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SEX BASED DIFFERENCES IN MUSCLE QUALITY RECOVERY FOLLOWING ONE WEEK OF LOWER LIMB IMMOBILIZATION AND SUBSEQUENT RETRAINING

by

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ABSTRACT

The present project represents the second part of a two-phase clinical trial designed to comprehensively examine the effects of knee joint immobilization and recovery on skeletal muscle size, strength, and central nervous system plasticity in healthy males and females. The purpose of this study was to examine differences between sexes in the recovery of muscle quality, size, and strength in response to a resistance training-based rehabilitation program following one week of knee joint immobilization. Twenty-seven participants (males: n = 16, age = 22 ± 3 years, BM = 81.3 ± 14.8 kg, BMI = 25.0 ± 3.4 kg/cm^2; females: n = 11, age = 20 ± 1 years, BM = 61.3 ± 9.4 kg, BMI = 23.3 ± 2.1 kg/cm^2) underwent one week of knee joint immobilization followed by twice weekly resistance training sessions designed to re-strengthen the left knee joint. Retraining sessions were conducted until participants could reproduce their pre-immobilization isometric MVC peak torque. Assessments of muscle quality, size, and strength were conducted prior to immobilization (Pre-), immediately after immobilization (Post-Immobilization), and until retraining was finished (Post-Retraining). Results suggested that both sexes experienced negative changes in MVC peak torque, specific torque, echo intensity, and ECW/ICW ratio, with females experiencing greater decrements in MVC peak torque and specific torque. The number of retraining sessions required was similar for males (median = 1, mean = 2.13) and females (median = 2, mean = 2.91). Following retraining, specific torque was the only "muscle quality" indicator that had fully recovered. This is the first study to examine sex differences in the recovery of muscle quality indices in response to a retraining program following lower-limb immobilization. The findings may have important implications for the development of evidence-based, sex-specific rehabilitation approaches following short-term knee joint immobilization.

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LIST OF ACRONYMS

1RMe	Estimated one-repetition maximum
ACL	Anterior Cruciate Ligament
AOMI	Action Observation / Mental Imagery
ANOVA	Analysis of Variance
AU	Arbitrary Units
BIS	Bioimpedance Spectroscopy
BF%	Body Fat Percentage
BMI	Body Mass Index
CM	Centimeters
CSA	Cross-Sectional Area
dB	Decibels
ECV	Extracellular water volume
ECW/ICW Ratio	Extracellular versus Intracellular Water Ratio
EI	Echo Intensity
FFM	Fat-Free Mass
FM	Fat Mass
ICC	Intraclass Correlation

ICV	Intracellular water volume
KG	Kilograms
MD	Minimal difference
MHz	Megahertz
MVC	Maximum Voluntary Contraction
Nm	Newton Meters
NMES	Neuromuscular Electrical Stimulation
PA	Physical Activity
RF	Rectus Femoris
SEM	Standard error of measure
VL	Vastus Lateralis

INTRODUCTION

Short periods of immobilization or disuse incur rapid declines in muscle mass and strength (Deitricket, 1948; Ingemann-Hansen & Halkjaer-Kristensen, 1980; Gibson et al., 1987; Deschenes et al., 2008; Deschenes et al., 2009; Clark et al., 2009; Deschenes, 2012; Wall et al., 2014; Dirks et al., 2014; MacLennan, 2020; MacLennan, 20201). A recent review examining the effect of immobilization on neuromuscular function noted that individuals undergoing lower-limb immobilization via a knee brace model experienced atrophy rates of 0.2% to 0.6% of thigh lean mass per day (Campbell, 2019). While decrements in muscle size and strength have been observed together, they occur at different rates during disuse (Campbell, 2019). The same review noted that more profound decrements have been observed for knee extensor strength, with losses ranging between 1.1% and 4% per day of brace immobilization, suggesting that strength loss during immobilization is influenced by underlying mechanisms besides atrophy (Campbell, 2019). This is even more evident in studies that have observed decrements in strength without changes in body mass (Deschenes, 2008; Deschenes, 2009; Deschenes, 2017). A more in-depth examination of the relationship between muscle size and strength may provide additional insight regarding the morphological and functional adaptations of skeletal muscle associated with immobilization and disuse.

The relationship between muscle size and force production capacity has been referred to as “muscle quality” (Goodpaster et al. 2006; Russ et al., 2012; Ivey et al. 2000). Muscle quality describes the specific force per unit of muscle mass and is considered a better marker of muscle function than absolute strength alone (Doherty, 2003). Because calculating muscle quality requires the measurement of muscle size, clinicians and researchers have been somewhat limited by the accessibility of imaging devices. More recently, however, ultrasound imaging has become an accessible and attractive means of anatomical imaging. Furthermore, ultrasound also provides the option to calculate echo intensity, which can be used to visually assess muscle composition. Echo intensity values represent the quantified brightness/darkness of the pixels within an image (Stock and Thompson, 2020). Based on their brightness/darkness, pixels are assigned a value on an arbitrary scale between 0 and 255 (AU) with black corresponding to 0 and white corresponding to 255. On this scale, darker pixels indicate hypoechoic anatomical structures such as contractile proteins, whereas hyperechoic anatomical structures such as intramuscular fat or connective tissue appear lighter in the image (Pillen, 2009). To date, only one study has tracked immobilization-related shifts in echo intensity (MacLennan, 2020) reporting more rapid changes in echo intensity compared to muscle cross-sectional area (CSA). Recently, segmental bioelectrical impedance spectroscopy has also been utilized to assess muscle quality via the ratio of extracellular water volume versus intracellular water volume (ECW/ICW) within a limb (Yamada, 2017; Taniguchi, 2017). Intracellular water volume provides a measure of muscle cell mass whereas extracellular water volume reflects interstitial fluid and blood plasma existing outside of the muscle cell membrane (Yamada, 2017; Taniguchi, 2017). Similarly to echo intensity, ECW/ICW may provide another means of examining the muscle composition in terms of non-contractile tissue relative to contractile tissue. Further examining muscle quality with a multifaceted approach via specific torque, echo intensity, and ECW/ICW may provide clinically relevant information regarding shifts in muscle function and morphology during periods of immobilization.

Individuals may undergo immobilization for a number of reasons related to illness or injury. In particular, knee joint immobilization is a common clinical intervention following orthopedic surgery such as anterior cruciate ligament (ACL) reconstruction. ACL tears are a common injury in the United States, with approximately 200,000 reported ACL reconstructions being reported on an annual basis (Mather, 2013). Females are especially susceptible with year-round athletes having an ACL tear rate around 5% (Renstrom, 2008). Female soccer and basketball athletes are three times more likely to undergo treatment for an ACL tear compared to their male counterparts (Prodromos et al., 2007; Renstrom et al., 2008). While critical during the initial stages of recovery from an adverse event such as ACL tear, immobilization results in deterioration of skeletal muscle size and strength that also seems to effect females disproportionately.

A small number of studies have been dedicated to investigating sex-based differences in skeletal muscle adaptations to short-term immobilization or disuse (Yasuda, 2005; Deschenes, 2009; Deschenes, 2012; Clark, 2009). While limited, this body of work provides strong evidence that alterations to the nervous system (e.g. reductions in voluntary activation, shifts in motor unit recruitment and firing behavior) underpin disuse-related decrements in strength that are more profound in females compared to males despite similar rates of atrophy (Deschenes, 2009, Clark, 2009; Yasuda, 2014; MacLennan, 2021). Furthermore, there is evidence suggesting that females require a longer period of time to fully recover strength upon returning to normal activity (Clark, 2009). Whether sex differences influence the time-course of strength recovery during post-immobilization rehabilitation has yet to be explored. Resistance training has been demonstrated to be an effective means of increasing both strength and muscle mass regardless of sex (Hubal et al., 2005, Roberts, 2020). While several studies have observed similar relative responses to resistance training between sexes (Hunter, 1985; Abe et al., 2000; Roth et al., 2001), others have indicated that the relative increases in strength associated with the introduction of a resistance training program may actually be more robust in females (Cureton et al., 1988; Hubal, 2005, Kell, 2011). Following a period of immobilization, a greater increase in relative strength associated with resistance training would be indicative of superior responses in muscle quality and may have clinical significance in the time course for strength recovery.

The few investigations focusing on sex differences suggest that the deleterious effects of short-term immobilization on muscle size and strength are more pronounced in females than in males (Deschenes et al., 2008; Deschenes et al., 2009; Clark et al., 2009). Additionally, there is evidence suggesting that males are able to recover strength at a faster rate than females upon returning to normal activity (Clark et al., 2009). It remains to be seen, however, if sex differences in muscle size, strength, or quality persist with the implementation of a resistance training based rehabilitation (retraining) program following a period of short-term immobilization. Considering the disproportionate rates of atrophy and strength loss during immobilization (Yasuda, 2005), the examination of muscle quality via echo intensity and force production relative to muscle mass (specific torque) may provide novel insight regarding muscle morphology and function recovery during a retraining program. As such, the purpose of this study is to examine sex differences in the recovery of muscle quality, size, and strength in response to a retraining program following one week of lower limb immobilization. It was hypothesized that males and females would both experience improvements in muscle quality along with strength as a result of retraining. Secondly, it was hypothesized that males would be less affected by the immobilization protocol and would thus require fewer retraining visits to fully recover strength compared to females.

METHODS

Experimental Approach to the Problem

This study represents Experiment 2 of a two-part investigation. During Experiment 1, participants were sorted into a control group and three intervention groups (1: immobilization only, 2: immobilization + neuromuscular electrical stimulation [NMES], and 3: immobilization + action observation and mental imagery [AOMI]) that underwent one week of lower limb immobilization wearing a fixed-angle brace over the left knee joint. Experiment 2 began immediately following the immobilization protocol. During Experiment 2, participants performed a resistance training based rehabilitation (retraining) program designed to restrengthen the lower limbs. Training took place two times per week. Retraining completion was marked by maximal isometric strength of the knee extensors returning to pre-immobilization baseline (± 2.5%). At Post-Immobilization and during each retraining session, participants underwent maximal isometric strength, ultrasound, and bioimpedance spectroscopy (BIS) testing.

Figure 1.Schematic representation of study procedures.

Post-Immobilization and Post-Retraining comparisons between sexes were made for MVC peak torque, specific torque, cross-sectional area, echo intensity, total body fat free mass, total body fat mass, total body water, segmental lean mass, ECW/ICW ratio, and the number of required retraining sessions. Complete descriptions for each assessment can be found in the Assessments section below.

Participants

For Experiment 1 and subsequently Experiment 2, a target sample of 60 healthy adults (age 18-35) from the university community was sought after. Prior to study enrollment, participants were required to complete a detailed in-house health questionnaire and Physical Activity Readiness Questionnaire + (PAR-Q+) to ensure no contraindications to participation. Exclusion criteria included BMI below 18.5 or above 30 kg/m^2, musculoskeletal issues (i.e., back, shoulder, or knee pain) that may interfere with the use of crutches, surgery on the hip or knee joints, family history of thrombosis, history of seizures or fainting.

All participants were informed of the study risks and completed an informed consent document prior to participation. All study protocols were approved by the University of Central Florida Institutional Review Board in accordance with the Declaration of Helsinki.

Immobilization

Participants were fitted with a knee joint immobilization brace. The brace was locked at 90° of knee flexion, to ensure that the foot was raised off the ground. This position prevented normal weight-bearing and allow the knee extensors to stay relaxed. Participants were instructed to remove the brace only in bed, so as not to disrupt sleep. During bathing, participants were asked not to remove the brace, but to keep it dry by covering it with a large plastic bag. For their safety and comfort, each participant was offered a shower chair. For ambulation, participants were provided with axillary crutches. Participants were fitted with and trained in the proper use of crutches, including navigation of curbs and stairs.

Participants were given a stocking to wear between the brace and their skin, which extended from the proximal thigh to the ankle. The stocking was intended to mitigate discomfort and minimize the risk of skin irritation from the brace. This was worn at all times, and only removed during sleep when the brace was removed. A compression stocking was also worn while sleeping, in order to reduce the risk of blood clots. In accordance with previous knee joint immobilization studies (Deschenes et al., 2008, 2009), participants performed light range of motion movements of the ankle and knee while lying in bed. These activities were performed twice daily (morning and evening) to minimize the risk of vascular or muscular issues due to immobilization. A video providing instructions was provided to participants, in addition to a handout with detailed descriptions of the exercises. To ensure compliance and safety, each participant was provided with a member of the research team to serve as their compliance officer. Each compliance officer checked in with their assigned participants daily and assisted with any concerns or issues that arose. Accelerometers (ActiGraph GT9X Link; ActiGraph Inc., Pensacola, FL) were worn throughout the week of immobilization to ensure participant compliance. Participants were fitted with an accelerometer device on both their right and left ankles and asked not to remove the devices except during showering or bathing. Accelerometer data monitored both overall wear-time compliance and compliance with the immobilization protocol via bilateral comparisons of steps and vigorous activity (PA) (Troiano, 2007; Cook et al., 2006)

Retraining

Prior to Experiment 1, participants performed unilateral repetition maximum testing of the left leg on the leg press, leg extension, and leg curl exercises. Following a brief dynamic warmup (e.g. five minutes walking, 10 body weight squats), Participants performed sets of each exercise at increasing load until they were only capable of performing 10 or fewer repetitions. Estimated one-repetition maximum (1RMe) was then calculated using the load and number of reps performed on the last set (Brzycki, 1993). Participants were first tested on the leg press followed by leg extension and leg curl. Each set was separated by at least three minutes of rest.

The first retraining session began immediately post-immobilization. Participants underwent retraining sessions twice per week, separated by at least 48 hours, until MVC peak torque returned to pre-immobilization baseline (±2.5%). Each retraining visit began with ultrasound and BIS assessments. Participants then performed three MVCs. If MVC peak torque reached pre-immobilization baseline (±2.5%), participants were deemed to have completed the study. If MVC peak torque did not meet baseline, they were immediately underwent a left leg unilateral retraining session consisting of leg press followed by leg extension and then leg curl. During the first retraining session, participants began each exercise with a load set to approximately 50% of their 1RMe. Participants performed three sets of each exercise to failure with a target range of 8-12 repetitions. If more than 12 repetitions were performed, the load was increased. If the participant was unable to perform at least eight repetitions during a set, the load was decreased accordingly for the next set. When multiple retraining sessions were required, training load was determined based on the last set of the exercise performed during the previous visit. Each set was separated by exactly three minutes of rest. The number of required retraining sessions was tracked for each participant. Relative volume for the first and last retraining sessions was calculated as the total load tonnage from each session normalized to the participants’ body mass.

Assessments

Anthropometrics

Height was assessed via stadiometer and mass were assessed using a scale (500KL Health O Meter, Alsip, IL, USA).

Ultrasound

Ultrasound images of the left vastus lateralis and rectus femoris muscles were collected with a portable B-mode imaging device (GE Logiq e BT12, GE Healthcare, Milwaukee, WI) and a multi-frequency linear array probe (12 L-RS, 5-13 MHz, 38.4 mm field of view; GE Healthcare, Milwaukee, WI). The panoramic function (LogiqView, GE Healthcare, Milwaukee, WI) was used to obtain images of the vastus lateralis and rectus femoris in the transverse plane. Prior to imaging, participants rested in the supine position for 5 min in order to allow the redistribution of fluids from their quadriceps muscles (Arroyo et al., 2018). Measurements for the RF were taken at 50% of the distance from the anterior, inferior suprailliac spine to the most proximal point of the patella. Vastus lateralis measurements were taken at 50% the straight-line distance between the greater trochanter and the lateral epicondyle of the femur. To ensure desired probe movement in the transverse plane, a high-density foam pad was secured across the thigh. Ultrasonography settings (Frequency: 12 MHz, Gain: 55 dB, Dynamic Range: 72) were kept consistent across participants. To ensure optimal image clarity, a standardized depth of 5.0 cm was utilized unless a greater depth was necessary to adequately capture the entire belly of the muscle (Girts et al., 2022). Depth for each participant was kept consistent across trials. A generous amount of water-soluble transmission gel (Aquasonic 100 ultrasonography transmission gel, Parker Laboratories, Inc., Fairfield, NJ) was applied to the skin to allow immersion of the probe surface during measurement to enhance acoustic coupling. Three images of each muscle were obtained, with mean values being used for statistical analyses.

The ultrasound images were digitized with ImageJ software (version 1.46, National Institutes of Health, Bethesda, MD) at the conclusion of the study. The polygon function was utilized to outline the borders of the vastus lateralis and rectus femoris muscles. After scaling the image units from pixels to cm, muscle cross-sectional area (cm^2) was determined with the polygon function. Echo intensity was assessed by computer-aided gray-scale analysis using the histogram function. Echo intensity values were determined as the corresponding index of muscle quality ranging between 0 and 255 arbitrary units (AU). A single individual performed all of the ultrasound analysis (CSA: ICC = 0.988, SEM = 0.68, MD = 1.87; Echo intensity: ICC = 0.997, SEM = 0.50, MD = MD = 1.34).

Bioimpedance Spectroscopy

Total body fat free mass, total body fat mass, total body water, segmental lean mass, and segmental ECW/ICW ratio were assessed by bioimpedance spectroscopy (BIS) using the ImpediMed SFB7 (ImpediMed Inc., Carlsbad, CA). Participants rested in the supine position with their arms ≥ 30 degrees away from their torso and their legs separated for at least five minutes prior to testing and remained in the position throughout testing. Electrodes were placed according to manufacturer recommendations. For total body measurements, one pair of electrodes were placed on the left wrist adjacent to the ulnar head, and another five cm distal. Another pair were placed on the left ankle at the level of the medial and lateral malleoli as well as five cm distal from that position. Height, weight, and sex information were entered into the device prior to each test. Two total body measurements were taken followed by two segmental measurements. Segmental extracellular water volume (ECV), segmental intracellular water volume (ICV), and segmental lean mass calculated using formulas described by Kaysen (2005). ECV and ICV (ECV/ICV) were used to calculate the ECW/ICW ratio. The average values derived from the two trials were used for analysis (Moon, 2008).

Isometric Strength

All isometric strength measurements were performed with the left knee extensors via a Biodex System 4 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). Before testing, participants were seated in the dynamometer with restraining straps placed over the trunk, pelvis, and thigh. The input axis of the dynamometer was aligned with the axis of rotation of the knee. Each participant's dynamometer chair settings were recorded during the first visit and replicated during subsequent testing sessions. The chair was adjusted so that isometric torque testing was performed at a knee joint angle of 90°. The lower leg was secured to an anti-shear attachment with the pad placed over the tibialis anterior, just superior to the malleoli. Prior to testing, participants were given a warm-up consisting of three 10-second contractions at 50% of their perceived maximal torque, one five-second contraction at 75% of their perceived maximal torque, and one three-second contraction at 90% of their perceived maximal torque. After the warm-up, participants performed three 5-second maximal voluntary contractions (MVCs) separated by three minutes rest. The highest value from the three trials was designated as the MVC peak torque value (Nm). MVC peak torque was measured using Delsys EMGworks software (Delsys Inc., Natick, MA). Isometric strength was tested during every visit. Prior to the week of familiarization, a baseline assessment was performed immediately prior to the immobilization protocol to be used as a benchmark during retraining.

Statistical Analysis

This study contained the following dependent variables of interest: MVC peak torque (Nm), specific torque (Nm/cm^2) cross-sectional area (cm^2), echo intensity (AU), total body water (L), total body fat-free mass (kg), total body fat mass (kg), segmental lean mass (kg), ECW/ICW ratio, and the number of required retraining sessions. Before statistical analysis, data was assessed for normality and sphericity. If the assumption of sphericity was violated, a non-parametric test was used. A two-way (sex [male, female] × time [Post-Immobilization, Post-Retraining]) mixed-factorial analysis of variance (ANOVA) was used to examine mean differences in dependent variables of interest between sexes after one week of immobilization and after the completion of a retraining program. The alpha level was set at 0.05. In the event of a significant interaction effect or main effect, Bonferroni adjusted post-hoc tests were used for pairwise comparisons. Effect sizes for pairwise comparisons were assessed using Cohen's *d*. Magnitudes of the standardized effects were evaluated with values of 0.2, 0.5, and 0.8 to characterize small, medium, and large differences, respectively (Cohen 1988). Sex and time effects for the ANOVA were further analyzed using partial eta squared (η_p^2). Interpretations of η_p^2 were evaluated in accordance with Cohen (1988) at the following levels: small effect (0.010–0.058), medium effect (0.059–0.137), and large effect (>0.138) (Cohen 1988). JASP software (version 0.14.0.1, JASP, Amsterdam, The Netherlands) was used for statistical analyses.

RESULTS

Participants

Thirty-three participants underwent the one-week immobilization period during Experiment 1. Five participants did not qualify for retraining as their MVC peak torque did not drop more than 2.5% following immobilization. One participant did not complete the retraining program due to scheduling conflicts and was removed from the final analysis accordingly. The results herein are based upon 27 participants (16 males, 11 females) that completed the Experiment 2 retraining program or reached the maximum number of possible retraining sessions (8). Participant demographics can be seen in Table 1. Within-sex responses to immobilization can be seen in Table 2.

Table 1. Participant Demographics

	Males n = 16	**Females n = 11**
Age (years)	22 ± 3	20 ± 1
Height (cm)	180.0 ± 8.0	162 ± 6.9
Body Mass (kg)	81.3 ± 14.8	61.3 ± 9.4
Fat-Free Mass (kg)	62.5 ± 9.1	41.5 ± 4.9
Fat Mass (kg)	18.8 ± 8.2	19.8 ± 4.9
BMI	25.0 ± 3.4	23.3 ± 2.1

Values reported as mean ± standard deviation.

Table 2. Responses to Immobilization

		MALES				**FEMALES**		
		Pre-	**Post-Immobilization**			**Pre-**	**Post-Immobilization**	
STRENGTH								
MVC peak torque		203.93 ± 39.2 Nm	181.93 ± 34.44 Nm	-10.79%		116.45 ± 20.54 Nm	98.78 ± 18.97 Nm	-15.17%
Specific Torque		5.61 ± 1.07 Nm/cm^2	5.06 ± 1.37 Nm/cm^2	-9.80%		4.48 ± 0.93 Nm/cm^2	3.89 ± 1.03 Nm/cm^2	-13.11%
ULTRASOUND								
CSA	VL	29.05 ± 5.81 cm^2	28.21 ± 6.60 cm^2	-2.88%		20.26 ± 6.75 cm^2	18.21 ± 6.36 cm^2	-10.11%
	RF	7.8 ± 2.11 cm^2	7.02 ± 2.21 cm^2	-10.35%		6.68 ± 2.02 cm^2	6.75 ± 2.15 cm^2	1.00%
Echo Intensity	VL	59.64 ± 13.50 A.U.	63.76 ± 15.68 A.U.	6.90%		66.18 ± 9.79 A.U.	70.11 ± 11.46 A.U.	5.94%
	RF	57.79 ± 14.77 A.U.	61.21 ± 11.70 A.U.	5.92%		57.92 ± 13.03 A.U.	59.14 ± 1117 A.U.	2.12%
BIS								
FFM		62.5 ± 9.15 kg	61.84 ± 8.93 kg	-1.06%		41.51 ± 4.92 kg	41.08 ± 4.25 kg	0.03%
FM		18.80 ± 8.21 kg	19.44 ± 8.13 kg	3.42%		19.79 ± 4.86 kg	18.77 ± 4.30 kg	-5.16%
TBW		45.75 ± 6.69 L	45.27 ± 6.54 L	-1.06%		30.39 ± 3.60 L	30.40 ± 3.12 L	0.03%
ECW/ICW		0.271 ± 0.04	0.292 ± 0.04	7.75%		0.355 ± 0.04	0.387 ± 0.05	9.01%
Segmental Mass		12.79 ± 1.57 kg	12.12 ± 1.37 kg	-5.23%		9.37 ± 0.69 kg	9.37 ± 0.69 kg	-3.28%

Pre- values reported as mean ± standard deviation. Post-Immobilization values reported as mean ± standard deviation, percent change from Pre-.

Immobilization Protocol Compliance

During the immobilization period from Experiment 1, 32 participants had at least 4 days of accelerometer data for a minimum of 10 hours. The mean number of days worn was 6.75.

Table 3. Immobilization Compliance

	Right leg	**Left leg**	***p*-value**
Vigorous PA	46.36 ± 44.30	1.59 ± 5.12	<0.001
Steps	20803.64 ± 8391.00	19024 ± 7833.00	0.008

Values reported as mean ± standard deviation.

Retraining

A Shapiro-Wilk test indicated that the retraining time course data was not normally distributed. A Mann-Whitney test indicated no significant difference between males (median = 1, mean = 2.13) and females (median = 2, mean = 2.91) for the number of retraining sessions needed to recover MVC peak torque (U = 67; $p = 0.26$). Within sex training data can be seen in Table 4.

Table 4. Retraining Performance

	Males	**Females**	***p***
Retraining Sessions Required	2.13 ± 2.0	2.91 ± 2.34	0.255
First Retraining Session Relative Volume (kg/kg body mass)	34.98 ± 11.02	22.75 ± 14.13	0.018
Final Retraining Session Relative Volume (kg/kg body mass)	38.76 ± 11.14	27.00 ± 15.13	0.028
Unilateral Leg Press 1RMe (kg)	83.52 ± 32.75	31.36 ± 25.91	<0.001
Unilateral Leg Extension 1RMe (kg)	42.98 ± 14.03	22.98 ± 5.75	<0.001
Unilateral Hamstring Curl 1RMe (kg)	25.20 ± 6.42	12.52 ± 2.70	<0.001

Values reported as mean ± standard deviation.

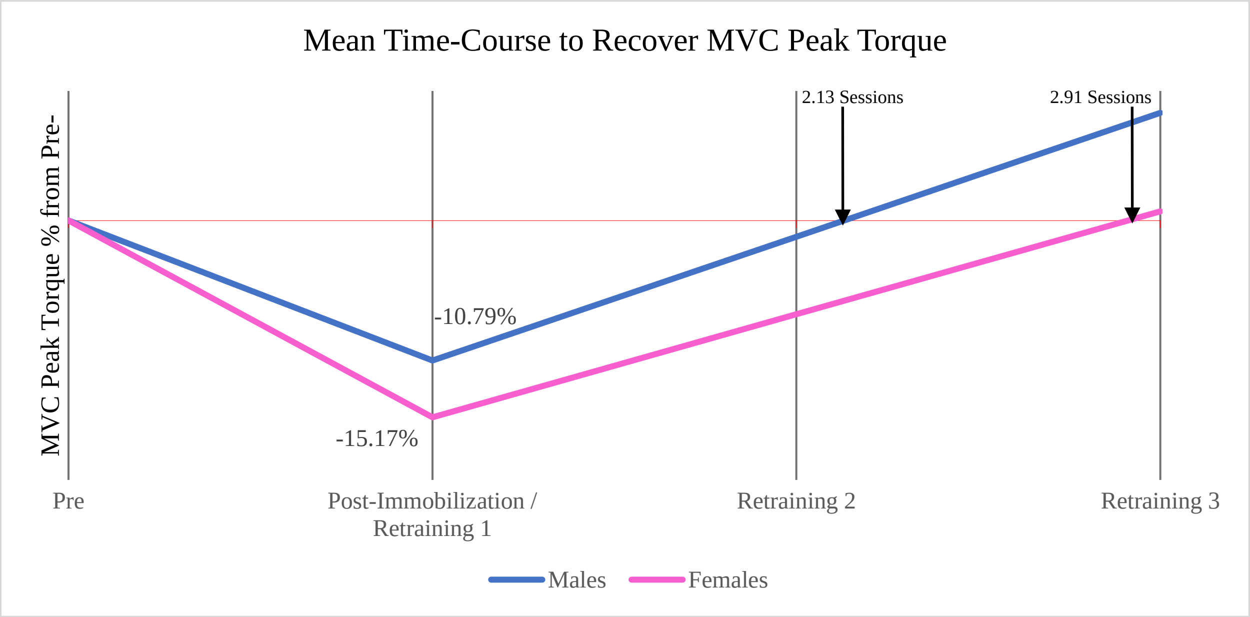


Figure 2. Retraining Time-Course.

Arrows indicate the time point representing the mean number of retraining sessions required in order to reproduce Pre- MVC peak torque.

Strength

MVC Peak Torque

For the males, the Post-Immobilization and Post-Retraining MVC peak torque values were 181.93 ± 34.44 and 223.51 ± 46.0 Nm, respectively, which represented a 22.85% increase. For the females, the Post-Immobilization and Post-Retraining MVC peak torque values were 98.78 ± 18.97 and 120.67 ± 26.86 Nm, respectively, which represented a 22.16% increase, Results indicated that the assumption of homogeneity was not violated (*p's* > 0.140). A significant sex × time interaction was observed, F (1,25) = 6.16, $p = 0.020$, $\eta_p^2 = 0.20$. Pairwise comparisons revealed that, for males, MVC peak torque was significantly greater at Post-Retraining compared to Post-Immobilization, $p < 0.001$, $d = 1.120$. For females, MVC peak torque was significantly greater at Post-Retraining compared to Post-Immobilization, $p = 0.009$, $d = 0.63$. Additionally, pairwise comparisons revealed that MVC peak torque was significantly greater for males at Post-Immobilization ($p < 0.001$, $d = 2.39$) and at Post-Retraining ($p < 0.001$, $d = 2.96$). Within sex changes can be seen in Table 5.

Specific Torque (MVC peak torque / CSA)

For the males, Post-Immobilization and Post-Retraining specific torque was 5.06 ± 1.37 and 6.25 ± 1.5 Nm/cm^2, respectively, which represented a 20.05% increase. For the females, Post-Immobilization and Post-Retraining specific torque was 3.89 ± 1.03 and 4.76 ± 1.2 Nm/cm^2, respectively, which represented a 22.37% increase. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.23). No sex × time interaction was observed, F (1,25) = 0.69, $p = 0.41$, $\eta_p^2 = 0.03$. A significant main effect for time was observed, F

(1,25) = 27.98, $p < 0.001$, $\eta_p^2 = 0.53$. When collapsed across sex, specific torque was significantly greater at Post-Retraining relative to Post-Immobilization, $p < 0.001$, $d = 1.02$. A significant main effect for sex was also observed, F (1,25) = 7.77, $p = 0.010$, $\eta_p^2 = 0.24$. When collapsed across time, males exhibited greater specific torque relative to females, $p = 0.010$, $d = 0.54$. Within sex changes can be seen in Table 5.

Ultrasound

Cross-Sectional Area

For males, cross-sectional area of the vastus lateralis was 28.21 ± 6.60 and 28.97 ± 6.30 cm^2 at Post-Immobilization and Post-Retraining, respectively, which represented a 2.7% increase. For females, cross-sectional area of the vastus lateralis was 18.21 ± 6.36 and 19.58 ± 5.65 cm^2 at Post-Immobilization and Post-Retraining, respectively, which represented a 7.49% increase. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.396). No sex × time interaction was observed, F (1,25) = 0.52, $p = 0.480$, $\eta_p^2 = 0.02$. A significant main effect for time was observed, F (1,25) = 6.43, $p = 0.018$, $\eta_p^2 = 0.21$. At Post-Retraining, VL CSA was significantly greater compared to Post-Immobilization, $p = 0.018$, $d = 0.49$. A significant main effect for sex was observed, F (1,25) = 16.02, $p < 0.001$, $\eta_p^2 = 0.21$. Males exhibited greater VL CSA relative to females, $p < 0.001$, $d = 0.77$. Within sex changes can be seen in Table 5.

For males, cross-sectional area of the rectus femoris was 7.02 ± 2.21 and 7.68 ± 2.27 cm^2 at Post-Immobilization and Post-Retraining, respectively, which represented a 9.45% increase. For females, cross-sectional area of the rectus femoris was 6.75 ± 2.15 and 6.44 ± 2.02 cm^2 at

Post-Immobilization and Post-Retraining, respectively, which represented a 4.7% decrease. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.706). No sex × time interaction, F (1,25) = 3.06, $p = 0.093$, $\eta_p^2 = 0.11$, main effect for time, F (1,25) = 0.40, $p = 0.535$, $\eta_p^2 = 0.02$, or main effect for sex was observed F (1,25) = 0.88, $p = 0.358$, $\eta_p^2 = 0.03$. Within sex changes can be seen in Table 5.

Echo Intensity

For males, echo intensity of the vastus lateralis was 63.76 ± 15.68 and 64.39 ± 15.17 A.U. at Post-Immobilization and Post-Retraining, respectively, which represented a 0.99% increase. For females, echo intensity of the vastus lateralis was 70.11 ± 11.46 and 71.18 ± 7.50 A.U. at Post-Immobilization and Post-Retraining, respectively, which represented a 1.52% increase. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.062). No sex × time interaction, F (1,25) = 0.02, $p = 0.88$, $\eta_p^2 = 0.01$, main effect for time, F (1,25) = 0.35, $p = 0.56$, $\eta_p^2 < 0.001$, or main effect for sex was observed F (1,25) = 1.69, $p = 0.205$, $\eta_p^2 = 0.06$. Within sex changes can be seen in Table 5.

For males, echo intensity of the rectus femoris was 61.21 ± 11.70 and 62.60 ± 15.60 A.U. at Post-Immobilization and Post-Retraining, respectively, representing a 2.26% increase. For females, echo intensity of the rectus femoris was 59.14 ± 11.17 and 64.64 ± 9.26 A.U. at Post-Immobilization and Post-Retraining, respectively, representing a 9.30% increase. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.134). No sex × time interaction, F (1,25) = 0.93, $p = 0.35$, $\eta_p^2 = 0.04$, main effect for time, F (1,25) = 2.59, $p = 0.12$,

$\eta_p^2 = 0.09$, or main effect for sex was observed, F (1,25) <0.001, $p = 0.997$, $\eta_p^2 < 0.01$. Within sex changes can be seen in Table 5.

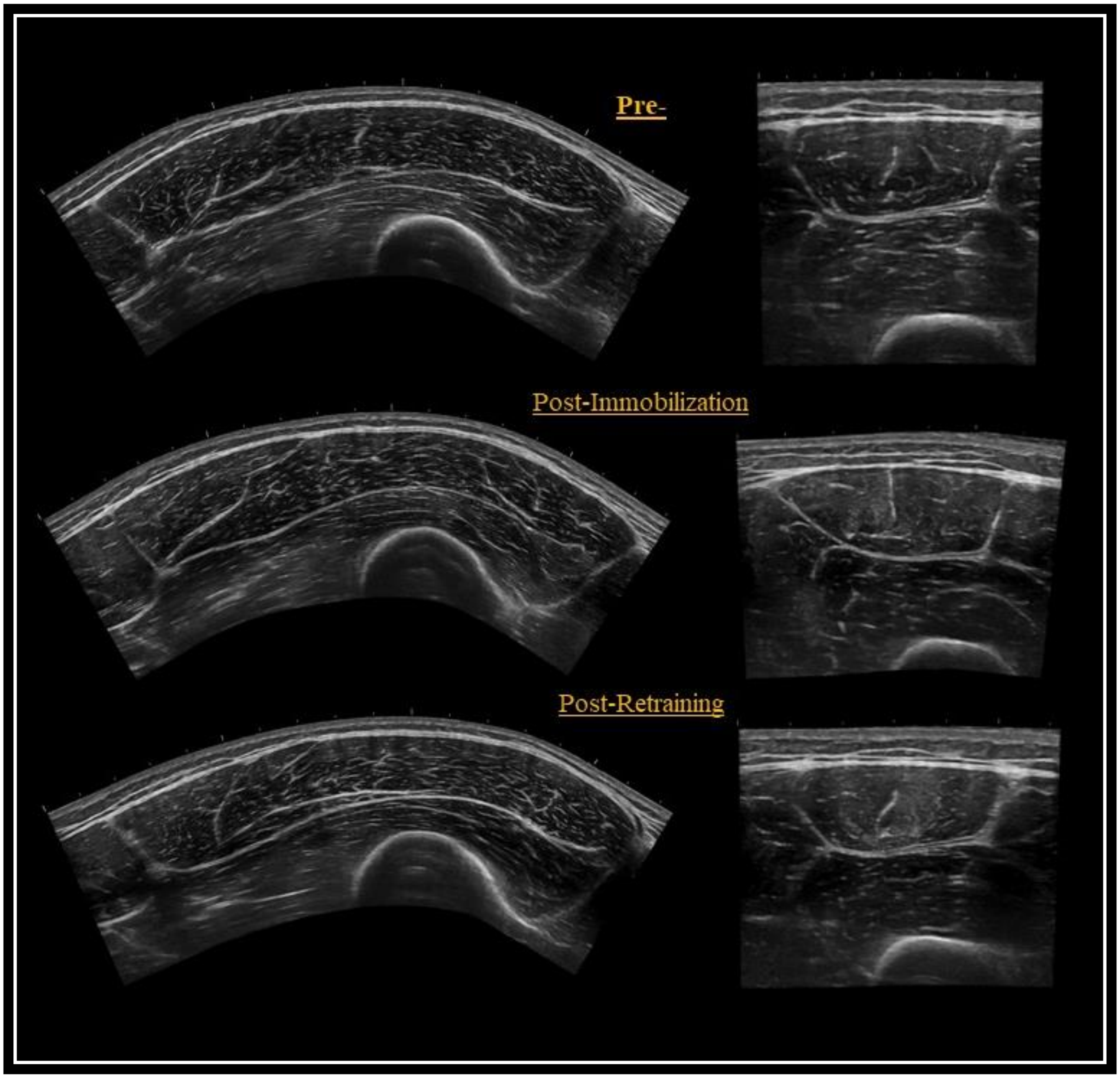


Figure 3. Example Ultrasound Images.

Examples of transverse ultrasound images of the vastus lateralis (right) and rectus femoris (left) muscles from a single participant at Pre-, Post-Immobilization, and Post-Retraining.

Bioelectrical Impedance Spectroscopy

Extracellular Water / Intracellular Water Ratio

For men, the mean ECW/ICW ratio was 0.292 ± 0.04 and 0.284 ± 0.04 at Post-Immobilization and Post-Retraining, respectively, representing a 2.8% decrease. For females, the ECW/ICW ratio was 0.387 ± 0.05 and 0.376 ± 0.04 at Post-Immobilization and Post-Retraining, respectively, representing a 2.9% decrease. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.503). No sex × time interaction, F (1,25) = 0.12, $p = 0.732$, $\eta_p^2 = 0.01$, or main effect for time was observed, F (1,25) = 3.67, $p = 0.067$, $\eta_p^2 = 0.13$. A main effect for sex was observed, however, F (1,25) = 44.58, $p < 0.001$, $\eta_p^2 = 0.64$. Compared to females, males displayed a significantly lesser and favorable ECW/ICW ratio, $p < 0.001$, $d = 1.29$. Within sex changes can be seen in Table 5.

Segmental Lean Mass

For males, segmental lean mass was 12.12 ± 1.37 and 13.24 ± 2.41 kg at Post-Immobilization and Post-Retraining, respectively, representing a 9.25% increase. For females, segmental leg mass was 9.37 ± 0.69 and 9.33 ± 0.89 kg at Post-Immobilization and Post-Retraining, respectively, representing a 2.94% decrease. The results indicated that the assumption of homogeneity was violated (*p's* < 0.043). Friedman's test showed that Segmental Mass was significantly greater following retraining, χ^2 (1) = 16.33, $p < 0.001$. Within sex changes can be seen in Table 5.

Fat-Free Mass (FFM)

For males, fat-free mass was 61.84 ± 8.93 and 63.19 ± 9.86 kg at Post-Immobilization and Post-Retraining, respectively, representing a 2.18% increase. For females, fat-free mass was 41.08 ± 4.25 and 41.93 ± 4.92 kg at Post-Immobilization and Post-Retraining, respectively, representing a 1.13% increase. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.095). No sex × time interaction was observed, F (1,25) = 2.26, $p = 0.145$, $\eta_p^2 = 0.08$. A significant main effect for time was observed with significant increases in FFM being observed at Post-Retraining F (1,25) = 7.94, $p = 0.009$, $\eta_p^2 = 0.24$. A main effect for sex was observed, however, F (1,25) = 46.22, $p < 0.001$, $\eta_p^2 = 0.65$. Compared to females, males displayed significantly greater fat-free mass, $p < 0.001$, $d = 0.54$. Within sex changes can be seen in Table 5.

Fat Mass

For males, fat mass was 19.44 ± 8.13 and 18.55 ± 7.56 kg at Post-Immobilization and Post-Retraining, respectively, representing a 4.6% decrease. For females, fat mass was 18.77 ± 4.30 and 19.54 ± 4.88 kg at Post-Immobilization and Post-Retraining, respectively, representing a 4.19% increase. The results indicated that the assumption of homogeneity was not violated (*p*'s > 0.142). No sex × time interaction, F (1,25) = 3.44, $p = 0.73$, $\eta_p^2 = 0.12$, main effect for time, F (1,25) = 0.02, $p = 0.90$, $\eta_p^2 < 0.001$, or main effect for sex was observed F (1,25) < 0.01, $p = 0.952$, $\eta_p^2 < 0.01$. Within group changes can be seen in Table 5.

Total Body Water

For males, total body water was 45.27 ± 6.54 and 46.25 ± 7.22 L at Post-Immobilization and Post-Retraining, respectively, which represented a 2.18% increase. For females, total body water was 30.40 ± 3.12 and 30.70 ± 3.60 L at Post-Immobilization and Post-Retraining, respectively, representing a 0.99% increase. The results indicated that the assumption of homogeneity was not violated (*p's* > 0.10). No sex × time interaction was observed, F (1,25) = 2.25, $p = 0.15$, $\eta_p^2 = 0.08$. A significant main effect for time was observed, F (1,25) = 7.92, $p = 0.01$, $\eta_p^2 = 0.24$. Relative to Post-Immobilization, TBW was significantly greater at Post-Retraining, $p = 0.009$, $d = 0.54$. A significant main effect for sex was observed, F (1,25) = 46.23, $p < 0.001$, $\eta_p^2 = 0.65$. Relative to females, TBW was significantly greater for males, $p < 0.001$, $d = 1.31$. Within sex changes can be seen in Table 5.

Table 5. Within-Sex Retraining Responses

		Males				Females		
		Post-Immobilization	**Post-Retraining**			**Post-Immobilization**	**Post-Retraining**	
STRENGTH								
MVC peak torque		181.93 ± 34.44 Nm	223.51 ± 46.0 Nm	22.85%		98.78 ± 18.97 Nm	120.67 ± 26.86 Nm	22.16%
Specific Torque		5.06 ± 1.37 Nm/cm^2	6.25 ± 1.51 Nm/cm^2	20.05%		3.89 ± 1.03 Nm/cm^2	4.76 ± 1.18 Nm/cm^2	22.37%
ULTRASOUND								
CSA	VL	28.21 ± 6.60 cm^2	28.97 ± 6.30 cm^2	2.70%		18.21 ± 6.36 cm^2	19.58 ± 5.65 cm^2	7.49%
	RF	7.02 ± 2.21 cm^2	7.68 ± 2.27 cm^2	9.45%		6.75 ± 2.15 cm^2	6.44 ± 2.02 cm^2	-4.70%
Echo Intensity	VL	63.76 ± 15.68 A.U.	64.39 ± 15.17 A.U.	0.99%		70.11 ± 11.46 A.U.	71.18 ± 7.50 A.U.	1.52%
	RF	61.21 ± 11.70 A.U.	62.60 ± 15.60 A.U.	2.26%		59.14 ± 11.17 A.U.	64.64 ± 9.26 A.U.	9.30%
BIS								
FFM		61.84 ± 8.93 kg	63.19 ± 9.86 kg	2.18%		41.08 ± 4.25 kg	41.93 ± 492 kg	1.13%
FM		19.44 ± 8.13 kg	18.55 ± 7.56 kg	-4.60%		18.77 ± 4.30 kg	19.54 ± 4.88 g	4.19%
TBW		45.27 ± 6.54 L	46.25 ± 7.22 L	2.18%		30.40 ± 3.12 L	30.70 ± 3.60 L	0.99%
ECW/ICW		0.292 ± 0.04	0.284 ± 0.04	-2.80%		0.387 ± 0.05	0.376 ± 0.04	-2.90%
Segmental Mass		12.12 ± 1.37 kg	13.24 ± .41 kg	9.25%		9.37 ± 0.69 kg	9.33 ± 0.89 kg	-2.94%

Post-Immobilization values are presented as mean ± standard deviation. Post-Retraining values are reported as mean ± standard deviation, percent change from Post-Immobilization. Green boxes indicate significant positive change from Post-Immobilization

DISCUSSION

To date, this is the first study investigating sex-differences in the recovery of muscle quality in response to a retraining program following lower-limb immobilization. The results of this study demonstrated that indicators of muscle quality respond differently to retraining immediately following one week of lower limb immobilization. specific torque, echo intensity, and ECW/ICW ratio were all negatively affected by one week of immobilization, however, specific torque was the only metric of muscle quality that significantly improved in the time required to recover baseline isometric leg extension strength. This time course was mostly similar between sexes, with females perhaps requiring slightly longer to recover MVC peak torque compared to males. Echo intensity and ECW/ICW ratio failed to improve or exhibited decreases between Post-Immobilization and Post-Retraining. Additionally, while males experienced a significant increase in total body fat-free mass and total body water with retraining relative to Post-Immobilization, the cross-sectional area of the vastus lateralis and rectus femoris muscles remained lower at Post-Retraining relative to Pre- for both sexes. Despite a lack of significant improvement in the structural qualities of the vastus lateralis and rectus femoris muscles, MVC peak torque still improved by greater than 22% for both sexes during the retraining program relative to Post-Immobilization, suggesting that the recovery of strength during retraining may have been mediated by neural factors. These retraining adaptions appear similar to those previously reported during the early phases of resistance training (Moritani and deVries, 1979; Blazevich, 2006).

Following one week of lower-limb immobilization, MVC peak torque was impaired by 10.79% and 15.17% for males and females, respectively. This falls in line with previous investigations reporting that, compared to males, females may be more sensitive to decrements in strength during periods of lower-limb disuse (Deschenes, 2009; Deschenes, 2012). One of the primary purposes of this investigation was to determine whether the time-course to recover isometric leg extension strength would differ between sexes, particularly if females did experience greater MVC peak torque decrements as previously observed. While not statistically significant, the mean (males: 2.13, females: 2.91) and median (males: 1, females: 2) number of retraining sessions performed seem to indicate that, compared to males, females required an additional retraining session to fully recover MVC peak torque. The only other study, to date, that has directly examined sex differences in the recovery of strength following immobilization reported a more rapid recovery of wrist flexion strength for males compared to females (Clark, 2009). It should be noted, however, that study did not include any rehabilitation program and instead had participants simply resume their normal daily activities (Clark, 2009). Having observed no sex differences in central activation, it was postulated that the discrepancies in recovery were not neural in nature, but most likely atrophy-related (Clark, 2009). It was unclear, however, whether these findings could be attributed to biological differences or behavioral factors related to the physical activity performed during the week following immobilization (Clark, 2009). In contrast, in the current study, the controlled resistance training intervention implemented immediately after immobilization appears to have induced more pronounced neural adaptations, which appear to have contributed more significantly to the restoration of MVC peak torque than changes in the mechanical properties of the vastus lateralis and rectus femoris muscles (i.e., echo intensity, ECW/ICW ratio, CSA). Previous investigations that have introduced a resistance training intervention have observed similar rates of improvement in relative strength for males and females that are initially driven by increased efficacy of the nervous system and not an increase in muscle size (Moritani and deVries, 1979; Ivey, 2000; Hubal, 2005; Blazevich, 2006.)

Of the muscle quality indicators, specific torque was the only one that fully returned to Pre- values with retraining. At Post-Retraining, MVC peak torque was actually greater than Pre- for both males and females while the summed CSA of vastus lateralis and rectus femoris at Post-Retraining had still not fully returned to Pre- values. These observed improvements in strength, despite the absence of significant hypertrophy of the quadriceps muscles, resulted in both sexes reporting their greatest specific torque at Post-Retraining, suggesting that the primary mediator of strength during retraining was not the reversal of atrophy, but instead was neural in nature for both males and females. Similar to this study, it has been reported that females experience greater relative decrements in isometric MVC peak torque and specific torque induced by immobilization (Yasuda et al., 2005). Reduction in motor cortical size, reductions in voluntary activation, and alterations in motor unit recruitment and firing frequency behavior have been underlying mechanisms reported to explain why muscle strength loss outpaces atrophy (Liepert et al., 1985; Berg et al., 1991; Dudley et al., 1992; Hortobágyi et al., 2000; MacLennan, 2021). Considering these alterations are quite plastic and that resistance training has been established as a potent stimulus for improving neuromuscular outcomes (Moritani and deVries, 1979; Enoka et al. 1988; Sale et al., 1988; Carroll et al., 2001; Folland, 2007), it was expected that relative strength would increase rapidly during the retraining program. In conjunction, the patterns of muscle atrophy and re-accrual between sexes likely contributed to the rapid increase in specific torque. There is evidence suggesting that immobilization-induced atrophy is more pronounced in type I muscle fibers which have been reported to make up a significantly greater proportion of the vastus lateralis muscle in females compared to males (Labarque et al., 2002; Staron et al., 2000). In the current study, females experienced much greater atrophy of the vastus lateralis compared to the rectus femoris during immobilization. At Post-Retraining, however, the slight recovery of vastus lateralis cross-sectional area was partially offset by further atrophy of the rectus femoris. Despite the inefficient recuperation of muscle cross-sectional area, MVC peak torque was still recovered and females experienced improvements in specific torque that were more pronounced than the decrements incurred by immobilization. In contrast, males experienced relatively greater atrophy of the rectus femoris muscle which represents a relatively smaller cross-sectional area than the vastus lateralis. While not complete, the recovery of vastus lateralis and rectus femoris cross-sectional area was more pronounced and uniform for males, compared to the females, allowing them to hypertrophy to a greater extent in the same time course it took to reproduce their original MVC peak torque. This enhanced hypertrophic capacity, while likely not occurring solely in the vastus lateralis and rectus femoris muscles, was further evidenced by a 9.25% increase in segmental lean mass and significant increases in fat-free mass and total body water at Post-Retraining. Hubal (2005) also reported a greater capacity for hypertrophy in males following unilateral training while females increased specific torque to a slightly greater degree.

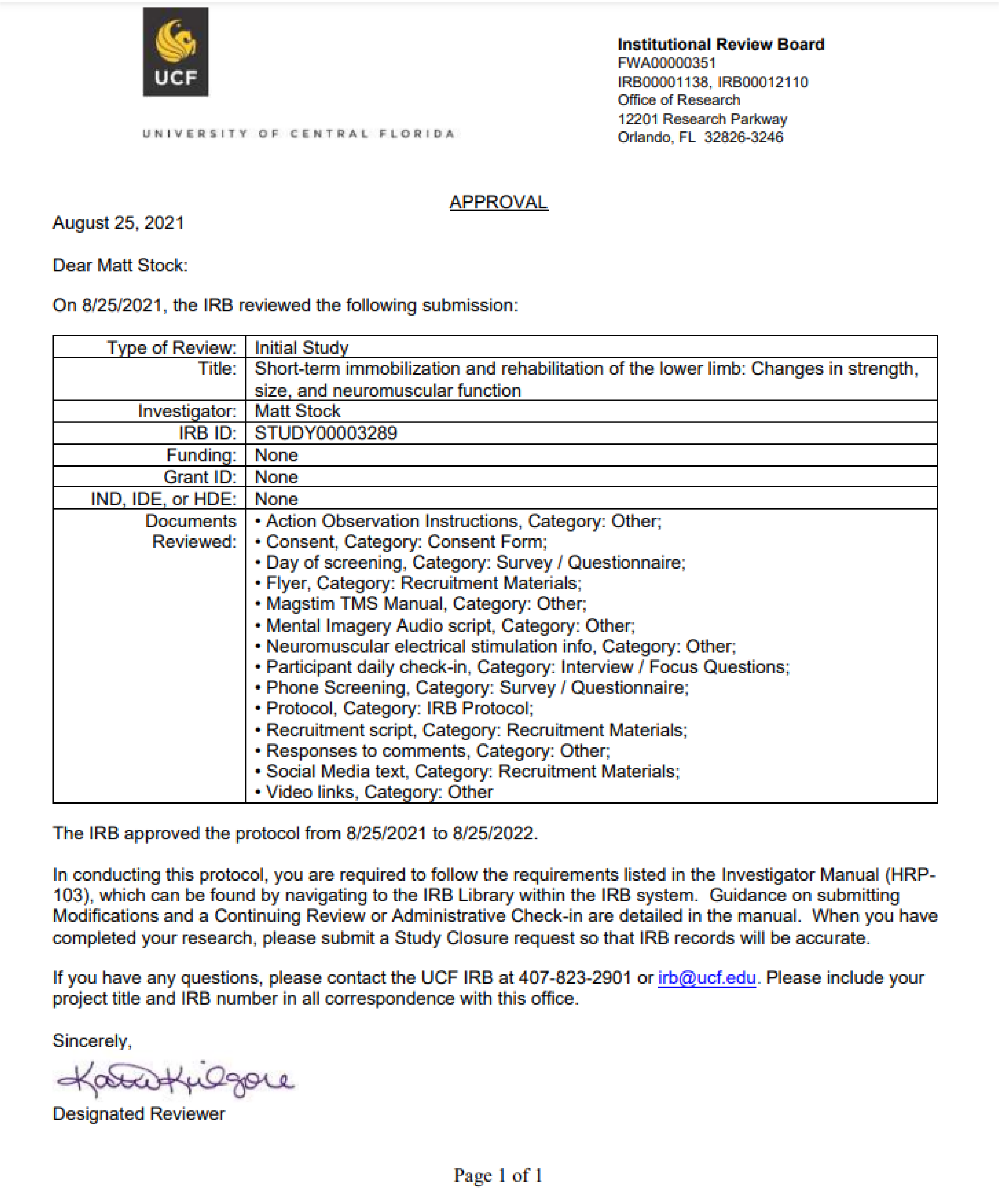
While specific torque increased alongside MVC peak torque with retraining, ECW/ICW ratio and echo intensity did not express significant improvements at Post-Retraining after being negatively affected by immobilization. The ECW/ICW ratio and echo intensity provide complimentary assessments of compositional muscle quality. ECW/ICW ratio expresses the amount of muscle cell mass (ICW) relative to interstitial fluid and blood plasma (ECW) within a limb effectively providing a ratio of non-contractile tissue versus contractile tissue (Yamada, 2017, Taniguchi, 2017). Considering no statistically significant improvements in cross-sectional area were observed for either sex and the significant increases in fat-free mass and total body water observed in males only constituted a 2.18% and 2.18% increase, respectively, it is not surprising that significant improvements in ECW/ICW ratio were not noted. Echo intensity more closely reflects the amount of adipose and connective tissue in the extracellular space within a target muscle (Taniguchi, 2017; Stock and Thompson, 2020). While the increases in echo intensity at Post-Immobilization were similar to previous findings from our lab (MacLennan, 2020), no differences were observed at Post-Retraining. It is possible, however, that any detectable shifts in muscle composition may have been masked by inflammation or muscle damage resulting from the previous training session (Nosaka and Clarkson, 1996; Radelli et al., 2012; Radelli et al., 2014). Although echo intensity has been shown to remain elevated up to 10 weeks after the start of a resistance training program, the improvements in MVC peak torque would suggest muscle damage was unlikely at this time point (Damas et al., 2016; Biazon et al., 2019).

There are several limitations of the current study that should be considered. Firstly, the findings of this study are inextricably linked to the results of Experiment 1. Within the male and female sex-groups were subgroups that experienced different variations of the immobilization protocol (i.e., immobilization only, AOMI, and NMES). While it is probable that this was non-influential on the results of Experiment 2, it cannot be dismissed completely. Another limitation of the current investigation related to Experiment 1 was the use of an isometric MVC as the singular benchmark for retraining completion when the retraining protocol was isotonic in nature. While the utilization of an isometric test to assess progress of dynamic retraining may be sub-optimal in terms of specificity, the use of isometric MVC peak torque to assess strength adaptations is common in the immobilization literature (Yasuda, 2005; Clark, 2009; Deschenes, 2009; Deschenes, 2012) and allowed for the utilization of the same assessment throughout Experiment 1 and Experiment 2, providing a full scope of the changes in muscular performance across all time points. Additionally, the current study did not track menstrual cycle. Some studies have indicated that strength and hypertrophy adaptations may be more pronounced during the follicular phase (Reis et al., 1995; Sung et al., 2014; Wikstrom-Frisen et al., 2017), whereas recovery may be inhibited during the luteal phase (Markofski et al., 2014). Future investigations are needed to determine whether menstrual cycle does influence the short-term recovery of strength and muscle quality immediately following immobilization. While it was collected during Experiment 1, the current study did not track calorie or protein intake during the time window in which retraining took place. Future studies should consider attempting to control protein intake to ensure consumption adequate to illicit potential hypertrophic responses with retraining. Lastly, the limited training experience of our sample should be considered a limitation. While only anecdotally reported, the sample in the current study lacked significant experience with resistance training making it somewhat difficult to distinguish whether the results of the current study are a product of implementing a retraining program immediately after immobilization or simply indicative of introducing resistance training as a new stimulus (Moritani and deVries, 1979; Blazevich, 2006). Future research is needed to elucidate the specific neural mechanisms responsible for the recovery of strength in the absence of hypertrophy immediately following immobilization. Such findings may provide insight into whether these adaptations fully mimic those observed in the early phases of traditional resistance training (Moritani and deVries, 1979; Blazevich, 2006), and may provide more insight into whether resistance training status may play an influential role in the recovery of strength following immobilization. Future studies are also needed to determine how the time-course for strength recovery changes with increased immobilization duration. The ratio of immobilization time to retraining time in the current study was approximately 1:1. Whether this remains consistent following longer bouts of immobilization requires further investigation.

Conclusion

In summary, males and females both exhibit negative changes in muscle quality as a result of one week of lower-limb immobilization. The results of this study indicate that with the implementation of a resistance training based retraining program, specific torque improved along with MVC peak torque, however, echo intensity and ECW/ICW ratio did not improve. The time-course for males and females to recover MVC peak torque was relatively similar with females perhaps needing an additional retraining session. This is the first study to examine sex-differences in the recovery of muscle quality indicators in response to a retraining program following lower-limb immobilization.

APPENDIX A: IRB APPROVAL LETTER



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Institutional Review Board
FWA00000351
IRB00001138, IRB00012110
Office of Research
12201 Research Parkway
Orlando, FL 32826-3246

APPROVAL

August 25, 2021

Dear Matt Stock:

On 8/25/2021, the IRB reviewed the following submission:

Type of Review:	Initial Study
Title:	Short-term immobilization and rehabilitation of the lower limb: Changes in strength, size, and neuromuscular function
Investigator:	Matt Stock
IRB ID:	STUDY00003289
Funding:	None
Grant ID:	None
IND, IDE, or HDE:	None
Documents Reviewed:	• Action Observation Instructions, Category: Other; • Consent, Category: Consent Form; • Day of screening, Category: Survey / Questionnaire; • Flyer, Category: Recruitment Materials; • Magstim TMS Manual, Category: Other; • Mental Imagery Audio script, Category: Other; • Neuromuscular electrical stimulation info, Category: Other; • Participant daily check-in, Category: Interview / Focus Questions; • Phone Screening, Category: Survey / Questionnaire; • Protocol, Category: IRB Protocol; • Recruitment script, Category: Recruitment Materials; • Responses to comments, Category: Other; • Social Media text, Category: Recruitment Materials; • Video links, Category: Other

The IRB approved the protocol from 8/25/2021 to 8/25/2022.

In conducting this protocol, you are required to follow the requirements listed in the Investigator Manual (HRP-103), which can be found by navigating to the IRB Library within the IRB system. Guidance on submitting Modifications and a Continuing Review or Administrative Check-in are detailed in the manual. When you have completed your research, please submit a Study Closure request so that IRB records will be accurate.

If you have any questions, please contact the UCF IRB at 407-823-2901 or irb@ucf.edu. Please include your project title and IRB number in all correspondence with this office.

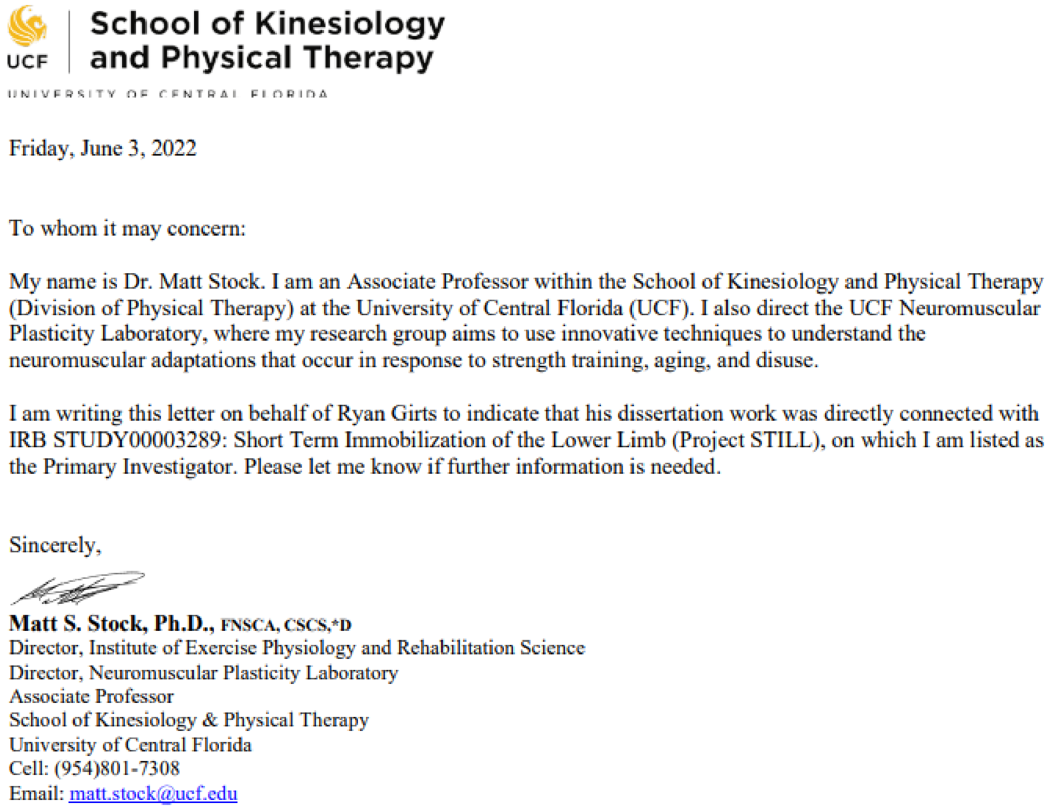
Sincerely,

Katie Kilgore

Designated Reviewer

Page 1 of 1

APPENDIX B: IRB AFFILIATION LETTER



UCF | School of Kinesiology and Physical Therapy
UNIVERSITY OF CENTRAL FLORIDA

Friday, June 3, 2022

To whom it may concern:

My name is Dr. Matt Stock. I am an Associate Professor within the School of Kinesiology and Physical Therapy (Division of Physical Therapy) at the University of Central Florida (UCF). I also direct the UCF Neuromuscular Plasticity Laboratory, where my research group aims to use innovative techniques to understand the neuromuscular adaptations that occur in response to strength training, aging, and disuse.

I am writing this letter on behalf of Ryan Girts to indicate that his dissertation work was directly connected with IRB STUDY00003289: Short Term Immobilization of the Lower Limb (Project STILL), on which I am listed as the Primary Investigator. Please let me know if further information is needed.

Sincerely,

Matt S. Stock, Ph.D., FNSCA, CSCS,*D
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Director, Neuromuscular Plasticity Laboratory
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