve strength with training is ideal for CE research because the CE transfer effect to the untrained limb are correlated to the amount of strength improvement in the trained limb (Carroll et al., 2006).

1.3.3 Specificity of Eccentric Training

Hawkins et al. (1999) found that ECC and CON isokinetic training produced equal changes in CON strength, but the ECC training produced greater adaptations in ECC strength. A systematic review and meta-analysis by Roig et al. (2009) compared ECC and CON training for differences in strength adaptations. Roig et al. (2009) found that strength was relatively specific to the trained muscle action; however, ECC training produced greater total strength (through multiple modes of contraction) and ECC strength compared to CON training. Seger et al. (1998) however found that training with ECC muscle actions resulted in more specific strength adaptations compared CON training with respect to contraction velocity and mode of exercise. These findings were confirmed in the review by Roig et al. (2009), which concluded that strength adaptations with ECC training were more pronounced when the strength test was specific to the training velocity, demonstrating that ECC training adaptations are velocity specific. In the context of CE of strength, Farthing and Chilibeck (2003a) investigated the specificity of strength transfer to the untrained contralateral limb with high (3.14 radians/second) and low (0.52 radians/second) velocity ECC and CON muscle actions. Farthing and Chilibeck (2003a) found that the CE of strength transfer was specific to contraction type and velocity when high velocity ECC muscle actions were used. Overall, ECC training especially at high velocities, appears to provide a greater global training effect - improving strength in other modes (ISO, CON) in the trained muscle group, compared to other training modes (Roig et al., 2009). And strength adaptations are more pronounced when measured specific to the mode and velocity used during training (Hawkins et al., 1999; Roig et al., 2009; Seger et al., 1998). Based on the previously mentioned literature in the context of CE transfer effects, contraction type specificity can be expected when training with high velocity ECC muscle actions, while the transfer effect is likely not as specific to contraction type when training with lower velocity ECC muscle actions (Farthing & Chilibeck, 2003a).

1.3.4 Neural Aspects of Eccentric Training

ECC muscle actions produce different neural activation strategies compared to ISO or CON actions (review; Duchateau & Enoka, 2016). The discharge rate of motor units is lower for ECC compared to ISO and CON (Duchateau & Enoka, 2016). Using surface EMG to assess muscle activity during contractions performed at the same velocity, Aagaard et al. (2000) demonstrated that ECC muscle actions produce lower EMG amplitudes compared to ISO or CON. Compared to CON actions the level of voluntary activation assessed via twitch interpolation is lower during ECC muscle actions in untrained individuals (Amiridis et al., 1996).

The specific mechanisms for ECC muscle actions producing different neural activation patterns compared to ISO and CON are currently unclear, but inhibition at spinal or supraspinal levels are likely responsible for the differences (Duclay, Pasquet, Martin, & Duchateau, 2011). An observed reduction in corticospinal excitability measured by lower amplitudes in MEPs and Hoffmann reflexes during ECC muscle actions provides an indication that corticospinal contributions are responsible for different activation patterns between contraction types (Duclay et al., 2011).

1.3.5 Eccentric Training and Cross-Education

ECC resistance training increases strength in the contralateral limb to a greater extent than CON resistance training (Farthing & Chilibeck, 2003a; Hortobágyi et al., 1997; Seger et al., 1998). The observed strength transfer of ECC compared to CON training is substantial; 77% versus 30% (Hortobágyi et al., 1997) and 15% versus 10% respectively (Seger et al., 1998), although the CE effect appears to be largely mode and velocity specific (Farthing & Chilibeck, 2003a; Hortobágyi et al., 1997; Seger et al., 1998). Hortobágyi et al. (1997) found ECC training had a greater global training effect than CON training in the contralateral limb, where ECC training improved ECC strength by 77% and ISO strength by 39%, while CON training improved CON strength by 30% and ISO strength by 22%. As mentioned earlier in Section 1.1, the larger transfer effects with ECC compared to other modes of contraction is likely due to the observed differences in how each muscle action alters cortical adaptations (Leung, Rantalainen, Teo, & Kidgell, 2015). The apparent global benefits of an ECC resistance training model with CE are intriguing from a rehabilitation perspective because ECC training may involve global training

benefits for an opposite injured or neurologically impaired limb. ECC training is an ideal exercise mode for research investigating specificity and sparing effects with CE. In the current study, high velocity (relative to the small moment arm of the wrist joint) ECC muscle actions were chosen for training to employ strong strength and size adaptations to the trained arm and to increase the potential magnitude of CE transfer. Further, based on the work by Farthing and Chilibeck (2003a) this mode of training is likely to result in contraction type specific adaptations in the untrained limb.

1.4 Objectives

The primary objective of this thesis was to test the specificity of sparing effects of CE in the untrained immobilized limb. Specificity effects are very convincing evidence of neural mechanisms with CE (Hortobágyi et al., 2011); but specificity of sparing effects with CE have never been studied. Muscle (i.e. homologous agonist, antagonist) and task (i.e. ECC, CON, ISO) specificity was investigated as evidence to support current theories of neural mechanisms contributing to sparing effects with CE. In addition, forearm muscle cross-sectional area (MCSA) was measured to gain insight into the possible peripheral or morphological mechanisms involved in sparing muscle size and strength in the contralateral untrained limb.

1.5 Purpose

The primary purpose of this study was to investigate muscle (size; wrist flexors, extensors), task (strength; flexion, extension) and type (strength; ECC, CON, ISO) specificity of CE sparing effects after four weeks of ECC unilateral training. The secondary purpose was to investigate muscle size sparing effects of CE using a measure of MCSA.

1.6 Hypotheses

The primary hypothesis was that CE sparing effects would be specific to both muscle (i.e. homologous wrist flexors, not extensors), task (i.e. flexion, not extension), and type (i.e. ECC strength not CON, ISO strength). The secondary hypothesis was that unilateral ECC training would result in the sparing of MCSA in the immobilized limb, as measure by pQCT.

CHAPTER 2

METHODS

2.0 METHODS

2.1. Participants

Sixteen participants from the University of Saskatchewan student population volunteered to participate in the study (table 1). Participants were randomly assigned to a control (n=8) or a training group (n=8). Participants were right handed, as determined by a handedness questionnaire, healthy (i.e. no physical injuries or neurological conditions), and were classified as currently untrained (less than six months resistance training experience in the previous year, where one month of experience is equal to resistance training on average three times per week for four weeks). Prior to beginning the study informed consent forms were signed. This study conformed to the standards set by the Declaration of Helsinki and was approved by the University of Saskatchewan Behavioural and Biomedical Research Ethics Board.

Table 1. Demographics

Group	Sex	Age (years)	Height (cm)	Weight (Kg)	WHQ	Training Exp (months)
Training n = 8	**M** = 1 **F** = 7	20 ± 2	170.3 ± 10.1	77.2 ± 19.2	18.3 ± 2.4	2.3 ± 4.1
Control n = 8	**M** = 2 **F** = 6	23 ± 5	169.3 ± 8.5	85.7 ± 22.7	17.1 ± 2.5	2.9 ± 4.3

WHQ = Waterloo handedness questionnaire

Means ± SD

2.2 Intervention and Design

The study began in September and ran through the fall and winter semesters at the University of Saskatchewan. All participants received a forearm cast on their left, non-dominant, forearm for four weeks according to our previous method (Farthing et al., 2009; Farthing et al., 2011). Casts were placed by a physician, and immobilized the wrist, hand, thumb and fingers up to the middle phalanges. Notches were cut out of the cast for placement of electrodes for EMG monitoring of

the wrist flexors during training sessions. The training group underwent strength training of the right wrist flexors three times per week while the control group did not train during the immobilization period. Training involved maximal effort ECC isokinetic contractions. Strength training was progressive, which commenced with two sets of eight maximal repetitions and progressed in volume up to six sets of eight, with a taper down to two sets for the last session. One-minute rest was given between each set. Participants were prompted to position their immobilized limb in the same pronated orientation as the training limb and to relax it during all testing and training sessions in order to minimize mirror activity (Hortobágyi et al., 2011). The mirrored positioning of the immobilized limb was important in controlling for a possible confounding effect of the orientation of the wrist and homologous or no-homologous mirror activity during unilateral movements (Post, Bakels, & Zijdewind, 2009).

2.3 Measures

2.3.1 Familiarization and testing sessions.

All participants underwent a familiarization session and were introduced to all of the strength and stimulation testing measures. Following familiarization, participants returned to the lab within seven days for two separate pre-testing sessions. Two pre-testing sessions were used to determine variance of measures and to establish a stable baseline, although, only the second pre-testing session was used in data analysis. Data collection occurred in two separate labs. Muscle thickness and strength measures were assessed by the primary researcher in one lab, while MCSA was collected in a separate lab by researchers blinded to group assignment. For pre- and post-testing, muscle thickness was always measured prior to strength. For pre-testing the order of testing for MCSA in relation to the other tests varied due to scheduling constraints; however, when possible, MCSA was scheduled first. If MCSA was scheduled after the first pre-testing session, a minimum of 48 hours was allotted for recovery. After the second pre-testing session participants received the non-dominant forearm cast which initiated the start of the intervention period. After the four-week intervention, participants returned the lab for cast removal followed by post-testing. The order of post-testing was consistent with MCSA measured immediately after cast removal followed by muscle thickness and strength testing.

2.3.2 Peak torque.

All testing and training sessions used an identical setup, were supervised and completed on an isokinetic dynamometer (Humac NORM, CSMi, Stoughton, MA) using an identical rotational velocity at the University of Saskatchewan. Testing sessions involved maximal effort isokinetic ECC, CON and ISO muscle actions of the wrist flexors and extensors. Peak torque was recorded for each contraction type over three sets of one repetition separated by 30 seconds and used as a measure of contraction specific strength. The highest torque value achieved over the three maximal attempts was used as the strength value on each occasion. For each contraction type wrist flexors were tested first followed by wrist extensors. The order of limb testing (left or right arm) was randomized and held constant for each participant for every testing session. One-minute rest was given between each test. ECC and CON muscle actions were performed through 80˚ of motion (40˚ flexion to 40˚ extension) with a fixed rotational velocity of 1.05 radians per second, with ISO muscle actions performed with a neutral wrist (three-second MVC at 0˚ of flexion). ISO contractions were assessed first followed by ECC and CON in a randomized order. Participants were seated in an upright position, with the elbow at 90˚, and the forearm resting on a pad with the wrist in a pronated position grasping the dynamometer handle. Participants were instructed to rest their immobilized limb on their lap with the forearm in a prone position, to be consistent with the wrist orientation of the training limb. Verbal encouragement was provided by the same experimenter for each test.

2.3.3 Peripheral quantitative computed tomography.

PQCT is commonly used to measure bone density, size and geometry of the scanned area of tissue. Although, muscle size, geometry and density measures are also becoming more commonly assessed via pQCT (Erlandson, Lorbergs, Mathur, & Cheung, 2016). PQCT is highly correlated to MCSA derived from MRI (Sherk, Bemben, Palmer, & Bemben, 2011). In this study MCSA (cm^2) and muscle density (mg/cm^3) were measured via pQCT (Stratec, Medizintechnik GmbH, Germany) and analyzed using the BoneJ plugin (Version 1.3.11) for open-source software ImageJ (Doube et al., 2010). The analysis used a 7 × 7 median filter to reduce noise, further, parameters were set at 0.4 × 0.4 × 2.4 mm pixel size with an air threshold of -30 mg/cm^3, fat threshold of <30 mg/cm^3, a muscle threshold of ≥30 mg/cm^3, and a soft tissue threshold of 280 mg/cm^3, with a scaling coefficient of 1.724 and a scaling constant of -322. This method was

previously found to have to a $CV\%_{RMS}$ of 1.8% for MCSA and 1.2% for muscle density in a healthy university student population (unpublished data). A similar method with a different muscle threshold (40 mg/cm^3) has previously reported a $CV\%_{RMS}$ of 2.7% in forearm MCSA and 1.5% $CV\%_{RMS}$ in forearm muscle density (Frank-Wilson, Johnston, Olszynski, & Kontulainen, 2015). Testing took place at two-time points. Pre- and post-testing MCSA was assessed in both arms of each group (intervention and control) to assess the changes in muscle volume that took place in the immobilized and non-immobilized limb over the duration of the study. Muscle density was also assessed as an exploratory measure but was not a primary outcome. During pre-testing pQCT was measured within seven days after the familiarization session, whereas post-testing pQCT was measured immediately post cast removal. PQCT was used to assess total MCSA and density of each limb but it is unable to differentiate between muscles (flexors, extensors). MCSA and muscle density were assessed at 65% of the length of the radius measured from the distal end of the radius (Frank, Lorbergs, Chilibeck, Farthing, & Kontulainen, 2010).

2.3.4 Muscle thickness.

Muscle thickness was assessed using ultrasound (LOGIQ e BTO8, GE Healthcare, Milwaukee, Wisconsin, USA). Ultrasound has previously been used in our lab and is a valid and reliable method of assessing muscle thickness (Candow, Chilibeck, Facci, Abeysekara, & Zello, 2006; Cartwright et al., 2013; Farthing & Chilibeck, 2003b; Farthing, Chilibeck, & Binsted, 2005; Krentz, Quest, Farthing, Quest, & Chilibeck, 2008). The procedure involved placing a probe with transmission gel on the surface of the skin over the bulk of the muscle while the limb is in a rested and neutral position. Anthropometric measures and landmarks on the arms (using non-toxic markers) and overhead transparency film were used to ensure the probe of the ultrasound machine was placed on the muscle of interest in the same spot each time muscle thickness was assessed. Muscle thickness was determined by measuring a linear distance of the muscle between the edge of the subcutaneous tissue to the edge of the bone. This method has been previously used in our lab on the wrist flexors (Farthing et al., 2011); however, in the current study this same method was also applied for the wrist extensors. Thickness measures were taken at a standardized location of 1/3 the distance between the medial epicondyle and radial styloid for the wrist flexors and 1/3 the distance between the lateral epicondyle and the ulna styloid for the wrist extensors. Thickness of the wrist flexor and extensor muscles during the pre- and post-testing

sessions was assessed to investigate the specificity of muscle size sparing effects. At each testing session, four measurements were recorded for each muscle, with the average of the two closest measures used for comparison.

2.3.5 Handedness.

Participant's handedness was determined with the Waterloo Handedness Questionnaire (Bryden, 1977). The questionnaire scores participants as either right handed (indicated by a positive score) or left handed (indicated by a negative score) with +20 representing strong right-handedness, and -20 representing strong left-handedness. Participants were required to be right-handed for this study because previous literature has demonstrated greater cross-education of strength in right-handed individuals when training their dominant arm (Farthing, 2009; Farthing et al., 2005).

2.3.6 Electromyography.

EMG was recorded at five-time points; during pre- and post-testing and during the first, seventh and twelfth training sessions. During pre- and post-testing surface EMG (Grass EMG P511 AC amplifier; Grass Technologies, Middleton, WI; Amplification of 1,000, bandwidth of 10 Hz to 1,000 Hz; and VERMED NeuroPlus; 2.5 cm^2, Ag/Ag chloride sensor) was used to measure muscle activity in the agonist and antagonist (wrist flexors and extensors) muscles and in the biceps brachii and triceps brachii of the trained and untrained limbs. Electrode placement for the flexor carpi radialis (FCR) muscle was placed 1/3 of the distance from the medial epicondyle to the radial styloid follow the recommendations from Buschbacher and Prahlow (2000) and Zehr (2002). The extensor carpi radialis (ECR) electrodes were placed on the medial side of the brachioradialis, at 1/5 (approximately three finger widths) of the distance from the lateral epicondyle on a line with the second metacarpal (Zehr, 2002). The electrode placement on the biceps brachii were placed one third of the distance from the fossa cubit on a line between the fossa cubit and the medial acromion. The triceps brachii long head electrodes were placed at the 50% mark between the posterior crista of the acromion and the olecranon at two finger widths medial to the line as per surface electromyography for the non-invasive assessment of muscles (SENIAM) guidelines (Stegeman & Hermens, 2007). The EMG data collected in the upper arm (biceps brachii and triceps brachii) was used during testing to visually monitor muscle activation in real time only; no offline analysis of these data was conducted. Participants were instructed to

relax the upper arm during contractions. During the first, seventh and twelfth training sessions EMG was measured in wrist flexors of each arm only and was used to determine the level of mirror activity occurring in the untrained limb during strength training of the opposite wrist flexors.

2.3.7 Data acquisition.

Custom software in LabVIEW (version 8.6) was used to obtain M-waves from evoked contractions and EMG and torque data during maximal voluntary contractions. All channels were acquired at a sampling rate of 1,000 Hz. To determine activation amplitude of the EMG data, the middle one second of the burst activity from each voluntary contraction was rectified to determine the mean absolute value and the greatest amplitude recorded from the three reps for each contraction type was used in analysis. An analog-to-digital converter (model PCI-6034E, National Instruments, Austin, TX) was used to convert the analog signals from each device to digital signals displayed in LabVIEW.

2.3.8 Maximum electrically evoked contraction.

A Constant Current High Voltage Stimulator (model DS7AH, Digitimer, Hertfordshire, England) was used to supramaximally activate the wrist flexors and extensors during a 10% isometric MVC background contraction (Lagerquist, Zehr, & Docherty, 2006). Electrodes (VERMED as above) were manually pressed into the median nerve above the elbow, under the muscle belly of the short head of the biceps brachii to ensure adequate contact between the electrode and the nerve for stimulation of the wrist flexors. Electrodes were also manually pressed into the radial nerve above the lateral epicondyle of the elbow for stimulation of the wrist extensors. A series of control twitches (0.5-ms pulses) were used to determine the current (mA) required to reach maximum M-wave (M_{max}). Stimulations started with a low level of current, barely detectable by the participant. The intensity was raised progressively until a plateau in the M-wave occurred. The milliamps required to evoke a plateau in the peak to peak magnitude of the M-wave plus 20% was used to ensure M_{max} was reached and was recorded. While maintaining a 10% isometric MVC background contraction the custom written LabVIEW software interface randomly administered five stimulations to the respective nerve. The average of the five evoked contractions was used as the M_{max}. M-wave data were filtered in MATLAB (MATLAB 2006b,

MathWorks, Natick, MA) with a fourth order Butterworth filter (high-pass filter of 100 Hz, low-pass filter of 250 Hz). The M_{max} was used as a reference to normalize the EMG data from the strength tests for the wrist flexors and extensor within each testing session. M_{max} was assessed during the two pre- and one post-testing sessions.

2.4 Data Analysis

The study was a 2 × 2 × 2 × 3 × 2 factorial design (group [training, control] × arm [right, left] × task [flexion, extension] × type [ECC, CON, ISO] × time [pre, post-training]). Strength data were analyzed with a 2 × 2 × 2 × 3 × 2 factorial ANOVA (group × arm × task × type × time) followed by further assessing the significant two- and three-way interactions appropriate for the research questions related to the contraction type and task specificity. Strength data were also split by arm (immobilized, non-immobilized) and task (wrist flexion, extension) for several breakdown analyses to better understand the trained limb vs. the CE sparing effect.

EMG data were analyzed with a 2 × 2 × 2 × 2 × 3 × 2 factorial ANOVA (group × arm × muscle [agonist, antagonist] × task × type × time).

In addition, an EMG analysis of the mirror activation in the left, immobilized arm, of the training group was conducted for the first, seventh and twelfth training sessions. The mirror activation analysis involved separate one-factor repeated measures ANOVA to investigate differences between sessions (1, 7, 12), repetitions (1-8) and sets (1-6).

Muscle cross-sectional area and muscle density (via pQCT) were analyzed with separate 2 × 2 × 2 (group × arm × time) factorial ANOVA tests and muscle thickness (via ultrasound) was analyzed with a 2 × 2 × 2 × 2 (group × arm × muscle [flexors and extensors] × time) factorial ANOVA followed by further assessing the significant two- and three-way interactions appropriate for the research questions related to the muscle specificity. Muscle size data were also split by arm (immobilized, non-immobilized) and muscle (for muscle thickness; flexors, extensors) for several breakdown analyses to better understand the trained limb vs. the CE sparing effect.

Greenhouse-Geisser (GG) adjustments were used for violations of sphericity. Breakdown analyses followed where significant interactions were detected. Data analysis was completed using SPSS version 24. Significance was accepted at $p < 0.05$.

CHAPTER 3

RESULTS

3.0 RESULTS

3.1 Demographics

There were no participant dropouts during the study and all participants maintained 100% adherence to the training protocol, having attended all 12 training sessions. One participant reported having eczema in the palm of the immobilized hand, the cast was trimmed (cut off approximately 1 cm at the distal end of the cast) to allow for treatment of the eczema without compromising the quality of immobilization. There were no significant differences between groups for height, $F(1,14)=.047$, $p=.831$, weight, $F(1,14)=646$, $p=.435$, training experience, $F(1,14)=.087$, $p=.773$, or handedness, $F(1,14)=.821$, $p=.380$ (table 1).

3.2 Muscle Strength

The five-factor interaction for strength data did not reach significance, $F(2,28)=3.151$, $p=.058$, $\eta_p^2=.184$. Significant three-way interactions were found for arm × type × task, $F(2,28)=3.447$, $p=.046$, $\eta_p^2=.198$, group × task × time, $F(1,14)=5.263$, $p=.038$, $\eta_p^2=.273$, and arm × task × time, $F(1,14)=7.027$, $p=.019$, $\eta_p^2=.334$.

The significant group × task × time interaction indicates that the CE effect was specific only to the trained homologous muscle group. To simplify the interpretation, data were separated by task and arm, and collapsed across type. Significant group × time interactions were observed for the left, $F(1,14)=4.653$, $p=.049$, $\eta_p^2=.249$, and right wrist flexors, $F(1,14)=5.236$, $p=.038$, $\eta_p^2=.272$. The mean changes for right wrist flexion strength were significantly different between the training (30.8%) and control (-7.4%) groups. For the immobilized left arm the changes for wrist flexion strength were significantly different between training (-2.4%) and control (-21.6%) groups. There were no group differences for the changes in wrist extension. These current data and three-way interactions suggest that CE was not specific to the trained contraction type (ECC), illustrated by the lack of the significant interactions including either group, time, or both

with the factor of type. Please refer to figure 1 and table 2 for strength results. Further analyses and detailed reporting of the five-factor design for strength are found in appendix D.

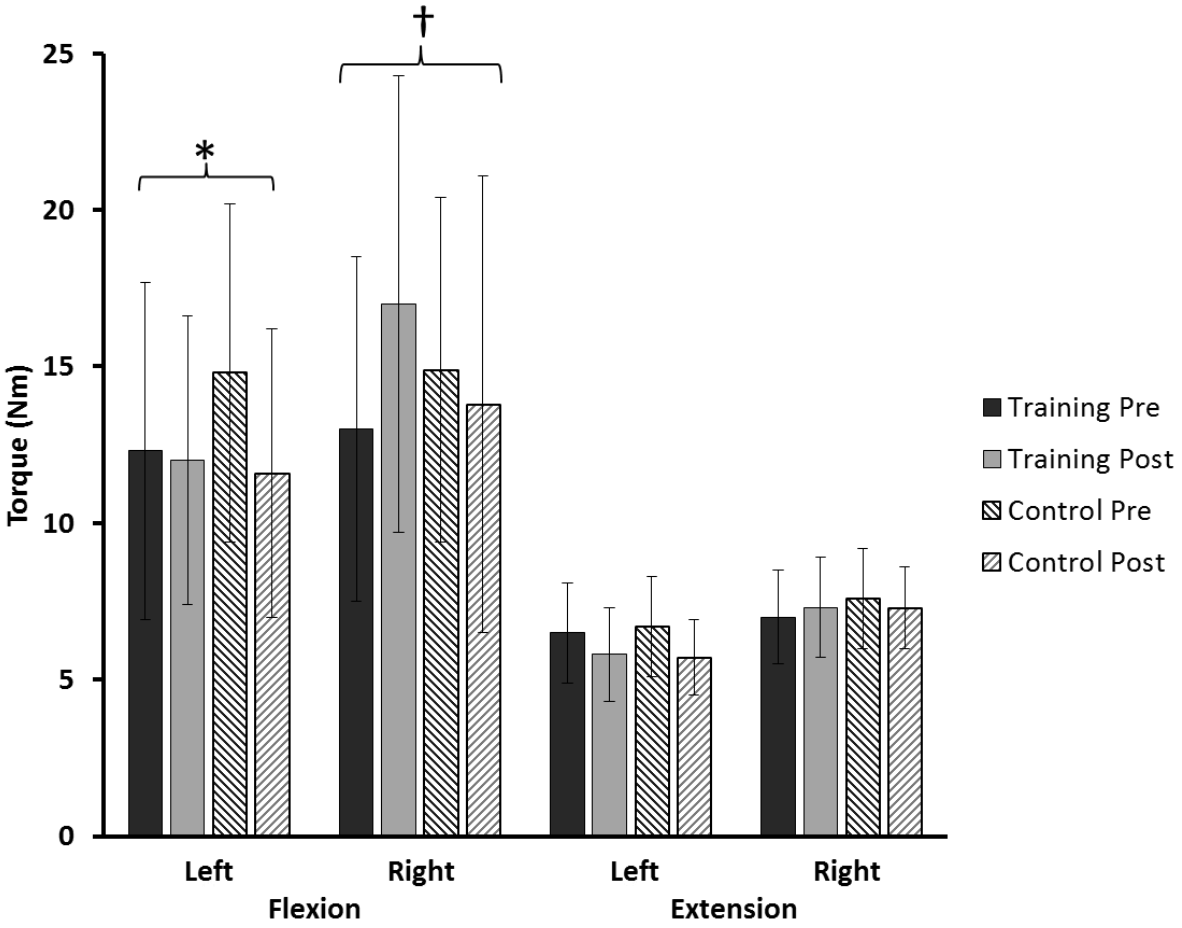

Figure 1. Torque changes for wrist flexion and extension tasks averaged across contraction types (ISO, CON, ECC) from pre- to post-testing for the training groups. The left arm was immobilized during training. Data represents mean ± SD in Nm.

* Significant group × time interaction, $p<0.05$

† Significant group × time interaction, $p<0.05$

Table 2. Strength Changes for Contraction Type and Task

Contractions			Training			Control		
Type	**Task**	**Arm**	**Pre**	**Post**	**% Δ**	**Pre**	**Post**	**% Δ**
ISO	Flexion	Left	12.5 ± 5.3	12.0 ± 4.0	-4.0	15.0 ± 6.7	11.2 ± 5.3	-25.3
		Right	12.3 ± 4.0	16.3 ± 7.1	32.5	14.2 ± 6.9	13.0 ± 6.1	-8.5
	Extension	Left	6.2 ± 1.6	5.3 ± 1.6	-14.5	6.3 ± 1.6	5.5 ± 1.1	-12.7
		Right	6.9 ± 1.6	7.1 ± 1.8	2.9	7.2 ± 1.6	7.1 ± 1.2	-1.4
CON	Flexion	Left	9.4 ± 2.7	9.5 ± 4.1	1.1	11.7 ± 5.4	8.7 ± 4.7	-25.6
		Right	10.9 ± 4.2	13.3 ± 6.2	22.0	11.8 ± 5.8	11.1 ± 6.3	-5.9
	Extension	Left	5.1 ± 1.3	4.5 ± 1.4	-11.8	5.6 ± 1.1	4.6 ± 1.1	-17.9
		Right	5.5 ± 1.2	5.9 ± 1.1	7.3	6.3 ± 1.5	6.1 ± 1.2	-3.2
ECC	Flexion	Left	14.9 ± 4.7	14.7 ± 4.7	-1.3	17.8 ± 7.6	15.0 ± 5.5	-15.7
		Right	15.7 ± 4.4	21.3 ± 9.6	35.7	18.7 ± 7.9	17.4 ± 9.1	-7.0
	Extension	Left	8.1 ± 2.4	7.5 ± 2.1	-7.4	8.3 ± 2.2	7.1 ± 1.8	-14.5
		Right	8.7 ± 1.8	9.1 ± 2.0	4.6	9.3 ± 1.9	8.9 ± 1.9	-4.3
Pooled	Flexion	Left	12.3 ± 5.4	12.0 ± 4.6	-2.4	14.8 ± 5.4	11.6 ± 4.6	-21.6
		Right	13.0 ± 5.5	17.0 ± 7.3	30.8	14.9 ± 5.5	13.8 ± 7.3	-7.4
	Extension	Left	6.5 ± 1.6	5.8 ± 1.5	-10.8	6.7 ± 1.6	5.7 ± 1.2	-14.9
		Right	7.0 ± 1.5	7.3 ± 1.6	4.3	7.6 ± 1.6	7.3 ± 1.3	-3.9
Pooled		Left	9.4 ± 3.3	8.9 ± 2.9	-5.3	10.8 ± 3.3	8.7 ± 2.9	-19.4
		Right	10.0 ± 3.5	12.2 ± 4.3	22.0	11.3 ± 3.5	10.6 ± 4.3	-6.2

Data represents Means ± SD in Nm and % change. There were no significant differences between contraction type.

3.3 Electromyography

The six-factor omnibus factorial ANOVA failed to reach significance, $F(2,28)=.513$, $p=.604$, $\eta_p^2=.035$. Significant interactions were observed for muscle × type × task × time, $F(2,28)=4.213$, $p=.025$, $\eta_p^2=.231$, and arm × time, $F(1,14)=5.526$, $p=.034$, $\eta_p^2=.283$.

There were no significant between-groups differences in muscle activation changes measured by EMG normalized to M_{max}. There were no significant interactions that included the group factor, indicating that there were no between group differences in the current study. The significant arm × time interactions indicated that the muscle activation in the left, immobilized limb declined due to immobilization (training: pre: .093 ± .03, post: .091 ± .03; control: pre: .120

± .03, post: .082 ± .03), compared to the right arm (training: pre: .100 ± .04, post: .111 ± .04; control: pre: .102 ± .04, post: .103 ± .04) regardless of contraction type, task, muscle, or group. Please refer to figure 2 for EMG results and appendix E for additional analyses. Raw data is included in table 3 (flexion EMG) and table 4 (extension EMG).

M-wave data was stable between pre- and post-testing sessions for the left wrist flexors (pre: 1.49 ± 1.03 mA; post: 1.57 ± 0.99 mA), left wrist extensors (pre: 1.65 ± 1.07 mA; post: 1.60 ± 0.73 mA), right wrist flexors (pre: 1.60 ± 0.96 mA; post: 1.49 ± 1.16 mA), and right wrist extensors (pre: 1.90 ± 0.95 mA; post: 2.04 ± 0.89 mA).

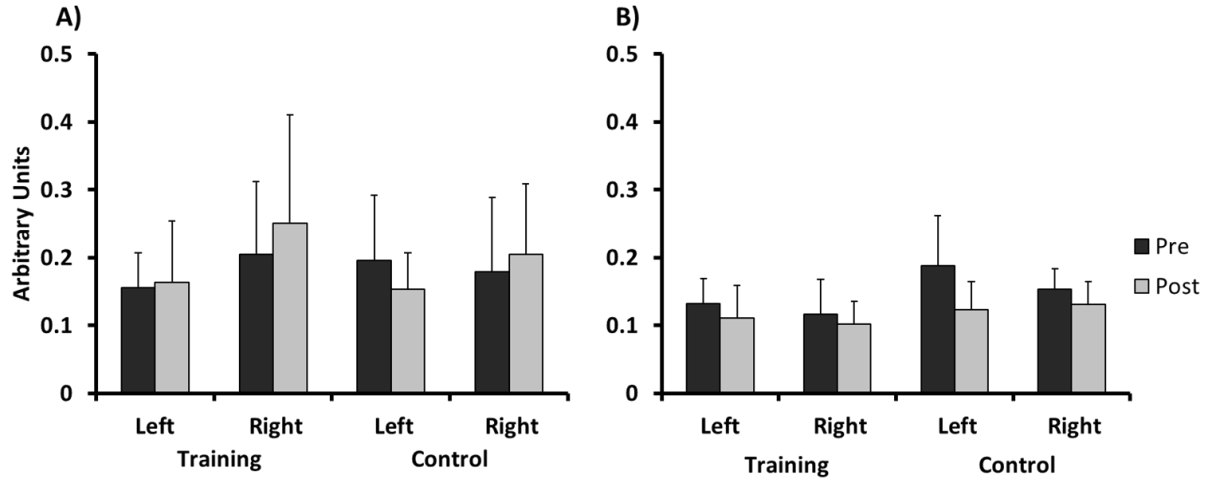

Figure 2. EMG of the agonist muscles normalized to M_{max} for **A)** wrist flexion and **B)** wrist extension tasks, collapsed across contraction type.

Table 3. Flexion EMG Changes

Flexion Contractions			Training			Control		
Type	**Muscle**	**Arm**	**Pre**	**Post**	**% Δ**	**Pre**	**Post**	**% Δ**
ISO	Agonist (FCR)	Left	.163 ± .067	.159 ± .080	-2.5	.189 ± .097	.155 ± .066	-18.0
		Right	.212 ± .122	.273 ± .182	28.8	.182 ± .127	.215 ± .110	18.1
	Antagonist (ECR)	Left	.050 ± .021	.049 ± .025	-2.0	.068 ± .052	.037 ± .027	-45.6
		Right	.046 ± .028	.049 ± .016	6.5	.055 ± .026	.047 ± .028	-14.5
CON	Agonist (FCR)	Left	.137 ± .036	.143 ± .100	4.4	.182 ± .086	.128 ± .057	-29.7
		Right	.190 ± .099	.214 ± .108	12.6	.168 ± .108	.184 ± .109	9.5
	Antagonist (ECR)	Left	.038 ± .020	.037 ± .029	-2.6	.063 ± .046	.025 ± .016	-60.3
		Right	.033 ± .022	.039 ± .013	18.2	.048 ± .017	.038 ± .029	-20.8
ECC	Agonist (FCR)	Left	.168 ± .062	.188 ± .105	11.9	.216 ± .109	.177 ± .054	-18.1
		Right	.215 ± .107	.266 ± .193	23.7	.188 ± .103	.216 ± .099	14.9
	Antagonist (ECR)	Left	.036 ± .018	.057 ± .046	58.3	.069 ± .050	.029 ± .016	-58.0
		Right	.033 ± .019	.047 ± .016	42.4	.047 ± .014	.037 ± .024	-21.3
Pooled	Agonist (FCR)	Left	.156 ± .051	.164 ± .090	5.1	.196 ± .096	.153 ± .054	-21.9
		Right	.205 ± .107	.251 ± .159	22.4	.179 ± .110	.205 ± .104	14.5
	Antagonist (ECR)	Left	.041 ± .017	.047 ± .034	14.6	.067 ± .048	.030 ± .020	-55.2
		Right	.037 ± .023	.045 ± .014	21.6	.050 ± .017	.041 ± .025	-18.0

Data represents means ± SD, % change in normalized units. There were no significant differences between groups regardless of muscle (flexors, extensors), task (flexion, extension) or contraction type (ECC, CON, ISO).

Table 4. Extension EMG Changes

Extension Contractions			Training			Control		
Type	**Muscle**	**Arm**	**Pre**	**Post**	**% Δ**	**Pre**	**Post**	**% Δ**
ISO	Agonist (ECR)	Left	.135 ± .046	.118 ± .060	-12.6	.183 ± .069	.128 ± .052	-30.1
		Right	.111 ± .053	.108 ± .041	-2.7	.158 ± .031	.135 ± .033	-14.6
	Antagonist (FCR)	Left	.046 ± .032	.036 ± .038	-21.7	.029 ± .017	.017 ± .011	-41.4
		Right	.045 ± .041	.051 ± .034	13.3	.025 ± .014	.033 ± .030	32.0
CON	Agonist (ECR)	Left	.128 ± .040	.097 ± .033	-24.2	.191 ± .084	.118 ± .035	-38.2
		Right	.125 ± .054	.106 ± .041	-15.2	.147 ± .028	.128 ± .029	-12.9
	Antagonist (FCR)	Left	.039 ± .024	.044 ± .043	12.8	.029 ± .018	.019 ± .009	-34.5
		Right	.035 ± .025	.036 ± .026	2.9	.024 ± .012	.032 ± .027	33.3
ECC	Agonist (ECR)	Left	.132 ± .039	.118 ± .070	-10.6	.191 ± .072	.123 ± .042	-35.6
		Right	.115 ± .057	.092 ± .031	-20	.153 ± .041	.130 ± .042	-15.0
	Antagonist (FCR)	Left	.041 ± .022	.043 ± .032	4.9	.032 ± .022	.025 ± .021	-21.9
		Right	.045 ± .029	.055 ± .045	22.2	.028 ± .015	.041 ± .029	46.4
Pooled	Agonist (ECR)	Left	.132 ± .037	.111 ± .048	-15.9	.188 ± .074	.123 ± .042	-34.6
		Right	.117 ± .051	.102 ± .034	-12.8	.153 ± .031	.131 ± .034	-14.4
	Antagonist (FCR)	Left	.042 ± .025	.041 ± .034	-2.4	.030 ± .017	.020 ± .014	-33.3
		Right	.042 ± .031	.047 ± .034	11.9	.025 ± .011	.035 ± .028	40.0

Data represents means ± SD, % change in normalized units. There were no significant differences between groups regardless of muscle (flexors, extensors), task (flexion, extension) or contraction type (ECC, CON, ISO).

3.4 Co-Activation of the Non-Training Limb (Mirror Activity)

The mean EMG activity of the left, immobilized, wrist flexors of the training group measured during the first, seventh and 12th training sessions was on average, 5.6% of pre-testing isometric MVC. The mirror EMG activity (normalized to baseline isometric MVC) was not significantly different between sessions one, seven, and 12 (range: .047 ± .017 to .085 ± .046), $F(1.2,8.3)=3.933$, $p=.077$, $\eta_p^2=.360$, between reps 1-8 (range: .051 ± .021 to .061 ± .024), $F(7,49)=.849$, $p=.553$, $\eta_p^2=.108$, or between sets 1-6 (range: .047 ± .026 to .061 ± .022), $F(1.9,13.5)=2.277$, $p=.142$, $\eta_p^2=.245$, during training sessions.

3.5 Muscle Size Measures

3.5.1 Muscle cross sectional area

A three-factor ANOVA revealed a significant interaction for group × arm × time, $F(1,14)=7.328$, $p = .017$, $\eta_p^2 = .344$. To breakdown the three-way interaction, data were split by arm and separate group × time ANOVA tests were run. A significant group × time interaction was detected for the left, immobilized arm only, $F(1,14)=8.383$, $p = .012$, $\eta_p^2 = .375$, indicating that the change in MCSA for the left arm for the training group (1.3%) was different from the change in the control group (-2.3%).

Further analysis involved splitting data by group and running separate arm × time ANOVA tests to compare between arm differences. These analyses revealed a significant arm × time interaction for the control group only, $F(1,7)=2.707$, $p = .019$, $\eta_p^2 = .566$, indicating that the change in the left arm (-2.3%) was different from the change in the right arm (1.2%). For the training group, the change in the left, immobilized arm (1.3%) was not significantly different from the change in the right, trained arm (1.2%). Please refer to figure 3 and table 2 for MCSA results.

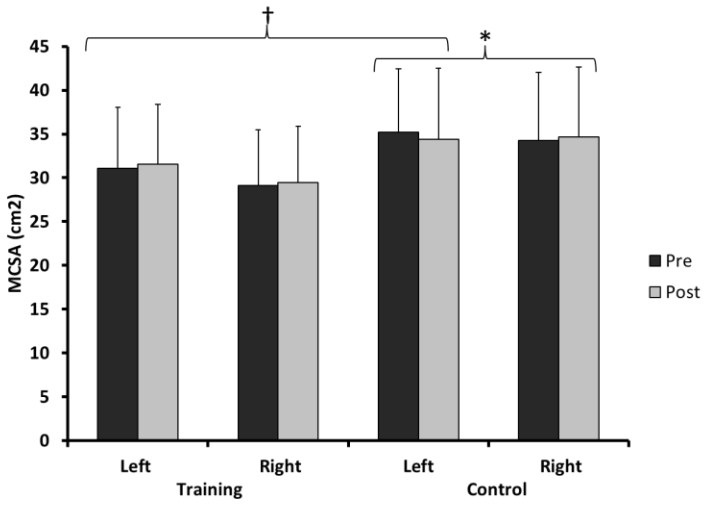

Figure 3. Muscle Cross-Sectional Area (cm^2) changes for the left, immobilized, and right arms of the training and control groups from pre- to post-testing.

* Significant arm × time interaction for control group only, $p < 0.05$

† Significant group × time interaction for the left arm only, $p < 0.05$

3.5.2 Muscle density

The three-factor ANOVA failed to reveal any interactions or main effects for muscle density, $F(1,14)=.008$, $p=.930$, $\eta_p^2=.001$. No changes in muscle density were observed for the left (pre: 75.5 ± 1.5 mg/cm^3; post: 75.9 ± 1.3 mg/cm^3) or right arm (pre: 75.5 ± 1.6 mg/cm^3; post: 75.5 ± 1.4 mg/cm^3) (table 2). Please refer to table 2 for muscle density results.

3.5.3 Muscle thickness

A four-factor omnibus factorial ANOVA revealed a significant interaction for group × arm × muscle × time, $F(1,13)=6.037$, $p=.029$, $\eta_p^2=.317$. To breakdown the four-way interaction, data were split by arm and by muscle, and separate group × time ANOVA tests were run. Significant group × time interactions were found for the right, $F(1,14)=5.825$, $p=.030$, $\eta_p^2=.294$, and left wrist flexors, $F(1,14)=8.864$, $p=.011$, $\eta_p^2=.405$, but not for either wrist extensor muscles, indicating sparing effects were specific to the trained homologous muscle group only.

Data were then split by group and separate arm × muscle × time ANOVA tests were run. A significant arm × muscle × time interaction was detected for the training group only, $F(1,7)=15.185$, $p=.006$, $\eta_p^2=.684$. Further breakdown of the three-way interaction for the training group involved splitting these data by muscle (flexors, extensors), but this did not reveal any arm × time interactions.

The ANOVA tests for muscle thickness revealed that the change in the untrained, immobilized left flexors of the training group (3.0%) was significantly different from control (-3.4%). The tests also revealed that the change in the right flexors of the training group (6.9%) was different from the control group (-2.9%). Please refer to figure 4 and table 2 for muscle thickness results and appendix D for further analyses and detailed reporting of the four-factor design for muscle thickness.

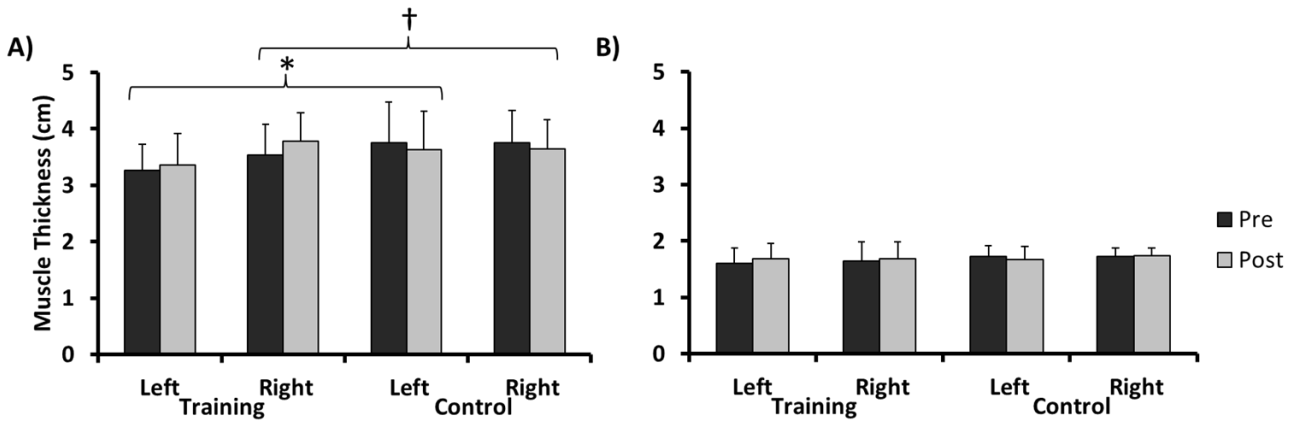

Figure 4. Changes in muscle thickness (cm) for **A)** wrist flexors and **B)** wrist extensors between groups.

* Significant group × time interaction for the left wrist flexors $p<0.05$

† Significant group × time interaction for the right wrist flexors $p<0.05$

Table 5. Muscle Size Changes

Group	Arm	Measure	Pre	Post	% Δ
Training	Left	Flexor MT	3.27 ± 0.46	3.36 ± 0.56	3.0
		Extensor MT	1.60 ± 0.27	1.68 ± 0.28	4.8
		MCSA	31.10 ± 6.95	31.52 ± 6.87	1.3
		MD	75.84 ± 1.17	76.42 ± 0.80	0.8
	Right	Flexor MT	3.53 ± 0.55	3.78 ± 0.51	6.9
		Extensor MT	1.65 ± 0.33	1.69 ± 0.29	2.7
		MCSA	29.13 ± 6.32	29.48 ± 6.40	1.2
		MD	75.94 ± 1.12	76.11 ± 1.15	0.2
Control	Left	Flexor MT	3.75 ± 0.73	3.63 ± 0.68	-3.4
		Extensor MT	1.72 ± 0.20	1.67 ± 0.24	-2.9
		MCSA	35.18 ± 7.23	34.38 ± 8.12	-2.3
		MD	75.07 ± 1.82	75.33 ± 1.65	0.4
	Right	Flexor MT	3.76 ± 0.57	3.65 ± 0.51	-2.9
		Extensor MT	1.72 ± 0.16	1.74 ± 0.14	1.5
		MCSA	34.29 ± 7.71	34.66 ± 7.96	1.1
		MD	75.02 ± 1.94	74.94 ± 1.64	-0.1

MD = Muscle density, MCSA = Muscle cross-sectional area, MT = Muscle thickness

Data are Mean ± SD and % change

CHAPTER 4

DISCUSSION AND CONCLUSION

4.0 DISCUSSION

A recent review by Hendy and Lamon (2017) identified the need for further investigation into the sparing effects of CE with novel approaches to understanding the CE phenomenon. By improving our understanding of how transfer effects for strength and size occur with immobilization, we can begin to bridge the gap between current CE research and its application in clinical practice.

This was the first study to investigate the specificity of CE sparing effects with immobilization and to identify muscle size sparing effects with pQCT; considered a robust measure for muscle size adaptations and recommended for researching CE sparing effects (Hendy & Lamon, 2017). In the present study, CE sparing effects of muscle strength were specific to the trained homologous muscle group in the contralateral limb. Although specific to the homologous muscle group, the transfer effects were not specific to contraction type. ECC wrist flexion training with the non-immobilized limb preserved ECC, CON and ISO strength in the contralateral, immobilized wrist flexors. Another important finding was the confirmation of muscle size sparing effects with both ultrasound and pQCT, supporting previous observations of muscle size preservation (Farthing et al., 2009; Magnus et al., 2010; Pearce et al., 2013).

4.1 Muscle Strength

In prior CE sparing studies, the average strength improvement in the trained limb was ~24%, which was accompanied by preservation of strength in the untrained, immobilized limb. The average decrease in immobilized limb strength for the non-training control groups in prior CE sparing studies was ~12%, therefore the CE sparing effect can be estimated as about half of the trained limb effect (Farthing et al., 2009; Farthing et al., 2011; Magnus et al., 2010; Pearce et al., 2013). As previously mentioned, the magnitude of CE of strength is typically ~50% of the trained limb gains in studies not involving immobilization models (Carroll et al., 2006). The current data merges well with prior CE sparing studies, where immobilized wrist flexors showed a non-significant change in strength of 0.3%, and the non-training control group showed a

significant strength decline of 13.2% in the immobilized limb (Farthing et al., 2009; Farthing et al., 2011; Magnus et al., 2010; Pearce et al., 2013). Therefore, the combined evidence suggests the sparing effect of CE amounts to ~12% for arm immobilization protocols of 3-4 weeks duration.

Previous research has found CE to be highly specific, transferring only to the contralateral homologous muscle group, with the same velocity or joint angle of the training in the opposite limb (Farthing & Chilibeck, 2003a; Seger et al., 1998; Weir, Housh, Weir, & Johnson, 1995). Novel to the current study, the intent was to investigate muscle size and strength in the wrist flexor and extensor muscles of the trained and contralateral, immobilized arm. CE attenuated strength loss in the contralateral homologous muscle group (i.e. immobilized wrist flexors). This finding is congruent with muscle specificity of CE effects in studies without immobilization and indicates that the mechanisms of CE specificity are likely unaltered in the presence of immobilization with respect to the contralateral homologous muscle group. It was postulated that immobilization could alter the specificity of transfer effects because of the known impact of immobilization on decreasing excitability and plasticity in several cortical regions including the primary motor cortex (M1) and primary somatosensory cortex (S1) (Burianová et al., 2016; Clark et al., 2008; Huber et al., 2006; Lissek et al., 2009; Opie et al., 2016); important regions of interest for mechanisms of CE (Ruddy & Carson, 2013).

Unilateral training with ECC muscle actions results in greater CE of strength in the contralateral homologous muscle compared to CON or ISO muscle actions (Farthing & Chilibeck, 2003a; Hortobágyi et al., 1997). Hortobágyi et al. (1997) observed a greater global training effect when training with ECC muscle actions compared to CON, meaning that both CON and ISO strength improved, albeit to a lesser extent, in the contralateral homologous muscle from the ECC training. Training with CON muscle actions did not transfer well to other contraction types. In the present study, participants in the training group attended three sessions per week of isolated ECC wrist flexion actions, which resulted in a similar increase in strength of the right wrist flexors across all contraction types (figure 1). Additionally, a preservation of strength regardless of contraction type was observed in the left, immobilized wrist flexors. In contrast, the control group declined across all strength types in the left immobilized limb for wrist flexion. ECC muscle actions compared to CON and ISO are known to cause greater

increases in intracortical facilitation and larger decreases in intracortical inhibition (Howatson et al., 2011; Kidgell et al., 2015). This could be one explanation for larger transfer effects with ECC, and may contribute to the observed global strength sparing in the contralateral homologous muscle group in the current study.

4.2 Electromyography

EMG findings of voluntary activation in previous CE sparing literature is mixed, with Farthing et al. (2009) not finding any significant differences over time for either arm in any group. Farthing et al. (2011) found a significant group × time interaction for the agonist muscle group, indicating that regardless of arm, the change in muscle activation between groups was different. Magnus et al. (2010) only observed changes in muscle activation in the non-immobilized limb between groups. Pearce et al. (2013) did successfully use TMS and EMG to identify a maintenance of corticospinal excitability to the immobilized limb after unilateral training in the opposite limb. The findings from the previous literature examining EMG of voluntary activation provide little evidence nor consistency to aid in the identification of CE sparing mechanisms. In the present study the EMG data did not reveal any significant differences between groups for any of the tested contraction types or tasks (i.e., flexion, extension). Of note, figure 2 displays a non-significant sparing effect of the left wrist flexors of the training group during muscle activation for flexion tasks pooled across contraction types compared to a non-significant decline in muscle activation for the control group. Although these changes in muscle activation were too small to reach significance, the direction of change supports CE specificity of sparing effects. It is important to note that these findings are inconclusive and do not provide evidence to support the observed strength and size specificity and sparing effects in this study, and further investigation with a larger sample size may shed light on specific mechanisms.

4.3 Mirror Activity

Although the concept of CE specificity is widely accepted amongst CE literature, one study found a strength increase in both the agonist and antagonist muscle of the untrained limb (Sariyildiz, Karacan, Rezvani, Ergin, & Cidem, 2011). Although a single study is not enough to alter the widely accepted hypothesis of homologous muscle specificity with CE, it was important to consider the possible mechanisms that contributed to this result. A study by Post et al. (2009)

observed that mirror activity in the contralateral limb from unilateral contractions reflected the direction of the target movement and was not confined to the homologous muscle. In the Sariyildiz et al. (2011) study, the contralateral, untrained arm was placed in the opposite orientation to that of the trained limb. It is possible that directional mirror activity could have impacted the strength adaptation in the contralateral antagonist muscle. Therefore, the current study took into account the possible impact directional mirror activity may have on CE and controlled for this by standardizing the contralateral limb placement during testing and training sessions.

Muscle activation at levels as low as 10% 1-RM have been shown to increase strength (Laidlaw, Kornatz, Keen, Suzuki, & Enoka, 1999). Farthing et al. (2011) proposed that, although unlikely, it is possible for the CE effects observed in past literature to be attributed to high levels (>10% MVC) of mirror activity during the unilateral training intervention in the opposite limb, because mirror activity was not monitored under the cast. The mirror activity reported in the current study averaged 5.6% of isometric MVC. Of the four studies to investigate the CE sparing effects in healthy participants, only Magnus et al. (2010) monitored mirror activity. Both Magnus et al. (2010) and the current data show CE training producing low levels of mirror activity in the immobilized limb. Although unlikely, it is still possible that the reported 5.6% in the current study and the 3.1% (Biceps brachii) and 6.1% (Triceps brachii) reported in the Magnus et al. (2010) study contributed to some, but not all of the observed sparing effects of size and strength in the immobilized limb. Therefore, the current belief that cortical contributions are primarily responsible for the transfer effects, particularly for strength, remains viable (Ruddy & Carson, 2013).

4.4 Muscle Size

Three of the previous four studies that investigated the sparing effects of CE with healthy immobilization found a preservation of muscle size (Farthing et al., 2009; Magnus et al., 2010; Pearce et al., 2013). Prior to this study, the muscle size sparing effect was only observed using ultrasound measures of muscle thickness. Although the use of ultrasound for muscle thickness assessment is valid, verifying muscle size sparing effects with a more precise measure is critical for confidence in interpreting previous findings (Hendy & Lamon, 2017). Investigating the CE sparing effects for muscle thickness revealed muscle specific effects to contralateral homologous

wrist flexors only (figure 4). However, the direction of mean change, large effect size (η_p^2=.206) and a p =.077 for the immobilized wrist extensors is not definitive and should be re-investigated with an increased sample size and a more precise measure that allows the investigation of individual muscle adaptations, such as MRI.

Further supporting the preservation of muscle thickness in the immobilized arm for the training group only was the confirmation of muscle size preservation with the MCSA analysis (figure 3). In the current study, MCSA data were collected by researchers from a collaborating lab who were blinded to group assignment. The blinding was a strength of this study and increases confidence in data interpretation. The observations that CE effects indeed impact muscle size changes with immobilization, confirmed by two measures (ultrasound and pQCT) from separate labs increases confidence and have substantial implications for clinical application of CE interventions. After analyzing the muscle density data as an exploratory measure, there were no changes in either group over the course of this study. To date only one other study has investigated the effects of resistance training on muscle density derived from pQCT (Duff et al., 2017). Duff et al. (2017) observed no change in forearm muscle density after participants performed resistance training three times per week for nine months. Considering the outcome from Duff et al. (2017) for forearm muscle density after nine months of training, it is not entirely surprising that no significant changes in forearm muscle density were detected with a relatively short, four weeks, intervention. Of note, the image resolution of pQCT prevents the reliable separation of specific muscle groups and caution is needed with the interpretation of these data because the origin of the muscle size sparing effects cannot be identified.

This study provides novel insight into possible mechanisms of CE sparing. Currently the dominant theories of CE effects do not account for the possibility of muscle size adaptations, and have proposed possible mechanisms for strength and skill transfer effects to reside primarily in the brain. This is understandable since CE effects typically do not present with evidence of alterations in muscle volume (Farthing, 2009; Lee & Carroll, 2007; Ruddy & Carson, 2013), especially without immobilization. There is no apparent candidate mechanism that accounts for muscle size changes with CE in the untrained limb, unless there is concurrent evidence of direct voluntary or involuntary muscle activation in the non-training limb greater than or equal to 15.5% of 1-RM (Holm et al., 2008). Holm et al. (2008) found that training with 15.5% 1-RM for

12 weeks was able to induce small amounts of hypertrophy in the trained limb. CE is thought to be a neural phenomenon where changes in cortical processes and motor engrams positively impact the neural drive to the contralateral limb (Farthing et al., 2007; Ruddy & Carson, 2013). With that, these findings shed new light on possible mechanisms of muscle size preservation in an immobilized homologous contralateral muscle after unilateral strength training. The possibility that a peripheral mechanism previously thought to not be involved in CE sparing effects may be neutrally or independently activated and contributing to the observed preservation of strength and size in the homologous contralateral muscle. At least it suggests the preservation of muscle strength and size via CE are related, but probably driven by both overlapping and independent mechanisms.

The regulation of muscle atrophy with immobilization or disuse occurs through two primary processes; muscle protein breakdown (MPB) and a decrease in muscle protein synthesis (MPS), with a decrease in MPS found to be the leading mechanism (Phillips & McGlory, 2014). While the mechanisms of muscle size preservation with CE are currently unknown, and a direct connection between neural contributions and the regulation of MPS and MPB is not clear, it remains possible that the unilateral ECC training of the wrist flexors in the present study led to muscle size preservation in the contralateral limb by influencing the balance of protein regulation. One possible mechanism may be in the neural regulation of the protein kinase B (AKT) and mammalian target of rapamycin (mTOR) pathway. AKT and mTOR are important protein complexes that play a role in the modulation of gene expression, cell development, growth and survival and are upregulated in the nervous system during cellular stress (Maiese, 2014; Zhao et al., 2005). The AKT and mTOR pathway is upregulated with skeletal muscle hypertrophy and downregulated with muscle atrophy caused by disuse (Bodine et al., 2001). Investigating AKT and mTOR pathway modulation with CE to an immobilized limb may aid in understanding the muscle size sparing effects observed. Another possible, yet unlikely, mechanism of muscle size sparing with CE is a systemic release of myokines after resistance training that can initiate satellite cells proliferation (Belizário, Fontes‐Oliveira, Borges, Kashiabara, & Vannier, 2016). However, if this potential mechanism was involved, it would not likely result in muscle specificity because the systemic release would have an effect on the wrist extensor muscles as well. In the present study there was a non-significant trend for wrist extensor muscle sparing in addition to the sparing of the wrist flexors. However, the muscle sparing

effects were found to be specific (wrist flexors not extensors) making the impact of myokines on CE sparing effects relatively unlikely. A logical next step in understanding these sparing effects is to investigate peripheral neuromuscular physiology through muscle biopsies and indwelling needle electromyography to precisely assess the changes in excitation-contraction coupling, MPS, MPB, gene-expression (AKT, mTOR), fibre type ratios and other morphological factors that may impact muscle size and strength of the untrained immobilized limb. Further to this, there is data from studies on the repeated bout effect after unilateral eccentric exercise to suggest that the contralateral homologous muscle's response to subsequent damaging eccentric exercise is altered by prior eccentric exercise of the ipsilateral limb (Chen, Chen, Lin, Yu, & Nosaka, 2016). Therefore, it is plausible to suggest that chronic unilateral eccentric training could involve protective mechanisms that attenuate the time course of disuse atrophy in an opposite immobilized limb by altering the balance of MPS and MPB, or by inducing cellular adaptations to transcription factors such as nuclear factor [kappa] B (NF-kB) which regulate cell survival (Xin, Hyldahl, Chipkin, & Clarkson, 2014). Chen et al. (2016) also suggest that eccentric exercise of the healthy limb (i.e. CE effects) prior to engaging in post-immobilization rehabilitation of an opposite injured limb might expedite recovery because immobilized muscles are more susceptible to eccentric muscle damage.

4.5 Limitations and Future Directions

There were several limitations in the present study that must be taken into consideration going forward. The sample size (n=16) was small and a larger sample size could decrease variability and increase confidence in findings. The use of EMG, even when normalized to M_{max}, failed to aid in the identification of precise mechanisms driving the CE sparing effects. EMG is a crude measure and it is difficult to extrapolate what is occurring within the nervous system from the muscle activation occurring under the surface of an electrode covering only part of a muscle. Data collection for muscle thickness and strength measures were conducted by the primary researcher whom was not blinded to group assignment. The potential for researcher bias for those measures is increased by the lack of blinding in the current study. The use of fMRI and TMS to investigate the specificity of CE sparing effects would provide clear evidence of the neural mechanisms contributing to the observed sparing effects of strength and size. Although the four-week immobilization period was longer than most of the previous CE sparing literature

(Farthing et al., 2009; Farthing et al., 2011; Pearce et al., 2013), a longer immobilization period would have been beneficial to increase the severity of strength loss and atrophy in the immobilized limb. Immobilization due to unilateral injury may be prescribed for durations longer than four weeks, and to improve the clinical relevance of these CE sparing effects, longer immobilization periods are recommended for future research. Due to the low resolution of pQCT, specific muscles cannot be differentiated in the MCSA images. Therefore, future research would benefit from using magnetic resonance imaging (MRI) to obtain higher resolution images of MCSA. The use of MRI to assess muscle size adaptations would allow for the use of a single measure to determine the specificity of the sparing effects, rather than the combination of ultrasound and pQCT as was done in the present study. While mirror activation in the immobilized limb was monitored during three testing sessions, the muscle activity was only monitored in the wrist flexors. Future research investigating the specificity of CE may benefit by recording EMG of both the contralateral agonist (wrist flexors) and antagonist (wrist extensors) muscles and the lack of wrist extensor monitoring is a noted limitation to this study. A final limitation to this study was the use of the wrist joint model for investigating specificity of CE sparing effects. Although the wrist is clinically relevant, there are several muscles that make up the wrist flexors and extensors, making data collection and analysis difficult for EMG recording and muscle imaging techniques. Specifically with regards to MCSA the images recorded by pQCT are pixilated and do not show individual muscles, making it impossible to differentiate between the flexors and extensors of a wrist/forearm. Future research should investigate the specificity of muscle size sparing with pQCT in an elbow joint where the elbow flexors and extensors are distinctly separated.

4.6 Conclusion

The present study provides novel insight into the specificity of CE sparing effects in an immobilized limb and draws a link between strength and size with CE sparing effects. The finding that immobilized limb strength was preserved across contraction types (i.e. ECC, CON, ISO) for the contralateral homologous muscle (i.e. wrist flexors) after only training with ECC muscle actions is intriguing from a clinical perspective and confirms that immobilization does not alter the CE specificity effects that have been previously reported in non-immobilization CE research. There were no direct measures of cortical (TMS or fMRI) or corticospinal (TMS)

adaptations in the present study, therefore, no definitive conclusions can be made with these data to support either the cross-activation hypothesis or the bilateral-access hypothesis (Ruddy & Carson, 2013). The lack of contraction type specificity coupled with a trend towards increased muscle activation in the agonist and antagonist pairs for the flexion movements measured by surface EMG may support the cross-activation hypothesis. Cross-activation is thought to be involved in increasing strength and corticospinal excitability to the untrained contralateral limb, whereas bilateral-access is predicted to be involved in motor learning tasks (Ruddy & Carson, 2013). Therefore, the lack of contraction type differentiation and apparent trend towards increased corticomotor drive to the agonist and antagonist pairs better aligns with the cross-activation hypothesis. These two findings have clinical implications, in that ECC muscle actions trained in a healthy limb will preserve strength across multiple contraction types and muscle size. While ECC training could be preferable to other modes, rehabilitative exercise should focus on complete joint symmetry by training both agonist and antagonistic pairs.

Importantly, this study confirms previous observations of muscle size sparing effects with CE in healthy immobilization (Farthing et al., 2009; Magnus et al., 2010; Pearce et al., 2013) with the use of pQCT (MCSA) and ultrasound. Although, the cause of the muscle size sparing remains unclear, the confirmation of this phenomenon brings new insight into possible contributing mechanisms of CE sparing effects. The possibility of a peripheral, muscle site specific, mechanism warrants further investigation. Regardless of the mechanisms at play, CE appears to be a relevant and practical exercise modality to attenuating the loss commonly associated with immobilization and is viable for consideration in clinical settings such as unilateral orthopedic or neurological injury.

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APPENDIX A

APPENDIX A

Table 6. Coefficient of Variations for Measured Variables

Measure	Limb	Task/Muscle	Type	CV %
Peak Torque	Left	Flexion	ECC	9.8
			CON	19.5
			ISO	7.7
		Extension	ECC	4.7
			CON	8.1
			ISO	6.4
	Right	Flexion	ECC	11.7
			CON	12.1
			ISO	9.7
		Extension	ECC	4.6
			CON	7.7
			ISO	11.1
Muscle Thickness	Left	Flexors		4.4
		Extensors		6.3
	Right	Flexors		3.4
		Extensors		4.6
MCSA	Based on prior testing from independent lab			1.8
Muscle Density	Based on prior testing from independent lab			1.2
M-wave	Left	Flexor		19.7
		Extensor		21.8
	Right	Flexor		29.6
		Extensor		22.7

APPENDIX B

APPENDIX B

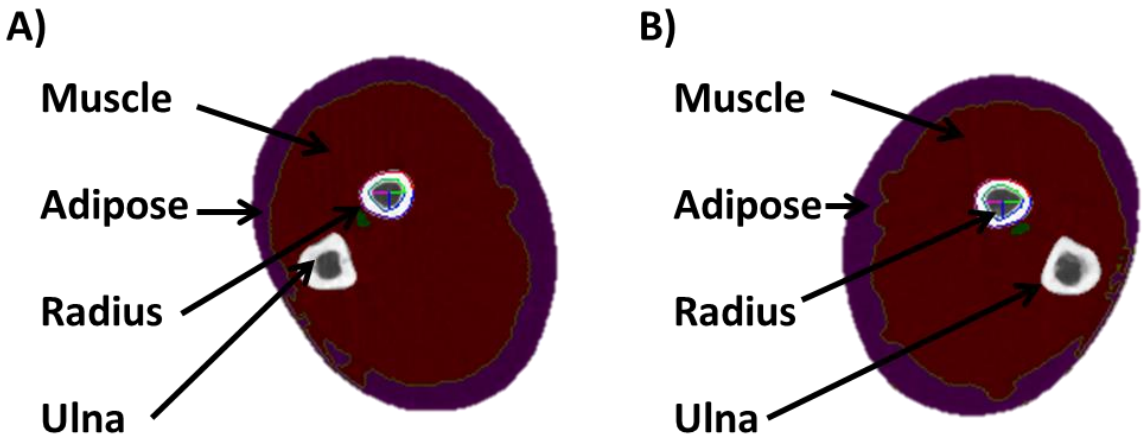

Figure 5. PQCT muscle cross-sectional area images measured of the forearm captured at 65% of the forearm length from the distance from the distal end of the radius for the **A)** left forearm and **B)** right forearm.

APPENDIX C

APPENDIX C

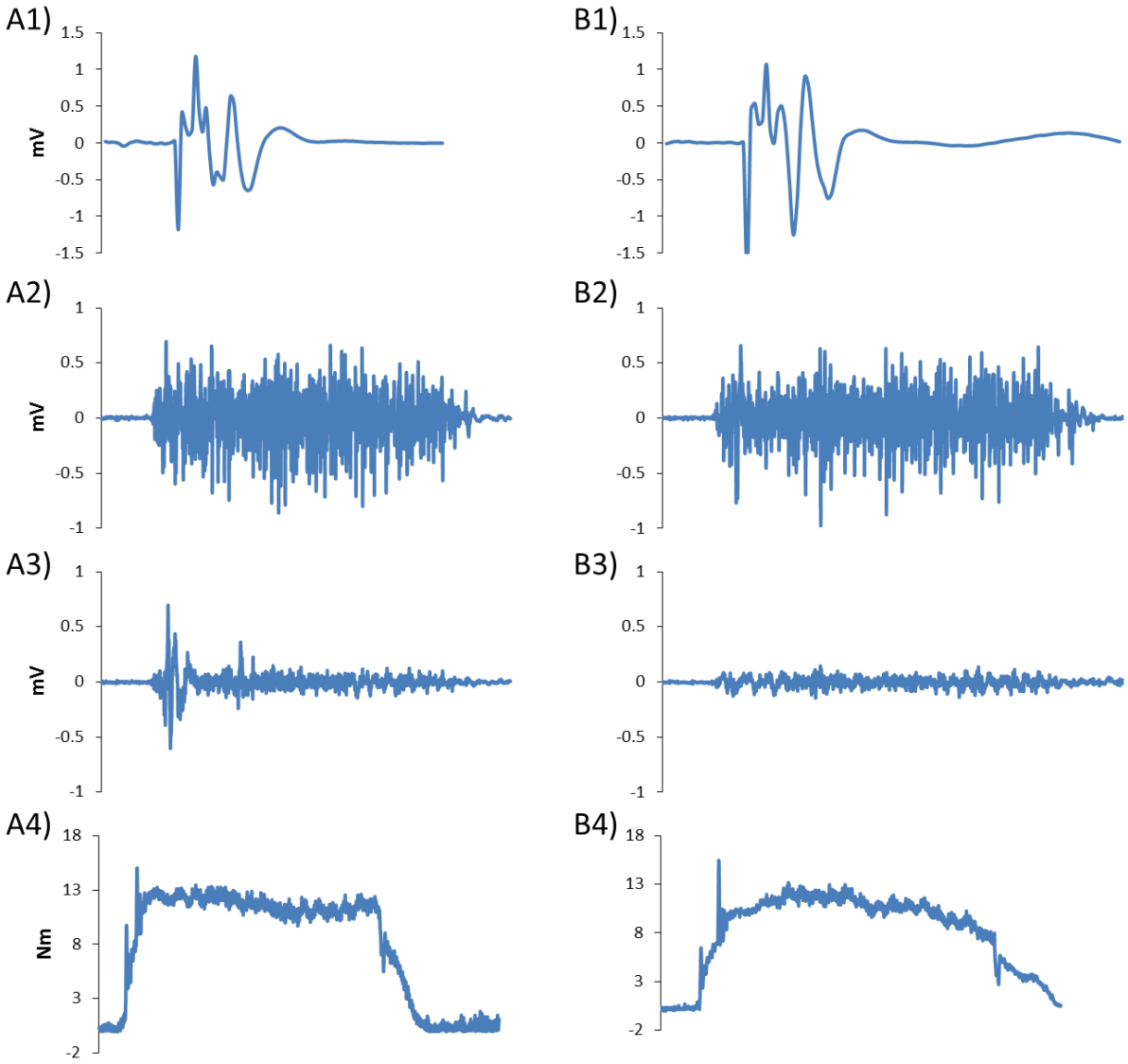

Figure 6. Representative data tracings of the left, immobilized limb from a participant in the training group from **A)** Pre-testing and **B)** Post-testing for **1)** Wrist flexor M-waves, **2)** Agonist (wrist flexors) EMG during an eccentric wrist flexion task, **3)** Antagonist (wrist extensors) EMG during an eccentric wrist flexion task, **4)** Torque tracing of the eccentric wrist flexion task.

APPENDIX D

APPENDIX D

Additional Reporting of Strength Analyses

Interactions and main effects from five-factor ANOVA not reported in main results:

Significant interactions were found for group × time, $F(1,14)=6.496$, $p = .023$, $\eta_p^2=.317$, type × task, $F(1,14)=6.496$, $p = .023$, $\eta_p^2=.317$, arm × time, $F(1,14)=22.671$, $p <.001$, $\eta_p^2=.618$. In addition, main effects of arm, $F(1.3,18.4)=21.043$, $p <.001$, $\eta_p^2=.600$ (GG adjusted), type, $F(1.4,19.3)=112.952$, $p <.001$, $\eta_p^2=.890$ (GG adjusted), and task, $F(1,14)=45.731$, $p <.001$, $\eta_p^2=.766$ were all found to be significant.

Four-factor ANOVA split by arm:

To assess the differences between immobilized and non-immobilized arms, a separate group × type × task × time factorial ANOVA was conducted for each arm separately. A group × time interaction was observed for the left, immobilized arm, $F(1,14)=4.848$, $p =.045$, $\eta_p^2=.257$, and the right arm, $F(1,14)=5.947$, $p=.029$, $\eta_p^2=.298$. The changes in torque between groups showed that the right arm of the training group increased (pre: 10.0 ± 3.5 Nm; post: 12.2 ± 4.3 Nm; 22.0%) while the left, immobilized arm showed no change (pre: 9.4 ± 3.3 Nm; post: 8.9 ± 2.9 Nm; -5.3%). For the control group, the right arm showed no change (pre: 11.3 ± 3.5 Nm; post: 10.6 ± 4.3 Nm; -6.2%) while the left, immobilized arm decreased (pre: 10.8 ± 3.3 Nm; post: 8.7 ± 2.9 Nm; -19.4%) with means pooled across contraction type and task for each arm.

Four-factor ANOVA split by task and then by arm:

The next step was to separate data by task (i.e. wrist flexion, wrist extension) and run separate group × arm × type × time factorial ANOVA. For wrist flexion, significant interactions were found for arm × time, $F(1,14)=14.146$, $p =.002$, $\eta_p^2=.503$, arm × type, $F(2,28)=3.650$, $p =.039$, $\eta_p^2=.207$, and group × time, $F(1,14)=6.008$, $p =.028$, $\eta_p^2=.300$. Main effects of arm, $F(1,14)=10.849$, $p =.005$, $\eta_p^2=.437$, and type, $F(2,28)=74.958$, $p <.001$, $\eta_p^2=.843$ were observed. For wrist extension, a significant arm × time interaction was found, $F(1,14)=30.335$, $p <.001$, $\eta_p^2=.684$, along with a main effect of time, $F(1,14)=12.692$, $p =.003$, $\eta_p^2=.476$, type, $F(1.342,21.293)=81.276$, $p <.001$, $\eta_p^2=.853$, and arm, $F(1,14)=66.562$, $p <.001$, $\eta_p^2=.826$. Subsequently these data were split by arm, and group × type × time factorial ANOVA tests were

run separately for the left and right wrist flexors and extensors. A significant group × time interaction was observed for the left, $F(1,14)=4.653$, $p=.049$, $\eta_p^2=.249$, and right wrist flexors, $F(1,14)=5.236$, $p=.038$, $\eta_p^2=.272$. For the wrist extensor, no group × time interactions were found for the left, $F(1,14)=.948$, $p=.347$, $\eta_p^2=.063$, or right arm, $F(1,14)=4.258$, $p=.058$, $\eta_p^2=.233$.

Additional Reporting of EMG Analyses

Interactions and main effects from the six-factor ANOVA not reported in main results:

Significant interactions were observed for muscle × type × task, $F(2,28)=4.587$, $p=.019$, $\eta_p^2=.247$, type × task, $F(1,14)=8.363$, $p=.001$, $\eta_p^2=.374$, muscle × task, $F(1,14)=160.06$, $p<.001$, $\eta_p^2=.920$, muscle × type, $F(2,28)=10.909$, $p<.001$, $\eta_p^2=.438$ and main effects of task, $F(1,14)=20.442$, $p<.001$, $\eta_p^2=.594$, type, $F(2,28)=15.376$, $p<.001$, $\eta_p^2=.532$, and muscle, $F(2,14)=6.497$, $p=.023$, $\eta_p^2=.317$ were also significant.

Five-factor ANOVA split by task:

Additional analyses included separate group × arm × type × muscle × time tests for each task (wrist flexion, extension). Significant arm × time interactions were found for wrist flexion, $F(1,14)=4.580$, $p=.05$, $\eta_p^2=.246$, and wrist extension tasks, $F(1,14)=5.514$, $p=.034$, $\eta_p^2=.283$.

Additional Reporting of Muscle Thickness Analyses

Interactions and main effects from the four-factor ANOVA not reported in main results:

Significant interactions were observed for group × arm × muscle, $F(1,13)=8.552$, $p=.012$, $\eta_p^2=.397$, group × time, $F(1,13)=10.005$, $p=.007$, $\eta_p^2=.435$, group × arm, $F(1,13)=7.414$, $p=.017$, $\eta_p^2=.363$, and main effects of arm, $F(1,13)=8.552$, $p=.012$, $\eta_p^2=.397$, and muscle, $F(1,13)=293.948$, $p=.000$, $\eta_p^2=.958$.

Three-factor ANOVA split by arm:

Separate group × muscle × time factorial ANOVA tests were run for each arm. For the left, immobilized arm a significant group × time interaction was found between training (pre: 2.4 ± 0.4 cm; post: 2.5 ± 0.4 cm; 4.2%) and control (pre: 2.7 ± 0.4 cm; post: 2.6 ± 0.4 cm; -3.7%),

$F(1,13)=8.492$, $p=.012$, $\eta_p^2=.395$. For the right arm, a significant group × muscle × time interaction was observed, $F(1,14)=4.610$, $p=.050$, $\eta_p^2=.248$, indicating that the right wrist flexors of the training group increased (pre: 3.5 ± 0.6 cm; post: 3.8 ± 0.5 cm; 8.6%), and decreased for the control group (pre: 3.8 ± 0.6 cm; post: 3.7 ± 0.5 cm; -2.6%). Concurrently no change was observed for the right wrist extensors of the training (pre: 1.6 ± 0.3 cm; post: 1.7 ± 0.2 cm; 6.3%) or control groups (pre: 1.7 ± 0.3 cm; post: 1.7 ± 0.3 cm; 0%).

APPENDIX E

APPENDIX E

Table 7. Muscle Strength Measures

	Immobilized		Non-Immobilized		
Study	**Training**	**Control**	**Training**	**Control**	**Strength Measure**
Farthing 2009	2.2%	-14.7%	23.8%	N/A	Ulnar deviation
Magnus et al 2010	N/A	N/A	18.9%	-1.6%	Elbow Flexion
Magnus et al 2010	32.2%	-6.1%	68.1%	1.3%	Elbow Extension
Farthing et al 2011	0.8%	-11%	10.7%	4.1%	Isometric MVC
Pearce et al 2013	2.7%	-5.7%	5.8%	0.2%	Isometric MVC
Pearce et al 2013	-0.1%	-19.9%	13.9%	-4.2%	1 RM Elbow Flexion
Current Study	0.3%	-13.2%	34.2%	-9.2%	Eccentric MVC

APPENDIX F

APPENDIX F

Table 8. Muscle Size Measures

Study	Immobilized		Non-Immobilized		Size Measure
	Training	Control	Training	Control	
Farthing 2009	-1.1%	-4.3%*	N/A	N/A	FCR MT
Magnus et al 2010	2.2%	-2.8%*	N/A	N/A	Biceps Brachii
Magnus et al 2010	3.4%	-5.2%*	7.10%	-1.9%	Triceps Brachii
Farthing et al 2011	-4.72%	-1.67%	-0.54%	0.81%	FCR MT
Pearce et al 2013	0%	-6.0%*	6.05%	-2.8%	Biceps Brachii
Current Study	1.4%	-2.7%*	1.02%	1%	MCSA

* Significantly different between groups

APPENDIX G

APPENDIX G

Table 9. EMG Mirror Activation

Study	Muscle	Immobilized Arm	Normalization Method
Farthing 2009	N/A	N/A	N/A
Magnus et al 2010	Biceps Brachii	3.1 %	Training homologous
Magnus et al 2010	Triceps Brachii	6.1 %	Training homologous
Farthing et al 2011	N/A	N/A	N/A
Pearce et al 2013	N/A	N/A	N/A
Pearce et al 2013	N/A	N/A	N/A
Current Study	Wrist Flexor	5.6 %	Pre-test Immobilized ISO MVC

APPENDIX H

APPENDIX H

WATERLOO HANDEDNESS QUESTIONNAIRE

Instructions: Please indicate your hand preference for the following activities by circling the appropriate response. Think about each question. You might try to imagine yourself performing the task in question. Please take your time.

- If you use one hand 95% of the time to perform the described activity, then circle right always or left always as your response.
- If you use one hand about 75% of the time, then circle right usually or left usually.
- If you use both hands roughly the same amount of time, then circle equally.

1) **Which hand do you use for writing?**
   Left Always Left Usually Equally Right Usually Right always

2) **With which hand would you unscrew a tight jar lid?**
   Left Always Left Usually Equally Right Usually Right always

3) **In which hand do you hold a toothbrush?**
   Left Always Left Usually Equally Right Usually Right always

4) **In which hand would you hold a match to strike it?**
   Left Always Left Usually Equally Right Usually Right always

5) **Which hand would you use to throw a baseball?**
   Left Always Left Usually Equally Right Usually Right always

6) **Which hand do you consider the strongest?**
   Left Always Left Usually Equally Right Usually Right always

7) **With which hand would you use a knife to cut bread?**
   Left Always Left Usually Equally Right Usually Right always

8) **With which hand do you hold a comb when combing your hair?**
   Left Always Left Usually Equally Right Usually Right always

9) **Which hand do you use to manipulate implements such as tools?**
   Left Always Left Usually Equally Right Usually Right always

10) **Which hand is the most adept to picking up small objects?**
   Left Always Left Usually Equally Right Usually Right always

APPENDIX I

APPENDIX I

RESISTANCE TRAINING EXPERIENCE & PREVIOUS INJURY QUESTIONNAIRE

1. If one month of resistance training is considered 3 times per week for 4 weeks, how much resistance training (in months) have you done?
    a. In the previous year? _____
    b. In the past month? _____

2. If you had previous resistance training experience, did this resistance training include any elbow flexion exercises?
    YES NO

3. If you had previous resistance training experience, did this resistance training include any wrist or hand gripping exercises?
    YES NO

4. A. Have you ever experienced an injury to your arm that required immobilization for an extended period of time (i.e. more than one week)?
    YES NO

    B. If yes, what was the injury, when did it occur and what was the duration of this condition?

5. A. Do you have any neurological conditions or injuries to the nervous system that have affected the arms?
    YES NO

    B. If yes, what was the injury, when did it occur and what was the duration of this condition?

APPENDIX J

APPENDIX J

Participant #: _____

Researcher: _____

Cross-Education Specificity of Sparing Effects – Immobilization Study
Humac NORM Dynamometer Participant Set Up Sheet

Chair

Slide				
Rotation	**Teal**		**Black**	
Fore aft				
Back Angle				
Back Pad Tilt				

Forearm Pad

Height	
Mediolateral Positioning	

Dynamometer

Shaft Height				
Shaft Rotation	**Teal**		**Black**	
Tilt				

Handle Grip Attachment

Length	
Grip Orientation	

Ultrasound Measures

Left – Wrist Flexors Pre-Testing #1 _____, _____, _____, _____, _____	Pre-Testing #2 _____, _____, _____, _____, _____	Post-Testing _____, _____, _____, _____, _____
Mean Score =_____	Mean Score =_____	Mean Score =_____
Left – Wrist Extensors Pre-Testing #1 _____, _____, _____, _____, _____	Pre-Testing #2 _____, _____, _____, _____, _____	Post-Testing _____, _____, _____, _____, _____
Mean Score =_____	Mean Score =	Mean Score =
Right – Wrist Flexors Pre-Testing #1 _____, _____, _____, _____, _____	Pre-Testing #2 _____, _____, _____, _____, _____	Post-Testing _____, _____, _____, _____, _____
Mean Score = _____	Mean Score = _____	Mean Score =_____
Right – Wrist Extensors Pre-Testing #1 _____, _____, _____, _____, _____	Pre-Testing #2 _____, _____, _____, _____, _____	Post-Testing _____, _____, _____, _____, _____
Mean Score =_____	Mean Score =_____	Mean Score =_____

Strength Measures

Left – Wrist Flexors ISO MVC Pre-Testing #1 _____, _____, _____	Pre-Testing #2 _____, _____, _____	Post-Testing _____, _____, _____
Mean Score =_____	Mean Score =_____	Mean Score =_____
Left – Wrist Extensors ISO MVC Pre-Testing #1 _____, _____, _____	Pre-Testing #2 _____, _____, _____	Post-Testing _____, _____, _____
Mean Score =_____	Mean Score =	Mean Score =
Right – Wrist Flexors ISO MVC Pre-Testing #1 _____, _____, _____,	Pre-Testing #2 _____, _____, _____,	Post-Testing _____, _____, _____,
Mean Score = _____	Mean Score = _____	Mean Score =_____
Right – Wrist Extensors ISO MVC Pre-Testing #1 _____, _____, _____,	Pre-Testing #2 _____, _____, _____,	Post-Testing _____, _____, _____,
Mean Score =_____	Mean Score =_____	Mean Score =_____
Left – Wrist Flexors CON Pre-Testing #1 _____, _____, _____	Pre-Testing #2 _____, _____, _____	Post-Testing _____, _____, _____
Mean Score =_____	Mean Score =_____	Mean Score =_____
Left – Wrist Extensors CON Pre-Testing #1 _____, _____, _____	Pre-Testing #2 _____, _____, _____	Post-Testing _____, _____, _____
Mean Score =_____	Mean Score =	Mean Score =
Right – Wrist Flexors CON Pre-Testing #1 _____, _____, _____,	Pre-Testing #2 _____, _____, _____,	Post-Testing _____, _____, _____,
Mean Score = _____	Mean Score = _____	Mean Score =_____
Right – Wrist Extensors CON Pre-Testing #1 _____, _____, _____,	Pre-Testing #2 _____, _____, _____,	Post-Testing _____, _____, _____,
Mean Score =_____	Mean Score =_____	Mean Score =_____
Left – Wrist Flexors ECC Pre-Testing #1 _____, _____, _____	Pre-Testing #2 _____, _____, _____	Post-Testing _____, _____, _____
Mean Score =_____	Mean Score =_____	Mean Score =_____
Left – Wrist Extensors ECC Pre-Testing #1 _____, _____, _____	Pre-Testing #2 _____, _____, _____	Post-Testing _____, _____, _____
Mean Score =_____	Mean Score =	Mean Score =
Right – Wrist Flexors ECC Pre-Testing #1 _____, _____, _____,	Pre-Testing #2 _____, _____, _____,	Post-Testing _____, _____, _____,
Mean Score = _____	Mean Score = _____	Mean Score =_____
Right – Wrist Extensors ECC Pre-Testing #1 _____, _____, _____,	Pre-Testing #2 _____, _____, _____,	Post-Testing _____, _____, _____,
Mean Score =_____	Mean Score =_____	Mean Score =_____

Stim Measures

Left Median Nerve - Wrist Flexors		
Pre-Testing #1 _____mA @ M_{max}	Pre-Testing #2 _____mA @ M_{max}	Post-Testing _____mA @ M_{max}

Left Radial Nerve - Wrist Extensors		
Pre-Testing #1 _____mA @ M_{max}	Pre-Testing #2 _____mA @ M_{max}	Post-Testing _____mA @ M_{max}

Right Median Nerve - Wrist Flexors		
Pre-Testing #1 _____mA @ M_{max}	Pre-Testing #2 _____mA @ M_{max}	Post-Testing _____mA @ M_{max}

Right Radial Nerve - Wrist Extensors		
Pre-Testing #1 _____mA @ M_{max}	Pre-Testing #2 _____mA @ M_{max}	Post-Testing _____mA @ M_{max}

APPENDIX K

APPENDIX K

PAWS Announcement

Neural Mechanisms of Sparing Effects in Humans: How can strength training of one arm prevent losses in strength and muscle size in an opposite immobilized arm?

If this question interests you we are currently seeking volunteers (age 18 and older) for a series of research studies to examine an effect called "cross-education". When you strength train one side of your body (arm or leg) the opposite side increases in strength as well. The strength increase in your untrained side is called **"cross-education"**. Recently we have discovered that cross-education can be used to prevent some of the strength and muscle loss that occurs when one arm is immobilized. These findings have important implications for rehabilitation from injury. We are interested in understanding why and how these effects occur.

If you choose to participate you will be asked to wear a cast or splint on one of your forearms that will prevent you from moving your wrist and hand joints. You will be randomly placed into different experimental groups. One or more of these groups will involve strength training. The immobilization will last for a minimum of 3 weeks but not longer than 6 weeks depending on which experimental group you are in. If you are immobilized for 5 or 6 weeks you will be asked wear a removable splint. You will receive an Honorarium for participating in this research.

The total time commitment for these studies ranges from 20-40 hours over the course of 4-8 weeks depending on the study duration and the measures conducted. If you do not do strength training your time commitment will be less. If you would like more information or are interested in participating, please contact:

Justin Andrushko (M.Sc. Student)

Email: justin.andrushko@usask.ca

College of Kinesiology

or

Doug Renshaw (Ph.D. Student)

Email: doug.renshaw@usask.ca

College of Kinesiology

or

Dr. Jon Farthing (966-1068) (Principal Investigator)

Email: jon.farthing@usask.ca

College of Kinesiology

APPENDIX L

APPENDIX L

Phone Call Script

Neural Mechanisms of Sparing Effects in Humans

Hello my name is _____ and I'm working with Dr. Jon Farthing, College of Kinesiology at the University of Saskatchewan, in conducting a series of experiments to examine an effect called "cross-education". When you strength train one side of your body (arm or leg) the opposite side increases in strength as well. The strength increase in your untrained side is called **"cross-education"**. Recently we have discovered that cross-education can be used to prevent some of the strength and muscle loss that occurs when one arm is immobilized. These findings have important implications for rehabilitation from injury. We are interested in understanding why and how these effects occur.

If you choose to participate you will be asked to wear a cast or splint on one of your forearms that will prevent you from moving your wrist and hand joints. You will be randomly placed into different experimental groups. One or more of these groups will involve strength training. The immobilization will last for a minimum of 3 weeks but not longer than 6 weeks depending on which experimental group you are in. If you are immobilized for 5 or 6 weeks you will be asked wear a removable splint. You will receive an Honorarium of $200 for participating in this research upon completion of this study.

The total time commitment for these studies ranges from 20-40 hours over the course of 4-8 weeks depending on the study duration and the measures conducted. If you do not do strength training your time commitment with be less.

At the present time, we are conducting the following experiments, where we are examining _____ *[insert description of details of the particular experiment and which measures will be included, time commitment, etc.].*

Do you have any questions about any of the studies?

Would you like us to send you a copy of the consent form that describes all procedures and risks for the study of your choice?

If you would like more information or are interested in participating, please contact:

Dr. Jon Farthing (966-1068) (Principal Investigator)

Email: jon.farthing@usask.ca

College of Kinesiology

APPENDIX M

APPENDIX M

ID:	INITIALS:	DOB:	SCAN DATE:
Right Arm _____ _____ _____	**Right Drawing:**		
Left Arm _____ _____ _____	**Left Drawing:**		

ID:	INITIALS:	DOB:	SCAN DATE:
Right Arm _____ _____ _____	**Right Drawing:**		
Left Arm _____ _____ _____	**Left Drawing:**		

APPENDIX N

APPENDIX N

UNIVERSITY OF SASKATCHEWAN
College of Kinesiology
KINESIOLOGY.USASK.CA

Neural Mechanisms of Sparing Effects in Humans: How can strength training of one arm prevent losses in strength and muscle size in an opposite immobilized arm?

If this question interests you we are currently seeking volunteers (age 18 and older) for a series of research studies to examine an effect called "cross-education". When you strength train one side of your body (arm or leg) the opposite side increases in strength as well. The strength increase in your untrained side is called **"cross-education"**. Recently we have discovered that cross-education can be used to prevent some of the strength and muscle loss that occurs when one arm is immobilized. These findings have important implications for rehabilitation from injury. We are interested in understanding why and how these effects occur.

If you choose to participate you will be asked to wear a cast or splint on one of your forearms that will prevent you from moving your wrist and hand joints. You will be randomly placed into different experimental groups. One or more of these groups will involve strength training. The immobilization will last for a minimum of 3 weeks but not longer than 6 weeks depending on which experimental group you are in. If you are immobilized for 5 or 6 weeks you will be asked wear a removable splint. You will receive an Honorarium for participating in this research.

The total time commitment for these studies ranges from 20-40 hours over the course of 4-8 weeks depending on the study duration and the measures conducted. If you do not do strength training your time commitment will be less. If you would like more information or are interested in participating, please contact:

Justin Andrushko (M.Sc. Student)

Email: justin.andrushko@usask.ca

College of Kinesiology

Dr. Jon Farthing (966-1068) (Principal Investigator)

Email: jon.farthing@usask.ca

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APPENDIX O

APPENDIX O

UNIVERSITY OF SASKATCHEWAN

PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE: Neural Mechanisms of Sparing Effects in Humans: Specificity of sparing effects of cross-education after eccentric strength training

PRINCIPAL INVESTIGATOR: Dr. Jonathan Farthing

College of Kinesiology

University of Saskatchewan

87 Campus Drive

Saskatoon SK, S7N 5B2

Email: jon.farthing@usask.ca

SUB-INVESTIGATORS and/or STUDENT RESEARCHERS

Dr. Saija Kontulainen (College of Kinesiology, University of Saskatchewan)

Dr. Ron Borowsky (Department of Psychology, University of Saskatchewan)

Justin Andrushko (M.Sc. Student)

Doug Renshaw (Ph.D. Student)

Funding Agency: NSERC Discovery Grant

CONTACT PHONE NUMBER: 306-966-1068 OR 306-290-5912 (JON FARTHING)

INTRODUCTION

You are invited to take part in this research study because you are healthy, 18 years of age or older, do not have history of major injuries or other neurological conditions that effect one arm or hand, and are not currently strength training your forearm and hand muscles.

Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you will not lose the benefit of medical care, employment, or academic standing to which you are entitled or are presently receiving. It will not affect your relationship with Dr. Farthing or any of the researchers.

Please take time to read the following information carefully. You can ask the researcher to explain any words or information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide.

WHO IS CONDUCTING THE STUDY?

This study is being funded by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to Dr. Jonathan Farthing, University of Saskatchewan. However, neither the institution nor any of the investigators or staff will receive any direct financial benefit from conducting this study.

WHY IS THIS STUDY BEING DONE?

This study is being done to better understand an effect called "cross-education". When you strength train one side of your body (arm or leg) the opposite side increases in strength as well. The strength increase in your untrained side is called "cross-education". Recently we have discovered that cross-education can be used to prevent some of the strength and muscle loss that occurs when one arm is immobilized using a cast or splint. These findings have important implications for rehabilitation from injury. This study will focus on why and how these effects occur.

WHO CAN PARTICIPATE IN THE STUDY?

You are eligible to participate in this study if you are 18 years of age or older, do not have history of major injuries or other neurological conditions that effect one arm or hand, and are not currently strength training your forearm and hand muscles. You might not be able to participate in you have metal in your body that prevents you from entering an magnetic resonance imaging (MRI) machine. A total of 120 individuals are going to be recruited across six different experiments.

WHAT DOES THE STUDY INVOLVE?

This study has six different experiments and you can decide to participate in one of them. Each experiment involves various combinations of strength training and muscle stimulation. In each of the experiments, you will be asked to wear a cast or splint on one of your forearms that will prevent you from moving your wrist and hand joints. The immobilization will last for a minimum of 3 weeks but not longer than 6 weeks. If you are immobilized for 5 or 6 weeks you will be asked wear a removable splint instead of a cast.

For the experiment we are currently running, you will be placed by chance into one of two groups exposed to 5 to 6 weeks of unilateral immobilization with a forearm cast: 1) Cross-education + Immobilization; 2) Immobilization only. The total time commitment for this study is estimated at 40 hours. This includes the testing sessions before and after the immobilization period. If you are placed in group 2, your time commitment is estimated to be less than 20 hours. All sessions take place at the Physical Activity Complex (PAC) in room PAC 353.

If you are in group 1 you will be asked to do strength training of your non-immobilized arm 3 days per week for up to 4 weeks using "eccentric" contractions. These contractions involve the muscle stretching while you are trying to contract. You will be asked to try to resist the machine but you won't be able to stop it from moving. Each session would take place at the lab and will last about 15 minutes and will be supervised by a trained research assistant. You will be asked to wear a short sleeve or loose fitting long sleeve shirt. If you are in group 2 you will be asked to wear the cast and attend the lab but you will not do strength training. Holes will be cut in your cast so we can record muscle activity using EMG electrodes. Your immobilized forearm will be fixed with straps onto an arm pad with the palm down during training sessions. Before, and after the immobilization period, we will measure the strength of your arm using isometric, concentric and eccentric contractions and use stimulation to generate small and larger twitches of your muscle. We will measure the size of your forearm muscles using an ultrasound machine before and after the immobilization. We will measure the size of your forearm muscles using a pQCT scanner 2 times during the study (before and after immobilization).

Testing sessions will be scheduled at a time convenient for you before, during and after the immobilization period. Each will take about 3 hours. The following measures will be done:

1. **Strength** of the muscles of the wrist and hand (forearm) will be assessed using handgrip devices or a strength machine. Your strength may be tested in various ways, using different types of contractions.
2. **Muscle thickness** will be measured using a muscle ultrasound machine. It can take pictures of your muscles. Some transmission gel will be placed on your skin.
3. **Muscle cross-sectional area** of the forearm muscles will be measured using a machine called a peripheral quantitative computed tomography (pQCT) scanner. You will be asked to sit still for 15 minutes with your arm in a scanner.
4. **Electromyography** (EMG) will record muscle activity for both your arms during strength training and during maximal effort contractions. Small electrodes (sticky tabs) will be placed on your skin surface.
5. **Nerve stimulation** will be used to cause muscle twitches while your muscle is resting and to cause low level involuntary contractions (10% of maximum effort). We might also deliver a higher amount of stimulation while you are doing a maximum effort contraction (a strength test) to see how well you can activate your muscles. Stimulation will be focused on nerves innervated muscles of the hand and forearm.
6. **Strength training** of hand and wrist muscles will take place supervised in the lab environment (PAC 353). We will provide you with access to the lab. Strength training will involve eccentric contractions of your forearm muscles (wrist).
7. **Immobilization** will be accomplished using a cast. You won't be able to remove the cast for bathing/sleeping.
8. **Questionnaires** related to hand preference and history of injury and strength training will be given to you to complete at your first visit.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

If you choose to participate in this study, there may or may not be direct benefits to you. It is hoped the information gained from this study can be used in the future to benefit other people recovering from injuries or neurological impairment that affects one side of their body such as a wrist or arm fracture or a stroke. If you participate in strength training your arm will probably increase in strength.

ARE THERE POSSIBLE RISKS AND DISCOMFORTS?

If you choose to participate in this study, the following are possible:

Strength training can cause some muscle soreness or discomfort up to 48 hours after a training session. Muscle injuries are very rare will be further prevented by providing a warm-up. Soreness is more common 48 hours after training with "eccentric" muscle contractions that also stretch your muscle but usually soreness goes away entirely after 5 or 6 sessions.

During the period of immobilization when you are wearing a cast, the hand/wrist will not be able to move freely. Some everyday activities will be more difficult for you while you are participating in the study. You will probably lose some muscle size and strength after immobilization and you will feel temporary stiffness in your hand/wrist muscles and joints after immobilization. You will be provided a chance to train your muscles after the immobilization period to regain strength. Someone will contact you one week after the study to see how your strength has recovered.

PQCT scans will be used to take pictures of your forearm muscles and bones. There is a small dose of radiation associated with pQCT scans. Trained grad student technicians will conduct pQCT scans. No more than three pQCT scans for each arm will be taken for any study. Effective radiation dose for all six pQCT scans is about 2.3 µSv (micro Sievert). This is less than the amount of background radiation a person receives in two days from naturally-occurring sources in Saskatchewan. Even in an unlikely situation, that would require all scans to be repeated once (due to movement artifacts), the total dose would be about 5 µSv. For reference, a cross-country flight could expose a person to about 30 µSv of radiation (http://www.hc-sc.gc.ca/hc-ps/ed-ud/respond/nuclea/measurements-mesures-eng.php). Due to the small amount of radiation, pregnant or breastfeeding women are not eligible to participate in this study.

Using small electrodes attached to your skin we will use small amounts of electrical current to stimulate your nerves and muscles. The stimulation will start at very low levels. At high stimulation levels your muscle will contract. Some people find this uncomfortable and it feels like a "pinch" but it is not harmful to you. The adhesive from the EMG and stimulation electrodes can cause mild skin reactions. Your skin will be shaved (disposable razors) and cleaned with alcohol before putting on the electrodes, and cleaned again after data collection.

WHAT IF NEW INFORMATION BECOMES AVAILABLE THAT MAY AFFECT MY DECISION TO PARTICIPATE?

During the course of this study, new information that may affect your willingness to continue to participate will be provided to you by the researcher.

WHAT HAPPENS IF I DECIDE TO WITHDRAW?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. There will be no penalty or loss of benefits if you choose to withdraw. Your future medical care, academic status or employment will not be affected.

If you choose to enter the study and then decide to withdraw later, all data collected about you during your enrolment will be retained for analysis.

WILL I BE INFORMED OF THE RESULTS OF THE STUDY?

The results of the study will be available 6 months after the completion of the study from Dr. Jonathan Farthing. A brief summary of findings using average data will be circulated to you via email after the study is complete. Individual data (baseline and post-testing test scores for muscle size and strength) can be made available upon your request via email. The researchers plan to publish the study in journals and as part of graduate student theses.

WHAT WILL THE STUDY COST ME?

You will not be charged for any research-related procedures. At the completion of the study, an honorarium of $200 will be provided to cover your time, out-of-pocket expenses such as travel, parking or meals, and to compensate you for the inconvenience of immobilization. If you choose to withdraw early from the study, you will not receive any compensation.

WHAT HAPPENS IF SOMETHING GOES WRONG?

In the unlikely event of an adverse effect arising related to the study procedures, necessary medical treatment will be made available at no additional cost to you. As soon as possible, notify the research team. By signing this document, you do not waive any of your legal rights

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

In Saskatchewan, the *Health Information Protection Act (HIPA)* defines how the privacy of your personal health information must be maintained so that your privacy will be respected.

Your confidentiality will be respected. A code number will be used on your study records instead of your name. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of NSERC, Health Canada, and the University of Saskatchewan Biomedical Research Ethics Board for the purpose of monitoring the research. No records, which identify you by name or initials, will be allowed to leave the Investigators' offices. The study data will be stored securely in a locked cabinet contained within a locked office under the supervision of the PI, for a minimum of 5 years after the termination of the grant funding period The results of this study may be presented in a scientific meeting or published, but your identity will not be disclosed.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY?

If you have any questions or would like further information about this study before or during participation, you can contact Dr. Jonathan Farthing at 306-966-1068 or 306-290-5912.

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Biomedical Research Ethics Board, at 306-966-2975(out of town calls 1-888-966-2975). The Biomedical Research Ethics

Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Biomedical Research Ethics Board.

APPENDIX P

APPENDIX P

UNIVERSITY OF SASKATCHEWAN

CONSENT TO PARTICIPATE

Study Titles: Neural Mechanisms of Sparing Effects in Humans

- I have read (or someone has read to me) the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I understand that I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my relationships with the researchers or my standing at the university.
- I give permission to the use and disclosure of my de-identified information collected for the research purposes described in this form.
- I understand that by signing this document I do not waive any of my legal rights.
- I will be given a signed copy of this consent form.

I agree to participate in this study:

Printed name of participant: Signature Date

Printed name of person obtaining consent: Signature Date