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ENDOCRINE AND CONTRALATERAL MUSCLE RESPONSES TO SHORT-TERM  
UNILATERAL RESISTANCE TRAINING

by

CARLEIGH H. BOONE

B.S. Sport & Exercise Science, University of Central Florida, 2012

A thesis submitted in partial fulfillment of the requirements  
for the degree of Master of Science  
in the Department of Educational and Human Sciences  
in the College of Education and Human Performance  
at the University of Central Florida  
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## ABSTRACT

**PURPOSE:** The purpose of this study was to examine the effects of short-term lower body unilateral resistance training on hormonal, muscle morphological, and performance measures in young men. **METHODS:** Seventeen healthy, untrained young men (Age:  $22.8 \pm 3.7$  y; BMI:  $26.5 \pm 4.9$  kg/m<sup>2</sup>) were randomly assigned to one of two groups (UT:  $22.9 \pm 4.6$  y,  $25.3 \pm 4.2$  kg/m<sup>2</sup>; CON:  $24.0 \pm 4.6$  y,  $27.7 \pm 5.1$  kg/m<sup>2</sup>). Resistance training consisted of 4 weeks of unilateral lower body and bilateral upper body exercises on 3 days per week. Each training session entailed unilateral countermovement jumps ( $3 \times 8$ ), unilateral leg press (LP), bilateral chest press (CP), unilateral leg extension (LE), and bilateral low row (LR). Strength exercises were performed for 3 sets of 8-10 repetitions; lower body exercises were performed with the dominant leg only. Muscle thickness (MT), pennation angle (PA), cross-sectional area (CSA), and echo-intensity (EI) of the vastus lateralis (VL) and rectus femoris (RF) muscles of both legs was assessed via ultrasound. Fascicle length (FL) was calculated as  $[MT / \sin(PA)]$ . Maximal dynamic unilateral LP and LE strength was assessed during one-repetition maximum (1RM) testing; CP and LR 1RM strength was estimated as  $[\text{repetition weight} / (1.0278 - 0.0278)(\text{reps})]$ . Maximal isometric knee extensor strength was isolaterally assessed via maximal voluntary contraction (MVC) testing. Mean and peak power output (Watts) was quantified during unilateral countermovement jumps via accelerometry. Fasting concentrations of total testosterone and growth hormone were obtained at baseline (PRE), immediately post (IP), 30-minutes post (30P), and 60-minutes post (60P) during both testing exercise sessions (Pre and Post). Following the 4-week intervention, all participants' maximal dynamic and isometric strength, mean and peak power output, muscle morphology, and hormonal responses were reassessed. Performance, ultrasound, and area under

the curve data were analyzed using ANCOVA to observe between-group comparisons while controlling for baseline (PRE) values. Endocrine data were analyzed using a two-way, mixed-factorial repeated-measures ANOVA. RESULTS: Participants in the UT group experienced significant strength improvements of the trained (28 to 150%) and untrained legs (12 to 160%). Training did not elicit significant improvements in maximal isometric strength or power output of the trained or untrained leg. The trained RF experienced significant increases in CSA and MT. The trained VL experienced a significant increase in CSA. Muscle size of the untrained leg was not significantly augmented. Training did not elicit changes in the acute hormonal response to exercise. CONCLUSIONS: Four weeks of unilateral lower body resistance training using the dominant leg appears sufficient to evoke strength gains of both the ipsilateral and contralateral legs. However, meaningful morphological changes were observed in the trained leg only. Differences in acute hormonal responses to resistance exercise did not appear to explain the observed differences. In addition, unilateral lower body resistance training did not appear to augment the acute endocrine response to an acute bout of resistance exercise. Current findings suggest that the cross-educational strength transfer during the early stage of training is attributable to factors other than changes in muscle morphology and circulating hormones.

## ACKNOWLEDGMENTS

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## TABLE OF CONTENTS

LIST OF FIGURES .....	vii
LIST OF TABLES .....	viii
CHAPTER I INTRODUCTION.....	1
Purposes .....	5
Hypotheses .....	6
Operational Definitions.....	6
Abbreviations .....	6
Delimitations.....	7
Assumptions.....	8
Limitations .....	8
CHAPTER II REVIEW OF LITERATURE .....	10
Mechanisms and Evidence of Cross-Education.....	10
Endocrine Mechanisms .....	10
Skeletal Muscle Adaptations.....	12
Neural Factors.....	14
CHAPTER III METHODS .....	17
Participants.....	17
Research Design.....	17
Familiarization and Testing Protocol.....	18
Training Protocol .....	19
Blood Collection .....	20
Ultrasound Measurement .....	20
Performance Measures .....	22
Blood Analyses .....	23
Statistical Analyses .....	24
CHAPTER IV RESULTS.....	25
Maximal Dynamic Strength.....	25
Maximal Isometric Strength .....	29
Relative Strength.....	29
Mean and Peak Power Output.....	30
Training Volume in Relation to Changes in Strength and Power.....	31
Muscle Morphology and Echo-Intensity .....	32
Total Plasma Testosterone .....	37
Growth Hormone .....	38

CHAPTER V DISCUSSION .....	41
Changes in Maximal Strength.....	41
Changes in Training Volume .....	42
Changes in Power Performance .....	43
Comparison of Contralateral and Ipsilateral Performance Gains .....	43
Changes in Muscle Morphology.....	44
Muscle Morphological Changes in Relation to Strength and Power Changes .....	46
Endocrine Response to Training.....	47
Endocrine Response in Relation to Changes in Morphology and Performance.....	48
Potential Limitations and Further Research.....	50
Conclusions.....	52
APPENDIX A: UCF IRB LETTER .....	53
APPENDIX B: NEIRB APPROVAL LETTER.....	55
REFERENCES .....	57

## LIST OF FIGURES

Figure 1. Changes in leg press 1RM strength of the trained leg from Pre- to Post-testing .....	26
Figure 2. Changes in leg press 1RM strength of the untrained leg from Pre- to Post-testing. ....	27
Figure 3. Changes in leg extension 1RM strength of the trained leg from Pre- to Post-testing ...	27
Figure 4. Changes in chest press 1RM strength from Pre- to Post-testing. ....	28
Figure 5. Changes in low row 1RM strength from Pre- to Post-testing .....	28
Figure 6. Changes in peak power output of the untrained leg from Pre- to Post-testing.....	31
Figure 7. Changes in cross-sectional area of the trained rectus femoris from Pre- to Post-testing .....	33
Figure 8. Changes in muscle thickness of the trained rectus femoris from Pre- to Post-testing ..	33
Figure 9. Changes in cross-sectional area of the trained vastus lateralis from Pre- to Post-testing. .....	34
Figure 10. Ultrasound image of rectus femoris muscle of the trained leg prior to exercise intervention measured.....	35
Figure 11. Ultrasound image of rectus femoris muscle of the trained leg following four weeks of resistance training .....	35
Figure 12. Total testosterone concentrations during Pre- and Post-testing .....	39
Figure 13. Changes in area under the total testosterone curve from Pre- to Post-testing. ....	39
Figure 14. Growth hormone changes during Pre- and Post-testing.....	40
Figure 15. Changes in area under the growth hormone curve from Pre- to Post-testing.....	40



## LIST OF TABLES

Table 1. Participant descriptive characteristics and dietary analyses at Pre- and Post-testing .....	25
Table 2. Changes in ipsilateral and contralateral absolute maximal dynamic (kg) and isometric (N) strength values in response to four weeks of unilateral lower body resistance training	26
Table 3. Changes in ipsilateral and contralateral mean and peak power output (w) in response to four weeks of unilateral lower body resistance training .....	30
Table 4. Changes in ipsilateral and contralateral muscle morphology and echogenicity following four weeks of unilateral lower body resistance training .....	36
Table 5. Changes in hematocrit, hemoglobin, and plasma volume during Pre- and Post-testing	37

## CHAPTER I INTRODUCTION

Resistance training is a potent stimulus for muscular adaptations resulting in strength gains, wherein early augmentation is typically attributed to improved neurological motor recruitment followed closely by changes to skeletal muscle morphology (Baechle, 2008; Moritani, 1979; Staron, 1994; Gabriel, 2006). Although traditional beliefs surmise that only the muscle fibers directly involved in activity will undergo specific adaptation, some research has shown that the adaptations may not be confined to the specific exercising muscle. In fact, recent evidence suggests that unilateral resistance training can cause strength gains to the opposite, contralateral limb when exercise is performed solely on one side of the body in a phenomenon known as cross-education (Munn J. , 2005; Lee, 2009; Lee, 2007).

Cross-education, or contralateral gain, is the ability of an untrained or immobilized limb to experience improvements in strength and/or neuromuscular activity when the contralateral limb is trained independently (Lee, 2007). The effects of cross-education are believed to be task-specific (Lee, 2009). Furthermore, strength gains of the contralateral limb during unilateral training are believed to affect only the homologous and neighboring muscle group (Scripture, 1894; Munn J. , 2004; Zhou, 2000). Numerous studies have shown that contralateral gains can occur following electrical stimulation (Oakman, 1999), autogenic contraction (Ranganathan, 2004), isometric (Carolan, 1992; Garfinkel, 1992; Kannus, 1992; Komi, 1978), isokinetic (Evetovich, et al., 2001; Hortobagyi, et al., 1996; Hortobagyi T. , 1999), and dynamic unilateral

training (Munn J. , 2005; Shaver, 1975; Shaver, 1970), but the precise mechanism(s) by which it occurs are currently unclear.

Since its first observation in 1894 (Scripture), many scientists have devoted research to understanding the mechanism(s) of cross-education. Although none have definitively identified the primary contributing factor, previous research suggests the effects of cross-education on strength gains may be attributed to neural factors (i.e. improved motor unit recruitment), circulating hormones, skeletal muscle morphology, or a combination of those factors (Carroll, 2006; Lee, 2009). Resistance training also presents a potent mechanical stimulus both locally on the exercising muscle and systemically on the endocrine system, often leading to muscle hypertrophy and changes in circulating anabolic hormones. Consequently, cross-education may also involve adaptations to contralateral muscle morphology influenced by the systemic circulation of hormones (Kraemer W. , 2005).

Skeletal muscle morphology includes physical characteristics such as cross-sectional area, muscle thickness, and myofiber arrangement (i.e., pennation angle and fascicle length). Cross-sectional area and muscle thickness are quantitative measures of muscle mass where increases in one or both of these characteristics parallel muscle hypertrophy. The length of a fascicle is determined by the number of serial sarcomeres within a particular muscle fiber, where a greater fascicle length represents greater contractile properties (Abe, 2000; Ranke, 2006). Pennation angle refers to the degree at which a fascicle inserts onto the deep aponeurosis and is highly predictive of a given muscle's force-generating capacity (Farup, 2012). Changes in muscle

morphology are specific to the imposed stimulus and have been observed following periods of training and disuse of varying lengths in both trained and untrained individuals (Blazevich A. , 2003; Seynnes, 2007; Shima, 2002). Detectable adaptations in muscle morphological characteristics have been reported following bilateral training bouts of as little as 20 days (Seynnes, 2007). However, it is unknown if four weeks of unilateral lower body resistance training will produce similar results among the trained and contralateral, untrained musculature.

In addition to the myofiber arrangement and muscle size, recent research has devoted an increasing amount of attention to echo-intensity as a qualitative measure of skeletal muscle in relation to performance (Fukumoto, 2012; Scanlon, 2014). Increased echo-intensity, as assessed via ultrasonography, is indicative of connective tissue and/or intramuscular adiposity within a given muscle – the latter is typically enhanced through disuse, advanced age, and increased body fat levels. As the proportion of contractile muscle cells to non-contractile tissue within a muscle is enhanced, so is the potential for force-production. Therefore, skeletal muscle of higher quality typically possesses a high proportion of muscle tissue and is associated with better strength and power performance (Cadore, 2012; Watanabe, 2013). Previous research has reported lower echo-intensity levels in athletes of varying ages (Sipila, 1994; Jajtner, 2013) suggesting that regular physical activity leads to improved muscle quality. However, it is unknown if a short-term unilateral training protocol will elicit similar benefits in untrained men. Additionally, it is unknown if changes in echogenicity are cross-transferrable.

The endocrine response to resistance exercise is dictated by exercise variables such as training status, exercise intensity, rest interval length, and amount of muscle mass used (Smilios, 2003; Migiano, et al., 2010; Kraemer W. J., 1988). Consequently, unilateral resistance training has been shown to elicit a similar, but lesser, endocrine response pattern when compared to a bilateral training program (Migiano, et al., 2010). There also appears to be a strong relationship between the level of training volume and the anabolic hormonal response, particularly testosterone and growth hormone (VanHelder, 1984; Smilios, 2003). Due to the greater impact on the hormonal response to exercise, it is expected that training-induced contralateral gains are greatest when more muscle mass (i.e., larger muscle groups) is employed (Carroll, 2006; Kraemer W. , 2005). Nonetheless, it is possible that the increase in anabolic hormones following unilateral resistance training could affect contralateral muscle performance and morphology due to the systemic nature of the endocrine response. With a sufficient exercise stimulus (i.e., training volume and intensity), it is plausible that the increase in circulating anabolic hormones following four weeks of lower body unilateral resistance training will evoke strength gains and muscle morphological adaptations of the trained and untrained leg.

Due to its beneficial implications on contralateral strength and function, unilateral training has gained attention as a potential rehabilitative mode of therapy during instances of disuse (i.e., injury or immobilization) (Lee, 2007). Strength decrements occur rapidly during immobilization – within the first two weeks (Vanderborne, 1998) – and are typically unaccompanied by atrophy of the affected musculature. Therefore, early strength losses may be attributed to a decrease in neuromuscular efficiency (Deschenes, 2002; Kitahara, 2003). In the event that only one limb is

affected by injury and/or immobilization, it is plausible that unilateral strength training of the contralateral, healthy limb may provide the means to maintain strength and function of the injured limb via a consistent neural stimulus.

Although previous researchers have examined cross-education, none have successfully clarified the potential muscular, neural, and/or hormonal mechanisms behind the contralateral strength training effect (Carroll, 2006). The extent of improvements in contralateral muscle morphology and/or maximal strength and power output following a unilateral training program are currently unknown. So, the purpose of this study was to examine the muscle morphological and endocrine factors potentially contributing to the contralateral gains obtained from a unilateral resistance training program.

### *Purposes*

1. To examine muscle morphological changes of the ipsilateral and contralateral leg after short-term lower body unilateral resistance training program via ultrasonography.
2. To assess the systemic endocrine response pattern before and after a short-term (4-week) lower body unilateral resistance training program.
3. To determine limb specific and contralateral strength and power adaptations from a short-term (4-week) lower body unilateral resistance training program.

### *Hypotheses*

It was hypothesized that four weeks of unilateral lower body resistance training would:

1. Increase maximal dynamic and isometric strength and power output of both the ipsilateral (trained) and contralateral (untrained) leg, but produce greater improvements in measures of strength than power.
2. Lead to changes in muscle morphological characteristics (i.e. increased cross-sectional area, muscle thickness, pennation angle, and fascicle length) of both the ipsilateral and contralateral knee extensors.
3. Decrease echogenicity of both the ipsilateral and contralateral knee extensors.
4. Augment the acute testosterone and growth hormone response to training.

### *Operational Definitions*

One-Repetition Maximum (1RM) – the maximum amount of weight that can be lifted in only one repetition of a given exercise with proper form.

### *Abbreviations*

1RM – one-repetition maximum

ACSM – American College of Sports Medicine

CON – control group

CSA – cross-sectional area

EI – echo-intensity

FL – fascicle length

GH – growth hormone

MPO – mean power output

MVC – maximal voluntary contraction

PA – pennation angle

PAR-Q – physical activity readiness questionnaire

PPO – peak power output

RF – rectus femoris

TES – total testosterone

UT – unilateral training group

VL – vastus lateralis

VO<sub>2</sub>max – maximal oxygen consumption

### *Delimitations*

Twenty men between the ages of 18 and 35 were recruited for this study. All participants completed a health history questionnaire, PAR-Q, medical and activity questionnaire, and a written statement of informed consent prior to any testing. To be eligible for inclusion in this study, participants must not have performed resistance exercise in the past year, must not have



completed more than the ACSM recommended guidelines for cardiovascular activity per week within the month prior to data collection (150 minutes of moderate intensity or 75 minutes of vigorous intensity exercise), and were free of any physical limitations as determined by the medical and activity questionnaire. The participants must have been free of any chronic illnesses that require continuous medical care and free of the use of medication and/or any ergogenic nutritional supplements within the three months prior to data collection. Lastly, participants must not have a history of medical and/or surgical events that may significantly affect the study outcome.

#### *Assumptions*

1. Participants answered questionnaires accurately and honestly.
2. All participants gave maximal effort during 1RM testing and countermovement jumps.
3. Participants consumed a consistent diet throughout the study duration.

#### *Limitations*

1. Dietary macronutrient consumption variations are inevitable between participants which may have influenced protein synthesizing capabilities.
2. Variation in training intensities/volume may have led to inconsistencies in training results.
3. Time constraints and scheduling conflicts caused a small number of participants to withdraw from the study.

4. Recruitment was done through word-of-mouth and flyer advertisement in the College of Education and Human Performance at the University of Central Florida, and therefore may not have been entirely random.

## CHAPTER II REVIEW OF LITERATURE

### *Mechanisms and Evidence of Cross-Education*

Contralateral gains in performance via cross-education have been evidenced by numerous reports employing unilateral resistance training interventions as brief as three weeks wherein strength gains of the untrained limb have been noted with (Malas, 2013) and without changes in myofiber arrangement or enzymatic activity irrespective of gender (Houston, 1983; Krotkiewski, 1979). Likewise, increased force production of the contralateral limb has been observed with (Hubal, 2005; Malas, 2013; Wilkinson, 2006) and without accompanying muscle hypertrophy (Houston, 1983; Narici, 1989; Housh, 1992) or increased limb circumference (Munn, 2005). Previous research reports gains in contralateral isometric (Shima, 2002; Komi, 1978; Carolan, 1992; Kannus, 1992) and isokinetic (Hortobagyi T. , 1997; Evetovich, et al., 2001; Hortobagyi T. , 1999) strength of the lower limbs. Improvements in force production of the contralateral limb have been reported with and without adaptations to muscle morphological characteristics, hormone secretion, and/or enzyme activity. It is currently alleged that neural, endocrine, and muscular adaptations are responsible for contralateral gains – whether individually or interrelated – but the exact mechanism(s) by which it operates remain unspecified.

### *Endocrine Mechanisms*

Muscle strength is partly influenced by circulating hormones, namely testosterone and growth hormone (Ahtiainen, 2003; Sato, 2014; Ranke, 2006). Supraphysiological doses of these anabolic hormones through exogenous administration have been shown to increase muscle

strength and protein synthesis (Martinez, 1984; Pell, 1987; Bhasin, 1996), but whether a rise in endogenous production improves net protein accretion or muscle mass in adult males remains highly debated (Ranke, 2006; Wilkinson, 2006; Kraemer, 2005; Harper, 1995). A heightened endocrine response to exercise (i.e., elevated post-exercise anabolic hormones) has been observed following bilateral (Kraemer W. , 1991) and unilateral (Migiano, et al., 2010) resistance training protocols. Yet even with the typical exercise-induced increases in circulating hormones, their efficacy is limited by the availability, frequency, and affinity of their respective receptors. As a powerful stimulus for increased muscle size and strength, resistance training has been shown to cause an up-regulation in androgen receptor content of the exercised muscle (Bamman, 2001). However, as this up-regulation is dependent on an imposed mechanical load, the overall influence of the endocrine system on contralateral strength and/or size gains remains unclear.

Because the strength of a muscle is partly determined by the internal arrangement of its fibers, it is speculated that anabolic hormones may produce changes in muscle morphology. Balzevich and colleagues reported increases in pennation angle following 12 weeks of strength training which were accentuated with the administration of testosterone (2001). However, more evidence is needed to conclude that the observed change in morphology was a direct result of testosterone administration. Alternatively, improvements in maximal dynamic and isometric strength and muscle size have been reported in the absence of acute elevations in endogenous testosterone or growth hormone concentrations following eight weeks of unilateral lower body resistance training (Wilkinson, 2006).

### *Skeletal Muscle Adaptations*

Adaptations to skeletal muscle tissue, whether biochemically, hormonally, or structurally-mediated, are closely associated with changes in performance. Strength training involving forceful muscle contractions relies primarily on anaerobic energy production (Tesch, 1987). Thus, resistance exercise requires greater intramuscular energy substrate availability (i.e., ATP, PCr, and glycogen) and enzyme activity – particularly lactate dehydrogenase (LDH), phosphofructokinase (PFK), and myosin ATPase (Barany, 1967). Myosin ATPase activity is associated with rapid muscle contraction and is greater in fast-twitch (type II) fibers (Barany, 1967). Type II muscle fibers also demonstrate higher glycogen content (Grichko, 1999) and enzyme profiles most suitable for anaerobic processes such as resistance training (Thorstensson, 1976). Therefore, type II muscle fibers are advantageous to strength training performance and may be predictive of one's exercise abilities. Chronic heavy resistance training has been shown to cause higher resting concentrations of intramuscular PCr (MacDougall, 1980). Further, increased activity of certain enzymes – PFK and LDH – associated with anaerobic processes has been observed following short-term, high-intensity exercise (Roberts, 1982).

From a structural standpoint, muscle strength has been strongly correlated to its respective cross-sectional area where greater muscle size typically equates to improved force-production capability (Maughan, 1984). In addition to whole muscle size, the force-generating capacity of a muscle is strongly influenced by the intrinsic arrangement and size of its myofibers (Abe, 2000; Kawakami, 1995). With the assumption that increased cross-sectional area of a muscle is the result of myofibrillar hypertrophy and not sarcoplasmic hypertrophy, a larger muscle will contain

additional contractile proteins capable of producing force. Potential muscle morphological adaptations include changes in whole muscle size, muscle fiber size, pennation angle, and/or fascicle length and have been reported following periods of training and disuse in both trained and untrained individuals (Blazevich A. , 2003; Kawakami, 1995; Seynnes, 2007; Moreau N. , 2013; Alegre, 2006). As muscle fascicles are comprised of serial sarcomeres containing contractile proteins, greater fascicle length is typically indicative of enhanced contractile properties of a given muscle (Abe, 2000; Ranke, 2006). The degree at which a fascicle inserts onto the aponeurosis (i.e., pennation angle) is highly predictive of a given muscle's force-generating capacity and typically increases synchronously with muscle thickness (Farup, 2012). Balzevich and colleagues reported a significant increase in pennation angle and fascicle length accompanied by greater force production following five weeks of concurrent lower body strength and power training among young competitive athletes (2003). In addition, significant increases in cross-sectional area, pennation angle, and fascicle length have been reported following three weeks of high-intensity resistance training among active adults (Seynnes, 2007). Further, previous research has identified the selective hypertrophy of type II fibers within a given muscle following long-term resistance training interventions (Houston, 1983; Tesch, 1987; Thorstensson, 1976).

Malas and colleagues observed significant improvements in contralateral knee extensor strength accompanied by increases in both muscle thickness and pennation angle of the untrained vastus lateralis following three weeks of isometric strength training (2013). By contrast, Ploutz and colleagues reported a 7% increase in 1RM strength of the untrained leg with no corresponding

change in muscle size following nine weeks of unilateral knee extensor-strengthening exercise (1994). Similarly, Munn and colleagues observed a significant increase in contralateral 1RM elbow flexor strength with no change in the untrained arm's circumference after six weeks of dynamic resistance training (2005). Houston and colleagues reported significant increases in contralateral peak torque, but observed no change in myofiber size or enzyme activity of the untrained leg musculature following 10 weeks of dynamic resistance training (1983). Additionally, Krotkiewski and colleagues reported significant improvements in isometric strength and isokinetic torque in the absence of significant changes in limb circumference, myofiber composition and area, or muscle thickness following five weeks of concurrent lower body unilateral isometric/isokinetic training (1979).

The inconsistency in findings suggest that early cross-educational effects may be the result of adaptations other than changes in muscle morphological characteristics, increased hormone production, and/or enzymatic activity. Changes in muscle size and myofiber arrangement (i.e., pennation angle, fascicle length) are not immediate and appear to occur only after at least three weeks of resistance training. Further, myofiber enzymatic adaptations within a given muscle may only be evoked upon direct mechanical stimulus and are likely not the sole causal property of contralateral gains during unilateral exercise.

### *Neural Factors*

Strength gains are influenced by adaptations to the central and peripheral nervous systems (i.e., improved neural drive, spatial recruitment, rate coding, and motor unit synchronization) and are

largely involved in increased force production of a muscle through enhanced neuromuscular efficiency (Gabriel, 2006). The early improvements in strength through resistance training are likely the result of increased neural drive causing enhanced voluntary activation of the exercised muscle or muscle group before increases in muscle size are detectable (Moritani, 1979; Gabriel, 2006). It is believed that the repeated performance of a unilaterally-executed activity enhances motor learning and skill acquisition of the opposite limb through familiarity (Lee, 2007; Farthing, 2005). Therefore, many researchers have concluded that the aforementioned neural factors are primary mechanisms influencing contralateral strength gains due to the inherent alliance of the nervous and muscular systems.

Given the previously discussed improvements in contralateral force-production in the absence of changes in muscle fiber area or enzyme activity, it is purported that cross-education is influenced by neural adaptations and/or improved myoelectric activity (Chen, 1997; Lee, 2009; Shima, 2002; Lee, 2007). Neural adaptations to the untrained limb have been observed following stimulation of only the opposite, trained limb (Magnus, 2010; Lee 2009; Narici, 1989; Garfinkel, 1992). Magnus and colleagues observed enhanced electromyographic activity in the untrained, immobilized arm following four weeks of unilateral isometric training of the mobile arm (2010). Similarly, Narici and colleagues reported increased contralateral isometric strength with an accompanying increasing in electromyographic activity following 60 days of unilateral strength training (1989). Additionally, significant contralateral strength improvements have been observed with synchronous changes in skeletal muscle electrical activity (Moritani, 1979; Shima, 2002). Previous research has reported significant increases in contralateral MVC and voluntary



activation via cortical stimulation following four weeks of unilateral isometric training of the wrist extensors (Lee, 2009) and six weeks of unilateral dynamic training of the plantar flexors (Shima, 2002).

From the current literature, it is apparent that cross-education occurs irrespective of age, gender, muscles trained, or mode of stimulation. With evidence of contralateral strength gains independent of changes in muscle morphological characteristics, enzyme activity, or elevated levels of circulating anabolic hormones, it is likely that neural factors play a primary role in cross-education. However, as many of the proposed underlying mechanisms of cross-education are interrelated, its effects are most likely mediated through a combination of neural, morphological, biochemical, and/or hormonal factors.

## CHAPTER III METHODS

### *Participants*

Twenty untrained men were recruited for this investigation. All subjects completed a health history questionnaire, PAR-Q, and medical and activity questionnaire to assess physical activity level, health status, and possible risk factors. Participants were asked to avoid any ergogenic supplement use (protein, creatine, etc.) and refrain from participation in any other clinical/investigational trials throughout the duration of this experiment. All participants were untrained as determined by the ACSM's guidelines for cardiovascular exercise. In addition, none had any lower body resistance training experience within the year prior to this experiment. The New England Institutional Review Board's approval was obtained before any data collection was conducted. All subjects completed a written informed consent form prior to any data collection and were randomly assigned to either a control (CON) or unilateral training (UT) group.

### *Research Design*

A randomized, controlled, mixed-factorial design was used to examine the effects of short-term unilateral resistance training on (a) muscle morphology [pennation angle, fascicle length, cross-sectional area, muscle thickness], (b) lower body power output, (c) maximal knee extensor strength [1RM], (d) maximal voluntary contraction [MVC], and (e) endocrine response. Hormones were analyzed acutely (pre-exercise [PRE], immediately post-exercise [IP], 30 minutes post-exercise [30P], and 60 minutes-post exercise [60P]) and chronically (pre-testing

[Pre] vs. post- testing [Post]) to assess the effects of short-term unilateral resistance training. All participants were asked to visit the university's Human Performance Lab on four separate occasions to complete Pre- and Post-testing; participants in the training group visited the facility a total of 16 times (four times to complete Pre- and Post-testing, 12 times to complete training sessions).

#### *Familiarization and Testing Protocol*

Pre-testing occurred during the week preceding the intervention period. Pre-testing assessments were completed on two separate days. The first day consisted of examination of the vastus lateralis and rectus femoris muscles via ultrasonography (General Electric LOGIQ P5, Wauwatosa, WI, USA), power testing via accelerometry during unilateral countermovement jumps (Tendo™ Power Units, Tendo Sports Machines, Trencin, Slovak Republic), maximal voluntary contraction of the knee extensors (Biodex Medical Systems, Shirley, NY, USA), exercise familiarization, and one-repetition maximum (1RM) testing of the chest press, low row, leg press, and leg extension exercises (Power Lift, Jefferson, IA, USA). Exercise familiarizations were conducted prior to 1RM testing, wherein all participants were instructed on proper form for each of the required exercises. The second day of testing occurred no less than 72 hours later to allow full muscle recovery. During day two of Pre-testing, participants reported to the Human Performance Lab following a 10-hour overnight fast to complete a simulated training session. On this occasion, blood was drawn to determine the acute hormonal response to exercise. In the week following the four-week intervention period, all participants returned to the Human Performance lab for Post-testing which mimicked the same two-day format as Pre-testing.

### *Training Protocol*

For the duration of the intervention period, each participant in the UT group reported to the Strength and Conditioning Lab on three nonconsecutive days per week for training sessions. In the event that a training session was missed, make-up sessions were scheduled with lab staff to ensure that 12 total sessions were completed during the four weeks while still maintaining appropriate rest periods between training sessions. Prior to each session, participants completed a general and specific warm-up. The general warm-up consisted of five minutes of non-fatiguing aerobic activity on a cycle ergometer at a self-selected resistance and cadence. The specific warm-up consisted of 10 body weight squats, alternating lunges, walking knee hugs, and glute kicks. During each training session, participants performed a unilateral lower body and bilateral upper body resistance training routine consisting of leg press, leg extension, chest press, and low row exercises. All exercises were completed for a total of three sets of 8-10 repetitions at 80% of the participant's previously determined 1RM. If a participant could not perform the minimum amount of repetitions during the first or second sets, the trainer decreased the weight accordingly while still ensuring a challenging intensity. Consequently, if the participant was able to perform all repetitions with proper form and minimal strain, weights were progressively increased during the subsequent training session. The rest interval between all sets was 90 seconds. Unilateral lower body exercises were performed by the dominant leg only; the nondominant, untrained leg remained relaxed throughout the exercise protocol. Training volume (repetitions  $\times$  load) was recorded after each training session for further statistical analysis. All participants were asked to refrain from any other form of structured resistive exercise and to maintain their usual recreational activities for the duration of this study.

### *Blood Collection*

Blood samples were obtained before exercise (PRE), immediately post-exercise (IP), 30 minutes post-exercise (30P) and 60 minutes post-exercise (60P) on the second day of Pre- and Post-testing. Following a 15-min equilibration period during which the participant laid supine, samples were obtained from a superficial antecubital vein using a Teflon™ cannula by an experienced lab technician whose abilities were previously approved by a University of Central Florida MD. The cannula was placed as not to interfere with the ability to perform the exercise routine. Further, a 1 ml infusion of a saline solution was administered after each blood draw to keep the cannula open. The total amount of blood drawn during the each testing session did not exceed 12 ml (6 ml per blood draw). Each participant's blood samples were obtained at the same time of day during each session to avoid diurnal variations in circulating hormones. Samples were drawn into serum or EDTA treated Vacutainer® tubes (Becton Dickinson, Broken Bow, NE) for further analysis. Whole blood samples were analyzed in duplicate for hematocrit via microcapillary technique and hemoglobin content at each time point. The remaining whole blood was centrifuged for 15 minutes at 1500g at 4°C. The resulting plasma and serum was aliquoted and stored at -80°C until further analysis. Samples were thawed only once for biochemical analysis.

### *Ultrasound Measurement*

During testing sessions, participants reported to the Human Performance Lab for non-invasive ultrasound examination of the quadriceps musculature. Participants were asked to lay supine on an examination table with both legs fully extended for a minimum of 15 minutes to allow fluid

shifts to occur. Images of the rectus femoris (RF) were captured midway between the anterior inferior iliac crest and proximal patellar border. Images of the vastus lateralis (VL) were captured on the midline halfway between the greater trochanter and lateral epicondyle. The following measurements were obtained from the images of the RF and VL: pennation angle (PA), muscle thickness (MT), cross-sectional area (CSA), and echo-intensity (EI). All measures were obtained by passing a 12MHz probe (General Electric LOGIQ P5, Wauwatosa, WI, USA) coated with water-soluble transmission gel (Aquasonic® 100, Parker Laboratories, Inc., Fairfield, NJ) over the surface of the thigh at the predetermined anatomical locations outlined above. Measures of CSA, PA, and MT were captured using B-mode ultrasonography with gain set at 50 and dynamic range set to 72 to optimize spatial resolution. Image depth was fixed at 5 cm<sup>4</sup>. Further analysis of all ultrasound images was performed via ImageJ (National Institutes of Health, USA, version 1.45s) to quantify CSA, PA, MT, and EI. Fascicle length (FL) was estimated using the following equation:

$$FL = MT / \sin(PA) \text{ (Kawakami, 1995)}$$

Echo-intensity (EI) was quantified through grayscale analysis using the standard histogram function in ImageJ. The same investigator performed all ultrasound measurements. Intraclass correlation coefficients and minimal differences (MD) were as follows: cross-sectional area (R = 0.93; MD = 1.68 cm<sup>2</sup>), muscle thickness (R = 0.95; MD = 0.20 cm), pennation angle (R = 0.93; MD = 1.94°), and echo-intensity (R = 0.92; MD = 8.76 au).

### *Performance Measures*

During testing sessions, each lower body exercise was tested unilaterally. Maximal isometric (MVC) strength of the dominant and nondominant leg was quantified using a Biodex™ isokinetic leg extension dynamometer. Each participant performed three separate maximal contractions at 110° with three minutes of rest separating repetitions. Lower body power output was then quantified via accelerometry during unilateral countermovement jumps. Each participant was asked to complete three maximal effort countermovement jumps on each leg with hands placed on his hips to rule out extraneous force generation. Power output was quantified using a Tendo™ Power Unit which consists of a transducer attached to the waist of the participant to measure linear displacement over time. Subsequently, velocity was calculated and power was determined. Mean and peak power output were recorded from each jump and used for later analysis. Test-retest reliability for the Tendo™ unit in our laboratory has consistently shown  $R > 0.90$ . One-repetition maximum testing of the lower body exercises followed methods previously outlined by Hoffman (2006). Upper body 1RM strength was predicted using a previously published formula:

$$1RM = \text{Repetition weight} / [1.0278 - 0.0278 (\text{repetitions to fatigue})] \text{ (Brzycki, 1993)}$$

Relative strength was calculated as strength relative to body weight. Specific strength, reported as strength relative to the sum of muscle cross-sectional areas, was calculated for MVC and leg press and extension 1RM strength:

$$[\text{Strength} / (\text{RF CSA} + \text{VL CSA})] \text{ (Kent-Braun, 1999)}$$

Prior to strength testing, each participant completed the previously described general and specific warm-up protocols. During 1RM testing, the trainer monitored and instructed proper exercise form to ensure that each participant met the desired range of motion for each exercise. Attempts not meeting the range of motion criterion for each exercise, as determined by the trainer, were discarded. All 1RM tests were completed under the supervision of a National Strength & Conditioning Association Certified Strength and Conditioning Specialist.

#### *Blood Analyses*

Plasma concentrations of total testosterone and growth hormone were assayed using commercially available ELISA kits (TES: KGE010; GH: DGH00, R&D Systems®, Minneapolis, MN, USA). The growth hormone ELISA focused on 20- and 22-kDa variants. Assay absorbance was read according to manufacturer specifications on a BioTek® Eon™ Microplate Spectrophotometer (BioTek Instruments, Inc., Winooska, VT, USA). All samples remained frozen until analysis, were thawed only once, and were measured in duplicate. The sensitivity of the testosterone assay was 0.041 ng/mL, and the intra-assay coefficient of variation was 5.3%. The sensitivity of the growth hormone assay was 7.18 pg/mL, and the intra-assay coefficient of variation was 5.8%. All assays procedures followed those outlined by the manufacturer.



### *Statistical Analyses*

Data were assessed for normality (Shapiro-Wilk test) and equality of variance (Levene's test). Data were considered normally distributed thus analyses proceeded with parametric statistical analyses. Between-group differences in performance, ultrasound, and hormone area under the curve (AUC) data were analyzed using one-way analysis of covariance (ANCOVA) to control for baseline measures recorded at the pre-exercise (PRE) time point. Baseline differences in hormone concentrations at Pre- and Post-testing were identified using independent sample *t*-tests. If no significant between-group baseline differences were identified, data were analyzed using a two-way, mixed-factorial [group (training [UT] vs. control [CON]) × time (pre-exercise [PRE], immediately post-exercise [IP], 30 min post-exercise [30P], 60 min post-exercise [60P]) repeated-measures ANOVA. In the event of significant between-group baseline differences, data were analyzed using two-way, mixed factorial repeated-measures ANCOVA to control for baseline measures. In the event of a significant *F* ratio, Tukey's post-hoc analysis was performed to determine the location of the group difference. In order to characterize directionality and relationships between changes in muscle morphology, endocrine response, and strength and power measures, Pearson product-moment correlations were used. Results were considered significant at an alpha level of  $p \leq 0.05$ . All data were reported as mean  $\pm$  SD. Data were analyzed via SPSS (Version 20.0, SPSS Inc., Chicago, IL).

## CHAPTER IV RESULTS

Twenty young ( $n = 20$ ) men volunteered to participate in this investigation. Seventeen men completed the study and were included in the analyses. Volunteers who did not complete the study reported personal reasons ( $n = 2$ ) and/or issues of time commitment ( $n = 1$ ). Participant characteristics are displayed in Table 1. Both groups were similar in BMI, age, relative protein (g/kg) and total caloric intake at Pre-testing. No significant changes in body mass, BMI, relative protein (g/kg), or total caloric intake were observed over the 4-week intervention period in either group. Participants in the CON group had significantly greater total body mass at Pre- and Post-testing than those in the UT group ( $p = 0.033$  and  $p = 0.046$ , respectively).

*Table 1. Participant descriptive characteristics and dietary analyses at Pre- and Post-testing*

	<b>Training Group (<math>n = 9</math>)</b>		<b>Control Group (<math>n = 8</math>)</b>	
<b>Age (y)</b>	$22.9 \pm 3.1$		$24.0 \pm 4.6$	
	Pre	Post	Pre	Post
<b>Weight (kg)</b>	$76.80 \pm 14.40$	$78.18 \pm 14.02$	$94.20 \pm 16.10^*$	$94.56 \pm 17.06^*$
<b>BMI (kg/m<sup>2</sup>)</b>	$25.25 \pm 4.24$	$25.72 \pm 4.22$	$27.70 \pm 5.14$	$27.81 \pm 5.46$
<b>Protein (g)</b>	$90.49 \pm 25.85$	$72.42 \pm 28.57$	$113.12 \pm 45.56$	$89.39 \pm 35.80$
<b>Pro(g)/BW(kg)</b>	$1.24 \pm 0.44$	$0.93 \pm 0.33$	$1.20 \pm 0.48$	$0.90 \pm 0.36$
<b>Total kCals</b>	$2001.03 \pm 394.16$	$1844.71 \pm 286.30$	$2372.79 \pm 495.65$	$2454.48 \pm 1138.90$

Values are means  $\pm$  SD. Training, resistance trained dominant limb. Control, no intervention. Pre, baseline measurements. Post, following 4-week intervention. Total kilocalories, daily average energy intake calculated from three-day dietary recall at Pre and Post. \*Significantly different from UT group at corresponding time point,  $p < 0.05$ .

### *Maximal Dynamic Strength*

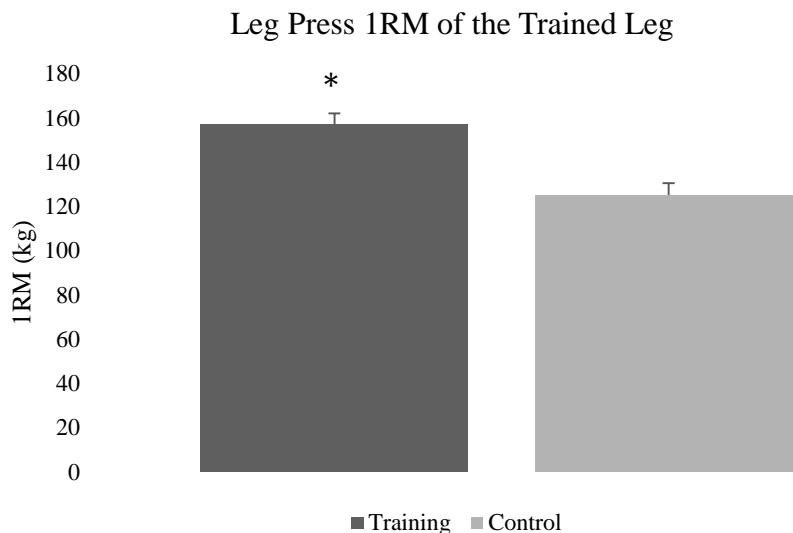
Data are displayed in Table 2. After controlling for Pre values, significant group differences were observed in leg press (trained:  $p = 0.001$ ,  $72.6 \pm 44.4\%$ ; untrained:  $p = 0.012$ ,  $60.4 \pm 52.4\%$ ), leg extension (trained:  $p = 0.006$ ,  $45.3 \pm 15.8\%$ ), chest press ( $p = 0.030$ ,  $24.7 \pm 17.2\%$ ), and low row ( $p = 0.008$ ,  $34.0 \pm 17.6\%$ ) 1RM strength in the UT group (Figures 1-5). No significant group

difference ( $p = 0.546$ ) was noted in leg extension 1RM strength of the untrained leg following training after controlling for Pre values.

*Table 2. Changes in ipsilateral and contralateral absolute maximal dynamic (kg) and isometric (N) strength values in response to four weeks of unilateral lower body resistance training*

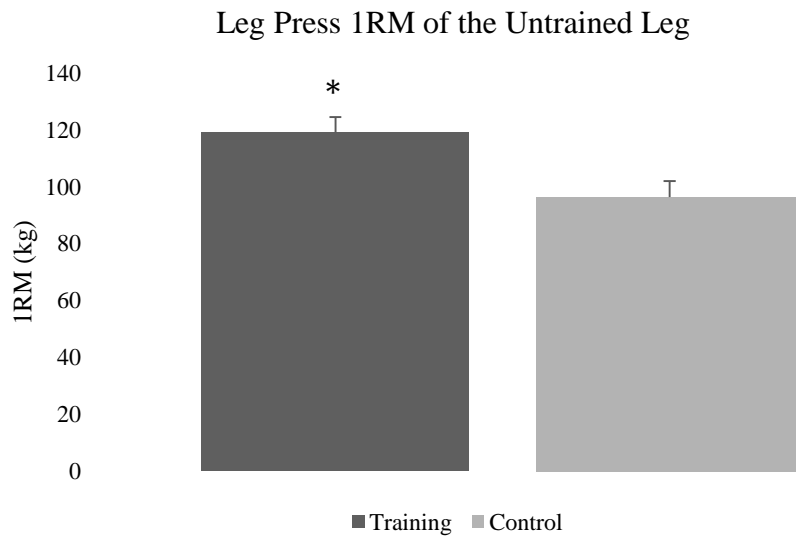
	Training Group (n = 9)		Control Group (n = 8)	
	Pre	Post	Pre	Post
<b>Unilateral Leg Press (kg)</b>				
Trained leg	92.99 ± 37.87	147.67 ± 33.30*	115.38 ± 32.88	135.79 ± 36.40
Untrained leg	80.13 ± 41.39	112.14 ± 35.54*	98.94 ± 25.49	104.33 ± 26.28
<b>Unilateral Leg Extension (kg)</b>				
Trained leg	42.84 ± 13.15	61.23 ± 15.42*	59.25 ± 12.21	63.50 ± 13.55
Untrained leg	43.60 ± 12.25	48.89 ± 10.02	56.42 ± 13.63	60.67 ± 9.13
<b>Chest Press (kg)</b>				
	26.13 ± 6.72	31.93 ± 6.54*	31.82 ± 7.18	33.85 ± 6.78
<b>Low Row (kg)</b>				
	117.32 ± 30.45	153.64 ± 29.66*	145.24 ± 18.83	151.90 ± 11.18
<b>MVC (N)</b>				
Trained leg	867.39 ± 253.32	932.22 ± 256.55	1002.43 ± 122.81	967.37 ± 119.91
Untrained leg	833.85 ± 227.62	831.01 ± 224.99	973.07 ± 127.37	980.27 ± 124.67

Values are means ± SD. Unilateral leg press & extension, 1RM. Bilateral chest press & low row, estimated RM via Brzycki formula. Pre, baseline measurements. Post, following 4-week intervention. Maximal isometric strength averaged from unilateral MVC (three per leg) via maximal isometric contraction during leg extension. Pre, baseline measurements. Post, following 4-week intervention.\*Significantly different from corresponding Pre value,  $p < 0.05$ .

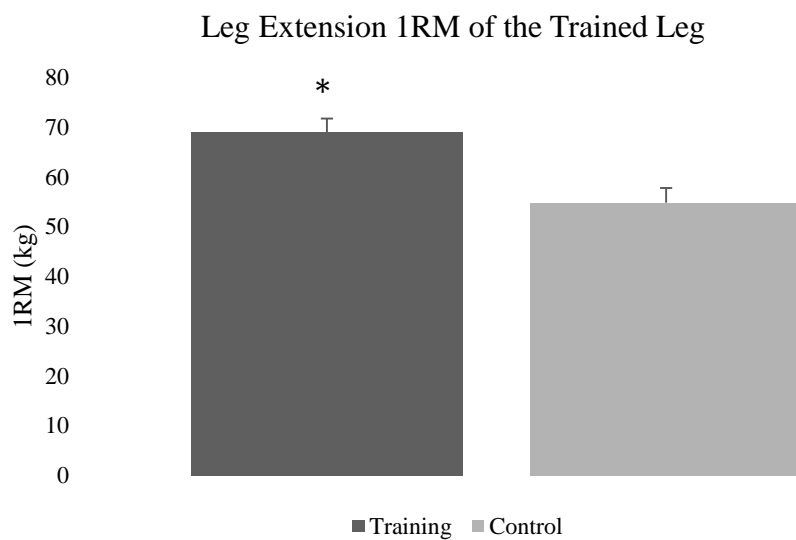


*Figure 1. Changes in leg press 1RM strength of the trained leg from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean = 103.5247).*

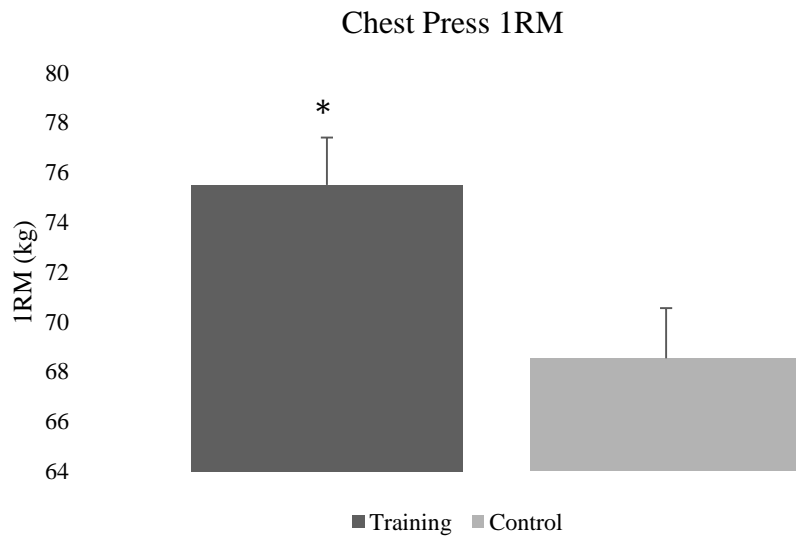
\*Significantly different from corresponding Pre value.



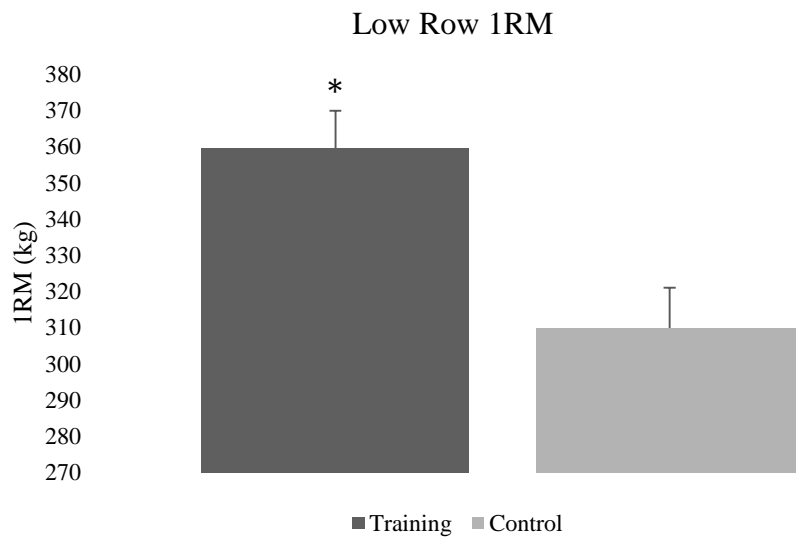
*Figure 2. Changes in leg press 1RM strength of the untrained leg from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean =88.9841). \*Significantly different from corresponding Pre value.*



*Figure 3. Changes in leg extension 1RM strength of the trained leg from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean =50.5612). \*Significantly different from corresponding Pre value.*



*Figure 4. Changes in chest press 1RM strength from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean = 63.3771). \*Significantly different from corresponding Pre value.*



*Figure 5. Changes in low row 1RM strength from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean = 287.0071). \*Significantly different from corresponding Pre value.*

### *Maximal Isometric Strength*

No significant between-group difference in maximal isometric strength of the trained ( $p = 0.113$ ) or untrained ( $p = 0.613$ ) leg was observed after controlling for Pre values.

### *Relative Strength*

After controlling for Pre values, a significant group difference was observed in relative leg press ( $p < 0.001$ ) and relative leg extension 1RM strength ( $p = 0.001$ ) of the trained leg where the UT group experienced a  $69.4 \pm 43.8\%$  and  $42.6 \pm 16.2\%$  increase, respectively. A significant group difference in relative leg press 1RM strength ( $p = 0.006$ ; UT:  $57.1 \pm 50.5\%$ ) of the untrained leg was observed after controlling for Pre values, but not in relative leg extension 1RM strength ( $p = 0.743$ ). Analyses controlling for Pre values determined significant group differences in relative chest press ( $p = 0.021$ ) and low row ( $p = 0.001$ ) 1RM strength where the UT group increased by  $22.3 \pm 16.4\%$  and  $31.6 \pm 17.8\%$ , respectively. After controlling for Pre values, no significant group difference was observed in relative MVC strength of the trained ( $p = 0.116$ ) or untrained leg ( $p = 0.608$ ).

### *Specific Strength*

After controlling for Pre values, a significant group difference was observed in specific leg press ( $p = 0.021$ ) and specific leg extension 1RM strength ( $p = 0.017$ ) of the trained leg where the UT group experienced a  $49.8 \pm 42.1\%$  and  $25.3 \pm 11.7\%$  increase, respectively. A significant group difference in specific leg press 1RM strength ( $p = 0.003$ ; UT:  $54.6 \pm 47.7\%$ ) of the untrained leg was observed after controlling for Pre values, but no significant group difference was identified

in specific leg extension 1RM strength ( $p = 0.730$ ). No significant group difference was observed for specific MVC strength of the trained ( $p = 0.786$ ) or untrained leg ( $p = 0.506$ ).

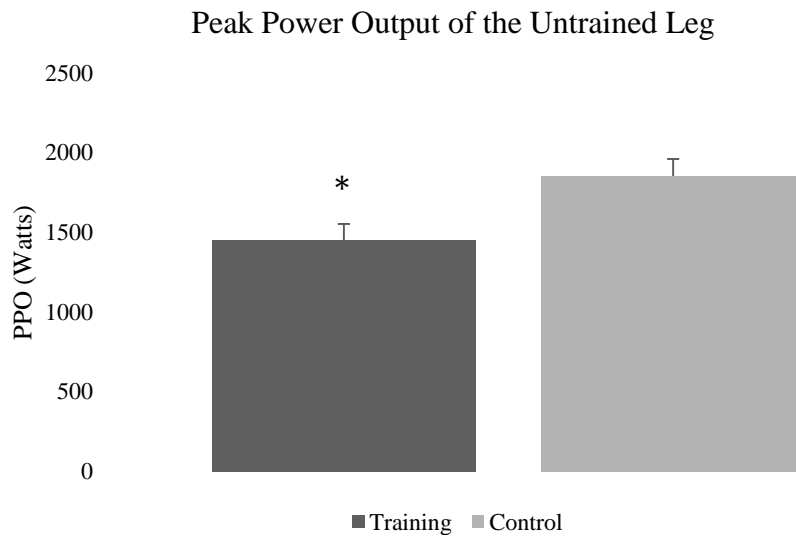
### *Mean and Peak Power Output*

Data are displayed in Table 3. Analyses controlling for Pre values indicated no significant between-group differences in mean power output of the trained ( $p = 0.163$ ) or untrained leg ( $p = 0.117$ ). A significant between-group difference in peak power output of the untrained leg ( $p = 0.018$ ; UT:  $-11.5 \pm 16.1\%$ ) was identified after controlling for Pre values (Figure 6), but no significant group difference was observed in peak power of the trained leg ( $p = 0.387$ ).

*Table 3. Changes in ipsilateral and contralateral mean and peak power output (W) in response to four weeks of unilateral lower body resistance training*

	<b>Training Group (n = 9)</b>		<b>Control Group (n = 8)</b>	
	Pre	Post	Pre	Post
<b>Peak Power</b>				
Trained leg	1623.4 ± 296.5	1620.9 ± 329.1	1975.6 ± 371.4	1943.2 ± 313.9
Untrained leg	1649.7 ± 348.8	1427.2 ± 261.6*	1841.1 ± 414.6	1880.4 ± 347.6
<b>Mean Power</b>				
Trained leg	696.4 ± 139.4	676.3 ± 181.6	811.9 ± 183.6	877.6 ± 198.8
Untrained leg	682.3 ± 134.3	609.2 ± 114.0	859.6 ± 229.7	885.3 ± 279.5

Values are means ± SD. PPO and MPO averaged from unilateral CMJ (three per leg) via Tendo accelerometers. Pre, baseline measurements. Post, following 4-week intervention. \*Significantly different from corresponding Pre value,  $p < 0.05$ .



*Figure 6. Changes in peak power output of the untrained leg from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean =1789.41). \*Significantly different from corresponding Pre value.*

#### *Training Volume in Relation to Changes in Strength and Power*

Participants in the UT group displayed a significant increase in training volume ( $p < 0.001$ ;  $57.9 \pm 19.2\%$ ) from Pre- to Post-testing. Pearson product-moment correlation coefficients determined significant ( $p < 0.01$ ) relationships between the change in training volume to changes in absolute leg press (trained:  $r = 0.780$ ; untrained:  $r = 0.714$ ), leg extension (trained:  $r = 0.663$ ), chest press ( $r = 0.674$ ), low row ( $r = 0.807$ ) 1RM strength, and MVC strength of the untrained leg ( $p < 0.05$ ;  $r = 0.533$ ). No significant correlations were observed between the changes in training volume and MVC strength of the trained leg ( $r = 0.512$ ), leg extension 1RM strength of the untrained leg ( $r = 0.229$ ), or mean or peak power output of the trained ( $r = -0.025$  and  $r = 0.090$ , respectively) or untrained leg ( $r = -0.046$  and  $r = -0.074$ , respectively).



### *Muscle Morphology and Echo-Intensity*

Data are displayed in Table 4. After controlling for Pre values, significant group differences were observed in CSA ( $p = 0.003$ ;  $16.3 \pm 7.7\%$ ) of the trained leg VL, and MT ( $p = 0.004$ ;  $16.6 \pm 8.5\%$ ) and CSA ( $p = 0.010$ ;  $15.3 \pm 7.4\%$ ) of the trained leg RF (Figures 7-9). No significant between-group differences in the trained leg were identified among PA (VL:  $p = 0.101$ ; RF:  $p = 0.948$ ), FL (VL:  $p = 0.854$ ; RF:  $p = 0.074$ ), or MT of the VL ( $p = 0.163$ ). Contralaterally, a significant between-group difference was observed in FL of the RF ( $p = 0.011$ ; UT:  $-4.66 \pm 17.26\%$ ) after controlling for Pre values. No significant between-group difference was observed in MT (VL:  $p = 0.069$ ; RF:  $p = 0.612$ ), CSA (VL:  $p = 0.735$ ; RF:  $p = 0.170$ ), PA (VL:  $p = 0.344$ ; RF:  $p = 0.071$ ), or FL (VL:  $p = 0.854$ ) after controlling for Pre values. No significant between-group differences in EI of the RF or VL of the trained ( $p = 0.608$  and  $p = 0.221$ , respectively) or untrained leg ( $p = 0.949$  and  $p = 0.643$ , respectively).

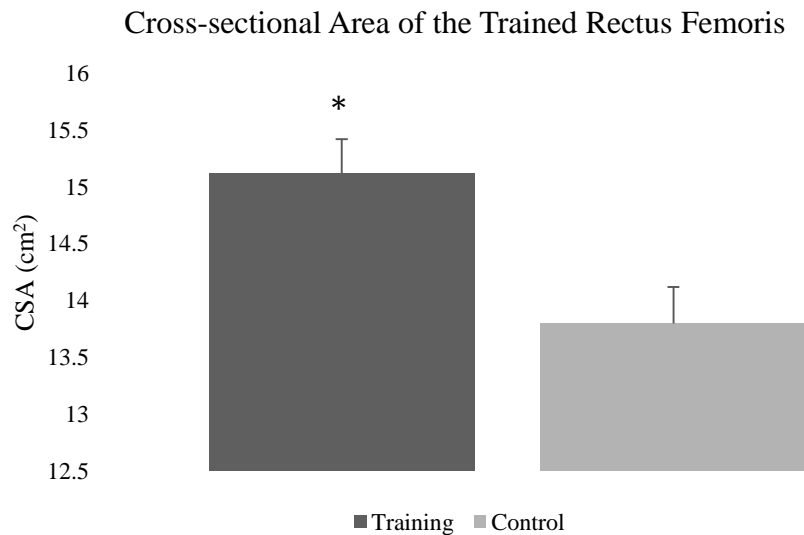


Figure 7. Changes in cross-sectional area of the trained rectus femoris from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean =13.1641). \*Significantly different from corresponding Pre value.

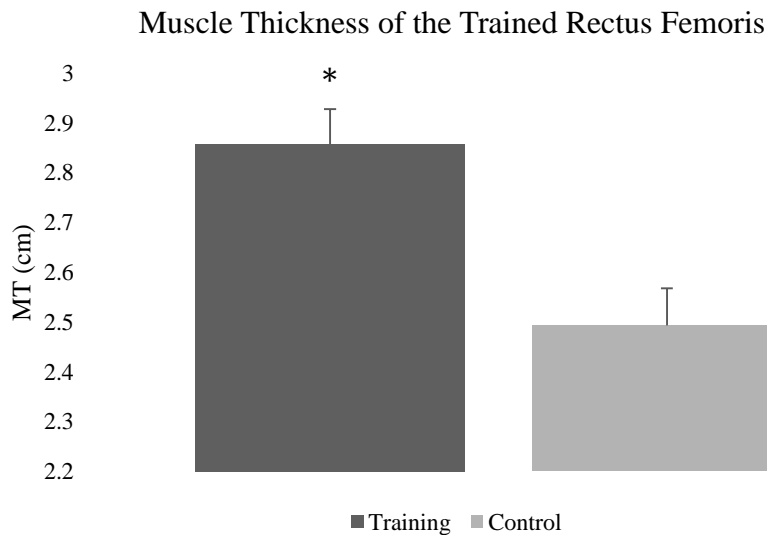
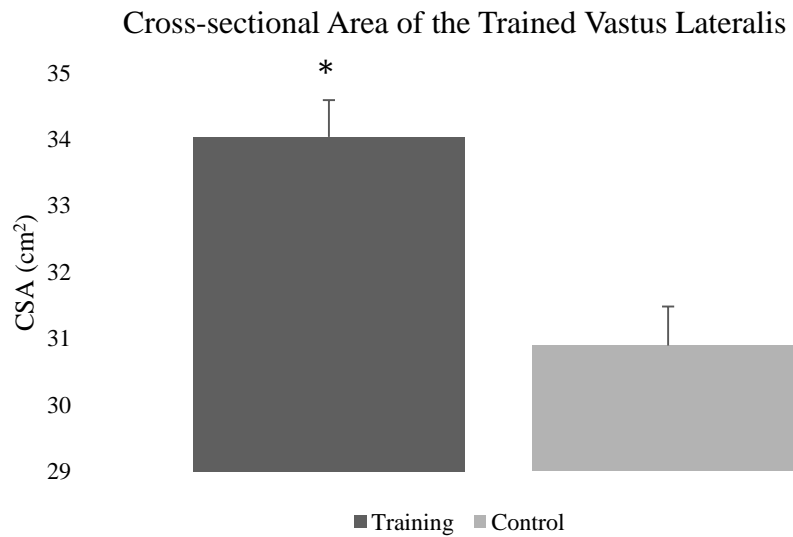
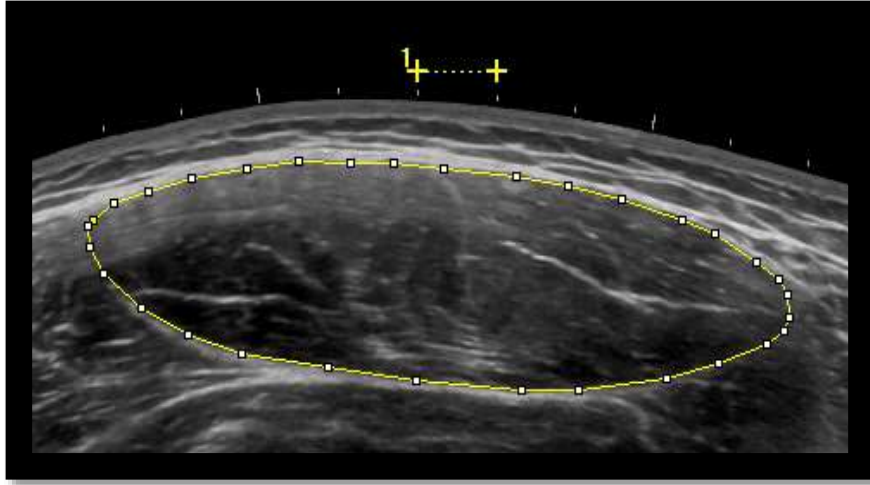


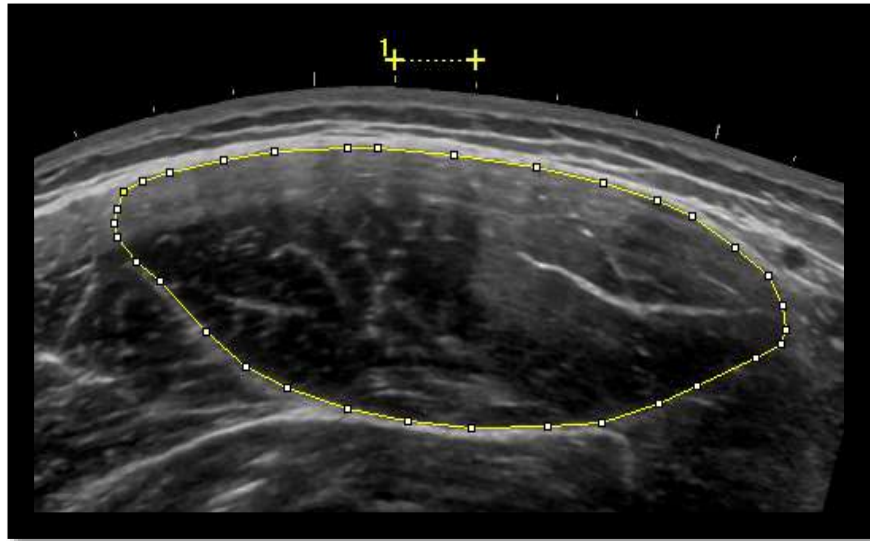
Figure 8. Changes in muscle thickness of the trained rectus femoris from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean =2.5341). \*Significantly different from corresponding Pre value.



*Figure 9. Changes in cross-sectional area of the trained vastus lateralis from Pre- to Post-testing. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean =29.8941). \*Significantly different from corresponding Pre value.*



*Figure 10. Ultrasound image of rectus femoris muscle of the trained leg prior to exercise intervention measured as 17.21 cm<sup>2</sup>.*



*Figure 11. Ultrasound image of rectus femoris muscle of the trained leg following four weeks of resistance training measured as 19.21 cm<sup>2</sup>.*

Table 4. Changes in ipsilateral and contralateral muscle morphology and echogenicity following four weeks of unilateral lower body resistance training

	Training Group (n = 9)		Control Group (n = 8)	
	Pre	Post	Pre	Post
Rectus Femoris				
<b>Cross-Sectional Area (cm<sup>2</sup>)</b>				
Trained leg	12.90 ± 2.86	14.84 ± 3.23*	13.47 ± 2.35	14.11 ± 2.40
Untrained leg	12.29 ± 1.85	12.28 ± 1.79	13.01 ± 2.12	13.55 ± 2.58
<b>Muscle Thickness (cm)</b>				
Trained leg	2.43 ± 0.26	2.82 ± 0.25*	2.65 ± 0.33	2.53 ± 0.19
Untrained leg	2.42 ± 0.32	2.47 ± 0.29	2.57 ± 0.38	2.63 ± 0.36
<b>Fascicle Length (cm)</b>				
Trained leg	11.82 ± 2.30	13.55 ± 3.40	12.05 ± 3.86	11.56 ± 3.35
Untrained leg	11.09 ± 3.07	10.18 ± 1.52*	13.01 ± 2.89	12.76 ± 1.98
<b>Pennation Angle (°)</b>				
Trained leg	12.26 ± 2.57	12.48 ± 2.12	13.46 ± 2.76	13.37 ± 2.95
Untrained leg	13.58 ± 3.50	13.94 ± 2.26	11.75 ± 2.18	12.03 ± 1.32
<b>Echo-Intensity (au)</b>				
Trained leg	56.06 ± 5.25	53.79 ± 6.75	51.03 ± 14.90	48.19 ± 14.00
Untrained leg	55.83 ± 6.88	56.14 ± 5.80	44.78 ± 13.09	47.80 ± 12.85
Vastus Lateralis				
<b>Cross-Sectional Area (cm<sup>2</sup>)</b>				
Trained leg	27.51 ± 5.59	31.83 ± 5.74*	32.58 ± 4.14	33.38 ± 3.52
Untrained leg	26.92 ± 6.64	28.07 ± 5.62	31.68 ± 4.12	32.11 ± 4.40
<b>Muscle Thickness (cm)</b>				
Trained leg	1.81 ± 0.42	2.17 ± 0.47	1.68 ± 0.16	1.86 ± 0.22
Untrained leg	1.72 ± 0.45	1.93 ± 0.40	1.64 ± 0.33	1.66 ± 0.14
<b>Fascicle Length (cm)</b>				
Trained leg	9.45 ± 1.73	10.31 ± 2.22	8.72 ± 1.20	9.60 ± 1.47
Untrained leg	9.09 ± 3.06	10.09 ± 2.53	8.56 ± 1.55	9.10 ± 1.15
<b>Pennation Angle (°)</b>				
Trained leg	11.19 ± 2.45	12.24 ± 1.84	11.63 ± 1.59	11.60 ± 1.30
Untrained leg	11.10 ± 1.26	11.18 ± 1.74	10.75 ± 1.71	10.43 ± 1.57
<b>Echo-Intensity (au)</b>				
Trained leg	63.35 ± 5.13	59.17 ± 6.86	55.58 ± 4.17	57.07 ± 6.01
Untrained leg	67.74 ± 7.91	65.15 ± 4.89	58.73 ± 5.32	61.35 ± 6.34

Values are means ± SD. Ultrasound images performed in triplicate. Pre, baseline measurements. Post, following 4-week intervention. \*Significantly different from corresponding Pre value,  $p < 0.05$ .

Table 5. Changes in hematocrit, hemoglobin, and plasma volume during Pre- and Post-testing

Pre				
	PRE	IP	30P	60P
<b>Hematocrit</b>				
Training group	46.46 ± 2.79	51.36 ± 3.21	46.75 ± 2.76	45.11 ± 2.67
Control group	46.34 ± 4.12	50.41 ± 2.86	45.78 ± 3.08	45.72 ± 3.57
<b>Hemoglobin</b>				
Training group	15.13 ± 1.32	16.87 ± 1.39	15.32 ± 1.22	14.78 ± 1.12
Control group	15.17 ± 1.12	16.50 ± 0.85	15.02 ± 0.94	14.84 ± 0.97
	PRE-IP	IP-30P	30P-60P	PRE-60P
<b>Plasma Volume (%Δ)</b>				
Training group	-17.90 ± 4.57	20.06 ± 4.78	6.00 ± 7.08	4.35 ± 8.43
Control group	-14.90 ± 4.94	20.28 ± 5.07	1.35 ± 4.64	3.46 ± 3.58
Post				
	PRE	IP	30P	60P
<b>Hematocrit</b>				
Training group	45.64 ± 1.60	51.43 ± 2.23	45.86 ± 0.90	44.07 ± 2.09
Control group	44.64 ± 4.80	49.14 ± 4.64	44.50 ± 4.50	44.29 ± 4.51
<b>Hemoglobin</b>				
Training group	14.73 ± 0.74	16.52 ± 0.88	14.84 ± 0.77	14.53 ± 0.75
Control group	14.69 ± 1.61	16.11 ± 1.54	14.74 ± 1.49	14.59 ± 1.53
	PRE-IP	IP-30P	30P-60P	PRE-60P
<b>Plasma Volume (%Δ)</b>				
Training group	-18.86 ± 4.85	23.09 ± 5.96	5.01 ± 5.79	4.55 ± 4.20
Control group	-16.26 ± 4.95	19.51 ± 5.05	1.46 ± 1.71	1.32 ± 2.81

Values are means ± SD. PRE, resting measurements. IP, immediately post-exercise. 30P, 30 minutes post-exercise. 60P, 60 minutes post-exercise. Pre, pre-testing prior to intervention. Post, post-testing following 4-week intervention.

### Total Plasma Testosterone

Data are presented in Figure 12. No significant between-group differences were identified at any time point during Pre- or Post-testing (Pre-testing: PRE [ $p = 0.783$ ], IP [ $p = 0.771$ ], 30P [ $p = 0.767$ ], 60P [ $p = 0.754$ ]; Post-testing: PRE [ $p = 0.778$ ], IP [ $p = 0.369$ ], 30P [ $p = 0.735$ ], 60P [ $p = 0.657$ ]). The two-way [group (CON vs. UT) × time (PRE, IP, 30P, 60P)] repeated-measures ANOVA indicated a significant ( $p < 0.001$ ) main effect of time but no significant group × time interactions. Post-hoc analysis indicated a significantly elevated plasma testosterone concentration at IP ( $p < 0.001$ ). Total testosterone concentrations at 60P were significantly lower

than PRE ( $p = 0.008$ ), IP ( $p < 0.001$ ), and 30P ( $p < 0.001$ ). After controlling for Pre values, no significant between-group difference ( $p = 0.496$ ) was observed in total area under the curve (Figure 13).

### *Growth Hormone*

Data are displayed in Figure 14. No significant between-group differences were identified at any time point during Pre- or Post-testing (Pre-testing: PRE [ $p = 0.401$ ], IP [ $p = 0.490$ ], 30P [ $p = 0.433$ ], 60P [ $p = 0.481$ ]; Post-testing: PRE [ $p = 0.083$ ], IP [ $p = 0.971$ ], 30P [ $p = 0.865$ ], 60P [ $p = 0.803$ ]). The two-way [group (CON vs. UT)  $\times$  time (PRE, IP, 30P, 60P)] repeated-measures ANOVA indicated a significant main effect of time ( $p = 0.002$ ), but no significant group  $\times$  time interactions. Post-hoc analysis indicated significant elevations in growth hormone concentrations at IP ( $p = 0.003$ ), 30P ( $p = 0.010$ ), and 60P ( $p = 0.037$ ). After controlling for Pre values, no significant between-group difference ( $p = 0.099$ ) was observed in total area under the curve (Figure 15).

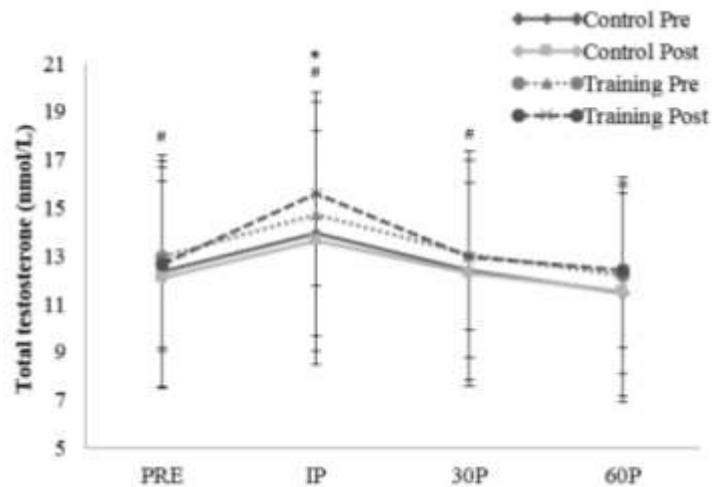


Figure 12. Total testosterone concentrations during Pre- and Post-testing. PRE, resting measurements. IP, immediately post-exercise. 30P, 30 minutes post-exercise. 60P, 60 minutes post-exercise. Pre, pre-testing prior to intervention. Post, post-testing following 4-week intervention. \*Significantly greater than PRE value,  $p < 0.001$ . #Significantly greater than 60P value,  $p < 0.05$ .

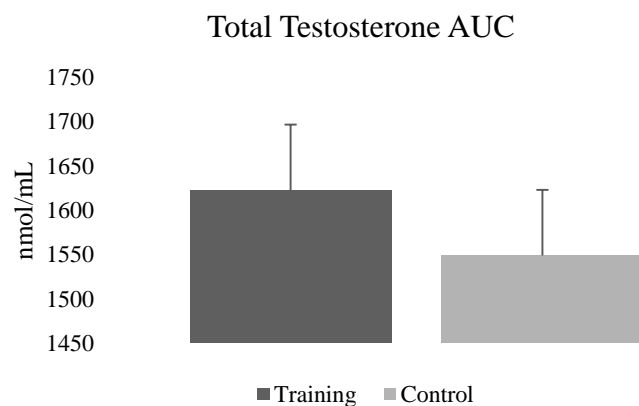


Figure 13. Changes in area under the total testosterone curve from Pre- to Post-testing. Pre, pre-testing prior to intervention. Post, post-testing following 4-week intervention. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean = 1584.17).



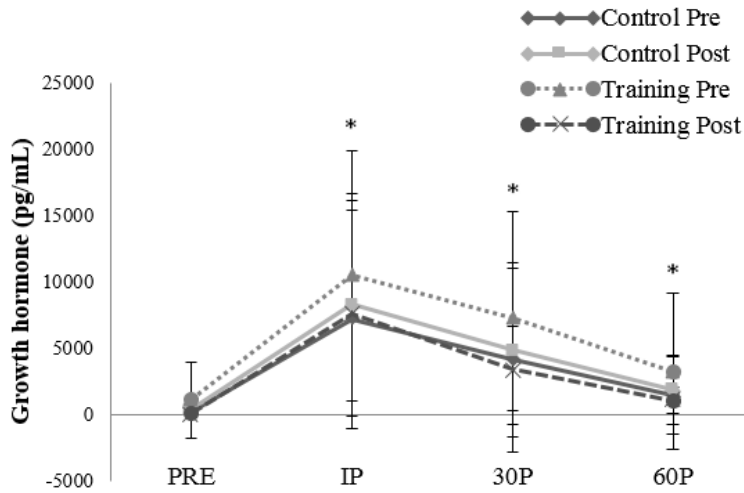


Figure 14. Growth hormone changes during Pre- and Post-testing. PRE, resting measurements. IP, immediately post-exercise. 30P, 30 minutes post-exercise. 60P, 60 minutes post-exercise. Pre, pre-testing prior to intervention. Post, post-testing following 4-week intervention. \*Significantly greater than PRE value.

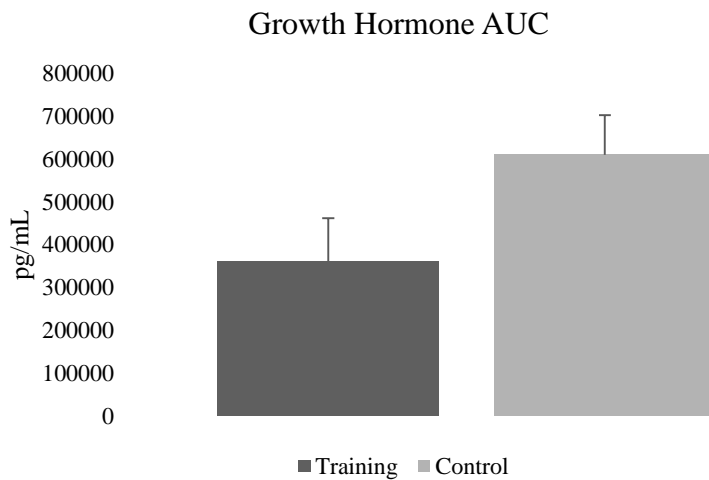


Figure 15. Changes in area under the growth hormone curve from Pre- to Post-testing. Pre, pre-testing prior to intervention. Post, post-testing following 4-week intervention. Mean values (+SEM) adjusted for the baseline differences (covariate; adjusted mean =634956.34).

## CHAPTER V DISCUSSION

Results demonstrate that short-term unilateral lower body resistance training of the dominant leg produced improvements in ipsilateral and contralateral leg strength accompanied by augmented size of the trained musculature. Alternatively, unilateral resistance training provided no cross-over effect on power performance. Contrary to our hypothesis, four weeks of resistance training did not significantly enhance the acute testosterone or growth hormone response to a single bout of exercise. While prior investigations have examined cross-educational strength transfer from unilateral resistance training, none to our knowledge have examined its implications on muscle morphology, echo-intensity, or power performance of the untrained limb in healthy young men. The current findings aim to further elucidate the underlying mechanisms and forthcoming of unilateral resistance training on contralateral gains.

### *Changes in Maximal Strength*

As hypothesized, four weeks of unilateral lower body resistance training led to significant increases in maximal leg press 1RM strength of both the trained ( $72.6 \pm 44.4\%$ ) and untrained ( $60.4 \pm 52.4\%$ ) legs. However, improvements in maximal leg extension 1RM strength were only significant in the trained leg ( $45.3 \pm 15.8\%$ ). Previous research employing isometric and isokinetic testing have reported contralateral lower body strength gains up to 48.3% following isometric training, 44.8% following isokinetic training, and 17.8% following dynamic training (Malas, 2013; Shima, 2002; Munn, 2004; Houston, 1983). While the strength improvements

observed in this study exceeded previous observations, the disparity may be due to differences in training and testing modalities.

Despite the improvements in dynamic strength, we observed no significant improvement in maximal isometric strength following training. This inconsistency may be explained by the nature of the training employed in the current study. While dynamic exercises, incorporating both concentric and eccentric contractions, have been shown to elicit greater strength gains than isometric training when performed together (Rasch, 1957), improvements in dynamic strength throughout a given range of motion may not be equivocally translated to maximal isometric strength at one specific degree of contraction.

Strength gains of the lower body were paralleled by significant improvements in upper body strength. Training resulted in a  $24.7 \pm 17.2\%$  increase in chest press 1RM strength and a  $34.0 \pm 17.6\%$  in low row 1RM strength. Abe and colleagues reported similar improvements in upper body strength (~20%) following four weeks of whole body resistance training program in untrained adults (2000). Our results add to previous reports which support the notion of high susceptibility and rapid training adaptations in untrained individuals during the early phase of resistance training (Hakkinen, 2000; Moritani, 1979; Kraemer W. , 1998).

### *Changes in Training Volume*

For this study, daily training volume was calculated as the product of weight lifted and repetitions performed. As expected, participants in the training group achieved a greater total

training volume from Pre- to Post-testing (~58%) after four weeks of progressive resistance training. Accordingly, a strong linear relationship between training intensity and volume has previously established and widely recognized (Baechle, 2008; Stone, 1982). Therefore, because all training sessions were supervised to ensure repetition compliance, the observed increase in training volume can most likely be attributed to the vast improvements in upper and lower limb strength.

#### *Changes in Power Performance*

Contrary to our hypothesis, unilateral resistance training imposed no benefit on power performance of the trained or untrained leg. Previous research explains that performance adaptations are specific to the velocities and movement patterns employed during training (Kannus, 1992; Malas, 2013). However, concurrent strength training and power training has been shown to prevent maximal adaptation to one or more of the skills being trained (Chtara, 2008). Therefore, it is understandable that changes in power performance were exceeded by improvements in strength. The power training employed in this study was auxiliary to the strength training protocol, suggesting that performance changes of the trained and untrained limbs favor the most demanding stimulus during concurrent strength and power training.

#### *Comparison of Contralateral and Ipsilateral Performance Gains*

A significant ( $p < 0.01$ ) correlation was determined between changes in leg press 1RM strength of the trained and untrained leg ( $r = 0.725$ ), but not leg extension 1RM strength. No other relationships were established between changes in maximal isometric strength or power

performance. The observed strength improvements are in agreement with the the previous claim that contralateral strength gains occur proportional to those observed in the trained limb (Zhou, 2000). Because we did not observe a significant correlation between changes in leg extension strength, it is possible that the extent of cross-education is magnified when training involves multi-joint exercises (i.e., leg press, squat) in comparison to exercises that isolate a particular muscle group (i.e., leg extension).

### *Changes in Muscle Morphology*

In agreement with prior reports of rapid muscle morphological adaptations in response to changes in training status, we observed marked alterations in certain measures of size and myofiber arrangement of the trained leg musculature following four weeks of resistance training (Seynnes, 2007; De Boer, 2007). The rectus femoris of the trained leg experienced a  $16.6 \pm 8.5\%$  increase in thickness and  $15.3 \pm 7.4\%$  increase in cross-sectional area, while only cross-sectional area of the vastus lateralis increased by  $16.3 \pm 7.6\%$ . Taking into consideration previous reports of non-homogenous morphological adaptations of the quadriceps muscle group following training (Ema, 2013; Wells, 2014), it is possible that adaptations occurring at a separate region of interest within the analyzed muscles went undetected. In the current study, no significant changes were observed for fascicle length or pennation angle of the trained leg musculature which is similar to previously reported results from a 3-week unilateral dynamic program, wherein no changes were reported in the length or angle of muscle fascicles (Malas, 2013). Alternately, prior investigation has reported an 11% increase in fascicle length and 13% increase in pennation angle of the vastus lateralis following 14 weeks of lower body resistance training in

older adults (Reeves, 2004), but it is plausible that the changes occurred sometime between the fourth and fourteenth week of the program. The only significant morphological change observed in the untrained leg was a 4.7% decrease in fascicle length within the rectus femoris. However, due to the large variance in results, we cannot conclude that this is a meaningful physiological adaptation. Interpretation of these results in comparison to previous research should take heed to the interdependent nature of pennation angle, fascicle length, and muscle thickness as well as the expression of dissimilar changes in thickness within a single muscle. Previous research has reported no change in contralateral pennation angle of the vastus lateralis following three weeks of unilateral knee extensor-strengthening exercise (Malas, 2013). Further, Blazeovich and colleagues observed no contralateral changes in muscle size, pennation angle, or fascicle length following five weeks of unilateral isokinetic leg extension training in untrained young adults (2007).

Although the current results indicated no change in echo-intensity of the trained musculature, Cadore and colleagues reported a decrease following six weeks of unilateral isokinetic training in men and women (2014). These differences may be attributable to differences in training/testing modalities, intervention duration, and/or participant gender. Additionally, the current study and the Cadore investigation employed inconsistent ultrasound devices and measurement settings (i.e., frequency) to capture images. Together, these findings suggest that changes in skeletal muscle echogenicity may require a training intervention in excess of four weeks and appear to be highly sensitive to the mode of assessment.

Collectively, results from present and previous investigations suggest that the magnitude of cross-educational effects may be limited by a minimal threshold of mechanical stimulus to the trained side. While it appears that a 4-week training intervention is sufficient to promote hypertrophy in the trained leg without any change in muscle fiber orientation, it is not enough to promote significant size gains of the untrained leg. Notably, the majority of research examining cross-education has implemented isometric and isokinetic testing which limits its interpretation to clinical and/or rehabilitative application. By contrast, the current investigation employed both dynamic training and testing which are more translatable to everyday functional strength and activity.

#### *Muscle Morphological Changes in Relation to Strength and Power Changes*

Strength improvements of the trained leg were accompanied by hypertrophy of the ipsilateral rectus femoris and vastus lateralis muscles. In fact, a strong relationship was established between the change in vastus lateralis cross-sectional area and change in leg extension 1RM strength ( $r = 0.858$ ) of the trained leg. The synchronous increase in knee extensor strength and hypertrophy of leg musculature from training is similar to previous results (Farup, 2012; Malas, 2013) and is consistent with the current literature explaining that force production of a given muscle is highly influenced by its morphological characteristics (Farup, 2012; Abe, 2000; Garfinkel, 1992).

The improved strength and decreased peak power output of the untrained leg occurred in the absence of any meaningful morphological adaptations (i.e. size, thickness, myofiber arrangement). Furthermore, no significant correlations were identified between the changes in

mean or peak power and any change of muscle morphology. Performance changes of the trained and untrained leg appear to suggest that muscle morphology may not be of primary influence on early performance changes. Though beyond the scope of this paper, it is plausible that significant short-term improvements in strength of the trained and untrained leg were the result of neural adaptations (i.e., improved motor unit recruitment, firing rate, and/or synchronization), skill acquisition, or some combination of the aforementioned variables (Lieber, 2000; Houston, 1983; Gabriel, 2006), yet the power adaptations remain unexplained.

#### *Endocrine Response to Training*

Participants in both groups elicited similar patterns of post-exercise elevations in total testosterone and growth hormone following a single bout of resistance training. Exercise during Pre and Post-testing resulted in a significant rise in post-exercise testosterone (IP) and growth hormone (IP, 30P, 60P) concentrations among all participants. Similar to our results, previous research reports that just one bout of whole body resistance training elicits a post-exercise rise in testosterone in non-strength trained men (Athiainen, 2004; Kraemer R. , 1992). Likewise, similar patterns of elevated post-exercise growth hormone concentrations have been reported regardless of participant gender or training status (Kraemer W. , 1991; Kraemer W. , 2005; Wideman, 2002).

Contrary to our hypothesis, four weeks of training did not alter the magnitude of the acute testosterone or growth hormone response to a single bout of resistance exercise. Although some researchers have reported that chronic training enhances the acute endocrine response to



exercise, the length of intervention periods was greater (10-21 weeks) and the training protocols involved more muscle mass than the current study (Kraemer W. , 1999; Hakkinen, 2001). With the understanding that the magnitude of an acute hormonal response is dependent on the amount of exercised muscle mass (Hansen, 2001), we must acknowledge the three-limb training protocol employed in the present study. The current results suggest that four weeks unilateral lower body/bilateral upper body resistance training is insufficient stimulus to evoke a greater acute hormonal response to resistance exercise. An intervention period greater than four weeks utilizing more muscle mass may be necessary to elicit significant alterations in total testosterone and growth hormone concentration following an acute resistance exercise stimulus.

#### *Endocrine Response in Relation to Changes in Morphology and Performance*

Interestingly, although both groups elicited similar patterns in post-exercise anabolic hormone concentrations and improvements in strength, muscle hypertrophy was only noted among the trained musculature of the training group. While we hypothesized that the anabolic hormones would correspond to uniform muscle hypertrophy in both the trained and untrained leg as both were exposed to a similar hormonal environment following exercise, our results did not support this hypothesis. Nevertheless, this study cannot rule out the mediating roles of endogenous testosterone or growth hormone secretion during the anabolic response to resistance exercise for two important reasons. First, the present study only measured two snapshots in time of the hormonal response to exercise. It is important to consider that those in the training group experienced an increase in systemic anabolic hormones seen during the testing sessions following each of the 12 training sessions. Secondly, we must consider the receptor's role in

facilitating the designated action of the hormone. Muscle hypertrophy, as a result of increased protein synthesis, is partially mediated by androgen receptors (Ranke, 2006). Thus, the impact of increased hormone concentrations is restricted by receptor availability and/or the number of interactions with the receptor (Ahtiainen, 2003). Previous research has explained that mechanical stress of resistive training leads to an up-regulatory response on the receptor cells within the exercised muscle (Bamman, 2001). Despite the systemic nature of the endocrine system, the lack of hypertrophy of the untrained leg musculature upholds that up-regulation of androgen receptors is heavily influenced by the imposed mechanical stress on the exercised muscle. Therefore, it is plausible that the exercised muscles of the trained leg had a higher affinity to bind with the more abundant availability of circulating testosterone and growth hormone following each training session. While we cannot exclude anabolic hormones as an influential factor in strength gains, the current results suggest that neural adaptations may be the predominant mechanism involved in the cross-education of early strength gains.

The increase in training volume before and after training did not correlate to any changes in anabolic hormone secretion. Prior reports have illustrated the strong association between post-exercise anabolic hormone production and total work performed (Gotshalk, 1997; Craig, 1994), thus a relatively greater metabolic stress would heighten the acute hormonal response to exercise following training (Hakkinen, 1993). The observed lack of differences in the current study suggest that training led to metabolic adaptations (i.e., improved lactate turnover, improved buffer capacity) which allowed for participants in the training group to perform at a higher absolute intensity while maintaining relative difficulty from Pre- to Post-testing. These results

further agree with the belief that a greater training stimulus is necessary in order to elicit a statistically significant rise in anabolic hormones following a single bout of exercise (Kraemer, 2005).

#### *Potential Limitations and Further Research*

To our knowledge, this study was the first to examine the effects of unilateral lower body resistance training on contralateral muscle morphology, muscle quality, and power output in previously untrained young men. In addition, our study is one of few to employ both dynamic training and testing. In light of their potential contributions, our results should be interpreted with some important considerations. First, we recruited men within the age range of 18 to 35 whom were free from regular, structured resistance training to participate in the study, thus the results of our study are only generalizable to similar populations. Second, while our within-subject results maintained relative consistency, it is difficult to conclude that all untrained men will respond identically to a short-term training intervention. Group selection was entirely randomized, but the range in baseline strength and power could be due to the ambiguity of our “untrained” inclusion criterion. Similarly, there was a high degree of variance among levels of basal testosterone and growth hormone which may have been attributed to differences in activity level, training status, or the age range of our selected participants.

During testing and training, the assumption was made that each participant was performing at his maximal effort. External factors such as participant mood, energy level, or facility environment were disregarded but may have impacted the extent of a participant’s performance. In the event

that maximal effort was not given for any reason, performance results may have been over- or underestimated.

Our results warrant further investigation into the contralateral muscle morphological adaptations to unilateral training. Although ultrasonography is a widely used timely mode of non-invasive intrinsic analysis of muscle tissue, we acknowledge that the use of a more sensitive, but less practical mode, (i.e., magnetic resonance imaging) may have provided slightly different results. Additionally, fascicle length was estimated rather than directly measured in this investigation. Although differences would be slight, a direct measurement of fascicle length may have led to different observations.

With the understanding that the efficacy of the endocrine system on skeletal muscle is restricted by the affinity and availability of receptors, the current results support the notion that up-regulatory processes of androgen receptors may only occur as a result of mechanical stress. However, receptor analysis was beyond the scope of the present study. Future research should focus on receptor activity, particularly of the untrained musculature, to delineate the role of androgen receptors in muscle hypertrophy.

An additional drawback to this study was the lack of dietary control. Although total energy and relative protein intake remained constant, participants were not given any nutritional coaching in regards to pre- or post-exercise nutrition. Nutrient timing is purported to be a leading influential factor in optimizing muscle protein synthesis and androgen receptor modification following

training, but without accounting for these variables we cannot rule out that nutritional practices elicited differences in results.

### *Conclusions*

Four weeks of bilateral upper body and unilateral lower body resistance training using the dominant leg is a sufficient time frame to evoke vast strength gains of the ipsilateral and contralateral legs but does not appear to augment the acute endocrine response to training. Despite the post-exercise rise in anabolic hormones we observed in both groups, hypertrophy was only evident in the trained leg musculature of the training group. Additionally, changes in trained and untrained muscle size were incongruent in spite of similar interlimb strength improvements following training. Therefore, early cross-educational effects may be more reliant on enhanced neuromuscular function than changes in circulating anabolic hormones or skeletal muscle structure. Results of this study suggest that strength of the contralateral, untrained leg can be enhanced through unilateral resistance training and may be a practical addition to a rehabilitatory program for individuals with an injured/immobilized limb.

APPENDIX A: UCF IRB LETTER



University of Central Florida Institutional Review Board  
Office of Research & Commercialization  
12201 Research Parkway, Suite 501  
Orlando, Florida 32826-3246  
Telephone: 407-823-2901, 407-882-2901 or 407-882-2276  
[www.research.ucf.edu/compliance/irb.html](http://www.research.ucf.edu/compliance/irb.html)

### Notice that UCF will Rely Upon Other IRB for Review and Approval

From : UCF Institutional Review Board  
FWA00000351, IRB00001138

To : Gerald T. Mangine

Date : October 31, 2013

IRB Number: SBE-13-09482

Study Title: **Short-Term Effects of Lower Body Unilateral Resistance Training on Muscle Morphology, Power, Strength, Neuromuscular Economy, and Endocrine Response**

Dear Researcher:

The research protocol noted above was reviewed by the University of Central Florida IRB Chair designated Reviewer on October 31, 2013. The UCF IRB accepts the New England Institutional Review Board's review and approval of this study for the protection of human subjects in research. **The expiration date will be the date assigned by the New England Institutional Review Board and the consent process will be the process approved by that IRB.**

This project may move forward as described in the protocol. It is understood that the New England IRB is the IRB of Record for this study, but local issues involving the UCF population should be brought to the attention of the UCF IRB as well for local oversight, if needed.

All data, including signed consent forms if applicable, must be retained for a minimum of five years (six if HIPAA applies) past the completion of this research. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

**Failure to provide a continuing review report for renewal of the study to the New England IRB could lead to study suspension, a loss of funding and/or publication possibilities, or a report of noncompliance to sponsors or funding agencies. If this study is funded by any branch of the Department of Health and Human Services (DHHS), an Office for Human Research Protections (OHRP) IRB Authorization form must be signed by the signatory officials of both institutions and a copy of the form must be kept on file at the IRB office of both institutions.**

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

Signature applied by Patricia Davis on 10/31/2013 03:51:27 PM EST

IRB Coordinator

APPENDIX B: NEIRB APPROVAL LETTER





September 13, 2013

Gerald Mangine  
University of Central Florida  
12494 University Boulevard  
Orlando, FL 32816

Re: **NEIRB # 13-266** SBE-13-09482 "Short-Term Effects of Lower Body Unilateral Resistance Training on Muscle Morphology, Power, Strength, Neuromuscular Economy, and Endocrine Response" and Amendment 1 dated September 10, 2013

**Date of Amendment Approval: September 13, 2013**

Dear Mr. Mangine:

This is to inform you that New England Institutional Review Board (NEIRB), via Expedited Review, Thursday Board, has reviewed **Amendment #1 dated September 10, 2013** for the above-captioned study. The changes to the study have been approved.

Please find the revised Informed Consent document, NEIRB version 2.0 enclosed. You will note that the date at the bottom right hand corner indicates an updated approval date of 9/13/2013. Only NEIRB-approved informed consent documents should be used. It must be signed by each subject who will participate in this study prior to the initiation of any protocol procedures. In addition, each subject must be given a copy of the signed consent form.

New England IRB has determined that all currently active subjects must be re-consented with the revised consent form.

The approval period for the study ends on 8/4/2014. Any additional modifications in the research protocol, study site/ personnel, or consent form during this time period must first be reviewed and approved by NEIRB.

Please feel free to call me if you have any questions.

Sincerely,

Shana R. Ross, MCJ, CIM, CIP  
Lead Administrator

cc: NEIRB Chair



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