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The acute effects of different blood flow restriction settings on blood lactate, electromyography and leg strength during bilateral knee extensions

Guillermo Eduardo Perez
University of Texas at Brownsville

THE ACUTE EFFECTS OF DIFFERENT BLOOD FLOW RESTRICTION SETTINGS ON BLOOD LACTATE, ELECTROMYOGRAPHY AND LEG STRENGTH DURING BILATERAL KNEE EXTENSIONS

BY

GUILLERMO EDUARDO PEREZ

A THESIS PRESENTED TO THE GRADUATE FACULTY OF THE COLLEGE OF EDUCATION IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN EXERCISE SCIENCE

APPROVED BY:

Murat Karabulut

Dr. Murat Karabulut
Thesis Director

Dr. Sue Anne Chew
Committee Member

Christopher M Ledingham

Dr. Christopher M. Ledingham
Committee Member

Charles Lackey

Dr. Charles Lackey
Dean of Graduate Studies

Graduate School
University of Texas at Brownsville
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DEDICATION AND ACKNOWLEDGMENT

This thesis is dedicated to the memory of my grandfather, Rodolfo Javier Cantu Sr. I strive to make you proud with everything I do. I hope the work displayed here does just that.

I would like to thank my principal supervisor, Dr. Murat Karabulut, who has been an excellent mentor and teacher. He truly understands what hard work and dedication can bring to one's life. Thanks also to Dr. Christopher Ledingham for his guidance and wisdom. Your mentorship provided me with valuable information to improve my organization and writing skills. I would also like to thank Dr. Sue Anne Chew for serving on my research committee and for all of the valuable assistance.

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To my family, I would like to thank you for your unconditional love, encouragement and pushing me to reach my potential. Finally, a sincere thanks to my wife Nicole. You are my world, thank you so much for your endless love and being my best friend through all the ups and downs.

Abstract

The current protocols being used for Blood Flow Restriction (BFR) exercise did not examine the effects of different Initial Restrictive Pressures (pressure applied to limb by cuff prior to inflation, IRP) during design. There are several research articles highlighting the importance of IRP and how it can create variability in results. Therefore it is necessary to evaluate the current BFR protocol against different IRP in order to determine reliability.

PURPOSE: The purpose of this study was to examine the acute effects of different blood flow restriction setting on 1) the level of blood lactate production, 2) neuromuscular activation (the changes in motor unit activation and median frequency), and 3) fatigue response during bilateral knee extension exercise.

METHODS: Thirty-three male (age= 26.63 (5.8), n=17) and female (age= 22.76 (3.3), n=16) participants performed four sets of bilateral knee extensions on four separate occasions separated by at least 48 hours. Each session was randomized into the following conditions: IRP50@FRP+20, IRP65@FRP-20, IRP50 (Control), and IRP65. During each condition, blood pressure (BP), heart rate (HR), rate of perceived exertion (RPE), discomfort/pain (DF), and blood lactate were recorded throughout the study. Maximal voluntary contraction (MVC) was recorded pre and post exercise. Electromyography was recorded at the vastus lateralis and rectus femoris with a focus on motor unit activation (RMS) and muscle firing rate (MDF).

RESULTS: No significant difference was found between conditions for BP, blood lactate or RPE. HR displayed a condition*gender interaction ($p=0.022$) and a trend for a condition*time interaction ($p<0.06$). IRP65@FRP-20 displayed a trend for increased levels of DF ($p=0.062$); it also produced significantly higher decreases in pre to post MVC in both males and females ($p<0.023$), showing a strong trend for a greater effect in males ($p=0.055$). IRP65@FRP-20

further displayed greater levels of overall motor recruitment in the vastus lateralis ($p=0.024$) as well as greater levels of motor recruitment throughout sets 2-4 in the vastus lateralis ($p<0.01$) and rectus femoris ($p<0.01$).

CONCLUSION:IRP65@FRP-20 created greater variations amongst the variables compared to the Control, despite having a lower final restrictive pressure. This suggests that the current protocol may not be the most reliable method of BFR. Further research is needed to develop a protocol that takes IRP into account.

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CHAPTER I

INTRODUCTION

Increased levels of strength have demonstrated positive effects on body fat, resting metabolic rate, blood pressure, glucose metabolism, and insulin resistance (Winett & Carpinelli, 2001). Many people, however, do not participate in strength training due to fear of re-injury, disabilities, and lack of time and/or motivation (Karabulut, Leal, Garcia, Cavazos, & Bemben, 2014). A novel exercise technique known as blood flow restriction (BFR) training that consists of restricting the circulatory pathways by way of pneumatic cuffs or elastic wraps has gained popularity. BFR technique reduces the amount of venous return, which causes venous blood to pool at the site of the exercising muscles (Fahs, Loenneke, Rossow, Thiebaud, & Bemben, 2012).

It has been found that BFR increases skeletal muscle activation and strength while using a relatively low intensity (20% 1RM) as well as low intensity walking and biking (Abe, Kearns, & Sato, 2006; Madarame et al., 2008; Takarada, Sato, & Ishii, 2002; Takarada et al., 2000; Takarada, Tsuruta, & Ishii, 2004; Yasuda, Fujita, Ogasawara, Sato, & Abe, 2010). The exact reasons for these adaptations are unknown, however, increases in metabolic accumulation, mechanical stress, and hormonal response have been recorded and are believed to contribute greatly to the findings (Cook, Clark, & Ploutz-Snyder, 2007; J. Loenneke, Fahs, Wilson, & Bemben, 2011).

Research has also found that BFR reduces the amount of time necessary to produce increases in cardio respiratory fitness to approximately fifteen to twenty minutes, compared to traditional training methods (Abe, Fujita, et al., 2010; Park et al., 2010). The convenience of decreased exercise intensity and time has made BFR a popular technique.

Aside from the benefits provided to a normal population, BFR also presents opportunities to individuals, who have contraindications to higher resistance and cardio respiratory training intensities (Fukuda et al., 2011; Nakajima et al., 2010). BFR may also prove to be beneficial to individuals of advanced age (Abe, Sakamaki, et al., 2010; Ozaki et al., 2011) as well as those undergoing the recovery process from recent injuries (Sata, 2005).

While these findings are significant in nature, there are other studies, which failed to find similar adaptations (Burgomaster et al., 2003; Laurentino et al., 2008; Teramoto & Golding, 2006). This may be a result of the use of different types of equipment being utilized for BFR research as well as the lack of a standardized protocol for setting restrictive pressures. Little is known about the protocol regarding restrictive pressure setting, and none of the manuscripts published have defined or explained the details about BFR protocol such as initial restrictive pressure (IRP, pressure created by the tightness of cuffs before inflating with air). IRP is the pressure applied to the circulatory pathways by the BFR cuff prior to their inflation. As reported in previous literature, IRP directly affects BFR regardless of the final restrictive pressure (FRP, highest restrictive pressure reached after inflation with air) (Karabulut, Mccarron, Abe, Sato, & Bemben, 2011; Karabulut & Perez, 2013). The inconsistencies in the reported literature may be a result of both the use of devices that are not able to measure IRP, and the lack of including IRP in the BFR protocol. The current BFR protocols being used in research vary depending on the instrument being utilized. Since there is a lack of a set protocol, an investigation of the current techniques is needed. Information regarding the effect of the BFR cuff

pressures on blood lactate, neuromuscular activation, and muscle strength will allow researchers to understand the importance of the variables related to the BFR protocol and to use this technique more effectively and properly.

Study Purpose

The purpose of this study was to examine the acute effects of different blood flow restriction setting on 1) the level of blood lactate production, 2) neuromuscular activation (the changes in motor unit activation and median frequency), and 3) fatigue response during bilateral knee extension exercise.

Research Questions

1. Will an IRP of 45-50 mmHg (IRP50) or 60-65 mmHg (IRP65) have greater effect on blood lactate, neuromuscular, and fatigue during the different BFR protocols?
2. Will a setting using the recommended FRP with an added 20 mmHg and an IRP of 45-50 mmHg have the same effects as the current method?
3. Will a setting using the recommended FRP with a subtracted 20 mmHg and an IRP of 60-65 have the same effects as the current method?
4. What changes in heart rate (HR), systolic and diastolic blood pressure (BP), discomfort (DF), and rate of perceived exertion (RPE) will be seen in the four conditions?

Hypotheses

1. An increase in blood lactate, differences in EMG activity, and decrease in MVC will be seen in both FRP conditions with an IRP65.

2. Using the recommended FRP with an additional 20 mmHg and an IRP50 will produce similar results as the current protocol.
3. Using the recommended FRP with a subtracted 20 mmHg and an IRP65 will produce similar results as the current protocol.
4. HR, BP, DF, and RPE will be greater in the condition using the recommended FRP setting with an IRP of 65 mmHg.

Significance of the Study

BFR is a novel technique that has been shown to be effective at producing strength training results similar to the current recommended intensities of >70% 1RM, made by ACSM (Abe, et al., 2006) while using relatively lower intensities (~20% 1RM). This technique is of extreme significance and may present an opportunity for those suffering from disabilities and other ailments to experience the benefits of healthy strength levels while exercising with low-risk training. While promising, there is no standard method of application for this new technique, and therefore cannot be used by the general population to improve health status in a safe and consistent manner. The goals of the proposed study aim to identify key factors that are needed to develop a proper protocol. The findings presented by this study will either confirm or invalidate the current popular method of application.

Delimitations

1. Only male and females between the ages of 18-50 were allowed to participate in the study.

2. Individuals suffering from diseases that prevent subjection to strength testing and training were excluded. These included hypertension, and cardiovascular disease.
3. Individuals suffering from disabilities that prevent subjection to strength testing and training were excluded. These included joint injuries or chronic pain.
4. Individuals who had recently adopted a strength training program were excluded.

Limitations

1. The study might not be representative of the population due to all participants being volunteers and not being randomly sampled.
2. Health history and medical information was gathered through self-report.
3. Participants were asked to refrain from changes to their current physical activity; however, physical activity performed outside of the study was not monitored.

Assumptions

1. Each subject performed all knee extension exercises to the best of their ability with maximal effort.
2. Each subject used correct form when performing knee extension exercise.
3. Each subject provided accurate health history and medical information.
4. The equipment used was reliable and provided accurate information for all testing sessions.

Operational Definitions

PAR-Q: PAR-Q (Physical activity readiness questionnaire) is a screening tool that is designed to determine whether a subject may perform the exercise in a safe and risk free manner.

Blood Flow Restriction (BFR): BFR is a technique that restricts venous return during exercise. This process involves cuff placement over the inguinal crease. The cuffs are then inflated to specific pressures. The cuffs are 5 centimeters wide and contain an inflatable bladder.

Isometric Strength (MVC): MVC is defined as the maximal force generated from a target muscle. It is measured during a voluntary, static contraction using a dynamometer.

Dynamometer: Is a device used to measure power, force, and moment force (torque).

Hemodynamic: Is the study of blood circulation and blood flow.

Blood Lactate: Is a metabolic by-product of anaerobic type exercise.

Initial Restrictive Pressure: The pressure applied to the limb by the cuff before inflation.

Final Restrictive Pressure: The pressure recorded inside the cuff post inflation.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this study is to examine the acute effects of different blood flow restriction settings on 1) the level of blood lactate production, 2) neuromuscular activation (the changes in motor unit activation and median frequency), and 3) fatigue response during bilateral knee extension exercise.

Blood Flow Restriction/KAATSU

The current blood flow restriction (BFR) protocols being used in research vary depending on the instrument being utilized (Karabulut et al., 2011). Since there is a lack of a set protocol, an investigation of the current techniques is needed. Information regarding the effect of the BFR cuff pressures on blood lactate, neuromuscular activation, and muscle strength will allow for future research to be done more effectively and use this technique properly.

As with any new training technique, proper protocols are necessary to achieve the full benefits of the technique. Currently, the protocols for BFR using different equipment are still being determined. BFR settings are broken down into two categories initial restrictive pressure (IRP) and final restrictive pressure (FRP). The tightness of the BFR cuff, pre-inflation, exerts an external pressure on the subject; this pressure is known as IRP. FRP is the greatest pressure exerted in the cuff post-inflation with air. As of now, there is no specific protocol for determining IRP and its necessity is a disputed factor (Karabulut & Perez, 2013; J. Loenneke et al., 2011). Two different protocols have been used for FRP one relying on the subject's arm systolic blood pressure and the other on the

subject's leg circumference. This review will go over all protocols in detail and outline supporting research.

Initial Restrictive Pressure

In a study examining the effects of different IRP on leg tissue oxygenation, it was found that IRP was inversely correlated with leg tissue oxygenation (Karabulut et al., 2011). The study used three different IRP. On different testing days, subjects were randomly subjected to IRP of 30, 50, or 70 mmHg for four minutes followed by a two-min deflation period. The cuffs were then inflated to the pressures of 120, 140, 160, and 180 with two-min deflation periods in between each inflation setting. Tissue oxygenation was measured using a near-infrared spectrometer probing exactly half the distance from the anterior superior iliac spine to the superior portion of the patella. Two-way repeated measures ANOVA determined the inverse relationship found between IRP and tissue oxygenation. The authors conclude that IRP is an important variable in BFR protocol and is directly related to the decrease in venous return and tissue oxygenation.

Karabulut et al. (2014) examined the effects of different IRP on leg strength, tissue oxygenation, and blood lactate. It was found that deoxygenated hemoglobin, tissue oxygenation and leg strength were affected more by the greater IRP. During the study participants were subjected to four sets of unilateral isotonic knee extensions. The first set consisted of 30 repetitions with the following three sets consisting of 15 repetitions. Leg strength was tested pre and post exercise through MVC, and tissue oxygenation and blood lactate were tested pre, mid, and post exercise. Two-way repeated measures ANOVA revealed that the IRP with 60-65 mmHg created greater impacts on the tested

variables than the IRP with 40-45 mmHg, regardless of both conditions being inflated to the same FRP. The authors conclude that IRP is an important factor to consider when using BFR and that higher IRP create higher hypoxic conditions.

Recently, another study showed that IRP is indeed an important variable to consider when determining BFR pressure protocols (Karabulut & Perez, 2013). The study utilized two different IRP (40-45 and 60-65 mmHg) during BFR combined with knee extension exercises. Electromyography values, Root Mean Square (RMS) and Median Frequency (MDF), were recorded at the vastus lateralis. Thigh circumference and sub-cutaneous fat were measured (measuring tape; ultrasound transducer) halfway between the right lateral epicondyle and greater trochanter. Repeated measures ANOVA revealed that IRP significantly affected RMS values pre- to post-exercise. It was also found that subcutaneous fat and not thigh circumference was the most influential factor affecting pressure applied by the cuff. The authors conclude by recommending that IRP be considered when setting BFR pressure protocols.

Another research team set out to determine which factors (limb composition, leg size, and blood pressure) should be considered when determining the prescribed pressure (J. P. Loenneke et al., 2012). The study found that thigh circumference alone was the number one factor affecting the pressures used with BFR. The study used two different cuff systems, KAATSU Master Cuffs and the Hokanson manual hand-held cuff inflator. The KAATSU system had the IRP set to 40-60 mmHg while the Hokanson system had no detectable IRP value. Both systems were inflated to 300 mmHg and pulse was measured. If there was still a detectable pulse at 300 mmHg, occlusion pressures were recorded as 300+ mmHg. Leg circumference was measured pre-inflation at one-third the

distance distal to the inguinal crease. Hierarchal linear regression determined statistical significance between thigh circumference and arterial occlusion pressure. It was concluded that the greatest determinant in arterial occlusion pressure was thigh circumference and suggested that only thigh circumference be considered when determining pressure protocol. However, the researchers failed to investigate the effects of different IRP as suggested by the previous two studies, IRP significantly affects test results.

Final Restrictive Pressure

Until recently, the most used protocol for setting the FRP during leg application has been multiplying the subject's arm systolic blood pressure by 1.44 (Cook et al., 2007). The supporting study used two different FRP, ~160 mmHg (based on arm systolic blood pressure multiplied by 1.44) and ~300 mmHg, were used on a population of 21 males and females. Subjects performed three sets of knee extensions till volitional fatigue. Pre- and post-exercise isometric strength (MVC) levels were taken with a dynamometer in order to assess muscular fatigue caused by BFR. Paired t-test on MVC, total repetitions, and force decrements revealed no significance between FRP conditions. Based on their findings, the authors suggest using the previously mentioned method of setting FRP based on arm systolic blood pressure multiplied by 1.44, since that technique produced comparative values to ~300 mmHg while being more comfortable to the subjects.

One study proposed the use of leg circumference in setting FRP, and the technique has begun to be utilized by several researchers in place of the previously

mentioned method (J. P. Loenneke et al., 2012). This study, previously discussed in the section above regarding IRP, found that thigh circumference was the determining factor for occluding blood flow. Because of this finding, the recommendations for setting FRP changed from using arm systolic blood pressure multiplied by 1.44 to setting FRP based on thigh circumference (<45–50 cm = 120 mmHg; 51–55 cm = 150 mmHg; 56–59 cm = 180 mmHg; and ≥60 cm = 210 mmHg).

Blood Lactate Responses to Exercise

It is evident that more research is needed to identify the correct protocol for BFR and examining the responses in blood lactate may help identify the most effective method. Lactate released during exercise, affects the levels of testosterone and growth hormone, both of which are responsible for increases in muscle mass and strength (Lin, Wang, Wang, & Wang, 2001; Reeves et al., 2006). When comparing different BFR protocols, identifying a condition that increases blood lactate more than the others may indicate that it is more effective. Blood lactate is continuously used in resistance exercise research in order to identify the effectiveness of a technique and therefore would be an ideal variable to include in the present study. In a study conducted by Reeves et al. (2006), it was reported that low intensity resistance exercise combined with partial occlusion increased blood lactate to levels similar to moderate intensity resistance exercise without occlusion. The researchers had subjects perform bicep curls and calf extensions at 30% 1RM. Cuff pressures were set to 20 mmHg below systolic blood pressure readings. Repeated-measures ANOVA revealed that there was a significant increase in lactate levels in both BFR and non-BFR trials with no significant difference

between trials. These findings the effectiveness of low intensity resistance exercise to elicit increases in muscular strength and hypertrophy as moderate intensity resistance exercise when partial occlusion is incorporated. This demonstrates how the use of blood lactate during analysis of the current BFR protocol may be beneficial for determining reliability.

Fujita et al. (2007) examined the effects of BFR combined with low intensity resistance exercise on muscle protein synthesis and S6K1 phosphorylation. Participants were subjected to 4 sets of bilateral leg extension exercise consisting of 1 set of 30 reps and 3 sets of 15 reps at 20% 1RM. The research team found that blood lactate was significantly higher during sessions with BFR compared to the control. This study aimed to identify the underlying causes of muscle protein synthesis during low intensity resistance exercise with BFR (S. Fujita et al., 2007). Here blood lactate was analyzed in order to identify synthesis-stimulating variables.

Fujita et al. (2008) set out to examine the acute effects of BFR, set at 200 mmHg, combined with unilateral knee extension on muscular hypertrophy. Subjects performed 4 sets of bilateral knee extension exercise consisting of 1 set of 30 repetitions followed by 3 sets of 15 repetitions. There were 30 seconds of rest in between each set of leg extensions. When comparing the results of BFR to the non-BFR condition, BFR produced significantly higher values for lactate. This study aimed to identify increases in muscle hypertrophy but examined blood lactate in order to identify a hypertrophic stimulating factor (T. Fujita, Brechue, Kurita, Sato, & Abe, 2008).

Fukuba et al. (2007) examined the combination of BFR and cycling. Subjects performed either 80% sub-Anaerobic threshold or 80% supra-Anaerobic threshold for 6

minutes, followed by a 6-minute rest. During the 6-minute rest period, subjects were subjected to BFR with a 15 cm wide cuff. It was found that blood lactate accumulated to significantly high levels during supra-anaerobic threshold, which helped confirm the target work intensity. This study aimed to analyze ventilation response to BFR but utilized blood lactate as a validation tool (Fukuba et al., 2007). This is yet another study that utilized blood lactate for confirmation of results.

The literature clearly shows that the analysis of blood lactate is an acceptable tool to include in the proposed research. Based on the discussed articles, blood lactate provides details on the level of intensity and exertion the subject is experiencing. The proposed research suggests comparing different BFR protocols to the current most used method. Examining blood lactate throughout each BFR protocol will provide insight as to whether the current method is the most effective at eliciting the desired effects of BFR.

Neuromuscular and Fatigue Response to Exercise

Examination of neuromuscular activation and fatigue response provides insight to the levels of intensity and exertion the subject is experiencing. Activation is measured through electromyography (EMG), which noninvasively reads the electrical activity of the muscles through surface electrodes. Previous research has focused primarily on two variables within EMG, root mean square and median frequency. Root mean square (RMS) provides information regarding changes in the levels of motor unit recruitment while median frequency (MDF) identifies changes in electrical conduction frequency throughout the active motor units (Cochrane, Coburn, Brown, & Judelson, 2014; Cresswell & Ovendal, 2002; Farina, Merletti, & Enoka, 2004; Jones, Howatson, Russell,

& French, 2013; Rahmani Nia, Farzaneh, Damirchi, & Shamsi Majlan, 2013). Fatigue response is measured through maximal voluntary contraction (MVC), usually performed on an isometric dynamometer (Cochrane et al., 2014; Cresswell & Ovendal, 2002; Jones et al., 2013). The inclusion of RMS and MDF into the proposed study would allow the research team to investigate the fatiguing effects of each of the proposed conditions and allow for determination of the effects of IRP on the current BFR protocol.

Farina (2004) utilized EMG to study the fatiguing effects of various power outputs during cycling on the vastus lateralis and rectus femoris. The participants pedaled at various RPM (45, 60, 90, and 120). These pedaling were conducted at both 50% and 100% lactate threshold. It was found that 100% lactate threshold created a greater neuromuscular response when compared to the 50% lactate threshold condition.

Cochrane et al. (2014) examined MVC and EMG response to various recovery interventions. Subjects completed two sets of 50 isokinetic leg extensions at maximum force production. Between the sets the subjects were given 2 minutes of rest, during which they performed one of 4 recovery treatments (passive, active, passive diverting, and active diverting). MVC and EMG activity was measured pre- and post-intervention and it was found that MVC decreased more during the passive condition. RMS was decreased post-intervention; however there was no significant difference between conditions. MDF was shown to decline more during one of the conditions.

Jones et al. (2013) examined the effects of the combination of strength and endurance training performed concurrently. During the research, MVC and EMG activity was monitored in order to identify significant changes between conditions. Subjects performed 6 weeks of exercise, three days a week, for 4 different conditions (strength

training alone (ST), strength-endurance, 3:1 (CT3); strength-endurance, 1:1 (CT1); and no training (CON)). It was found that ST and CT3 increased MVC significantly more. There was no significance found for EMG activity.

Cresswell et al. (2002) compared bilateral knee extension to unilateral knee extensions, while examining the responses in MVC and EMG. Subjects performed unilateral and bilateral isokinetic leg extensions at maximal force. The movement pace was made at sixty degrees per second and had a ninety-degree range of motion. The researchers found that the bilateral condition resulted in greater decreases in MVC lower EMG activity compared to the unilateral condition.

Rahmani et al. (2013) studied EMG response during eccentric leg extension exercise in order to examine the effects of L-glutamine on muscle injury markers. Participants were subjected to 6 sets of eccentric leg extension, at seventy-five percent of their one repetition maximum, performed till exhaustion. Three minutes of rest were given in between sets. Subjects were broken up into two groups in which they either consumed the supplement or placebo. Both groups were instructed to consume the treatment three times per week for a total of four weeks. The research team found no significant difference between the two groups in EMG activity.

Conclusion

This review highlights the lack of proper protocols for BFR. While it seems that using thigh circumference to determine FRP is the best method, IRP is still a debatable variable within protocol. The study conducted by Karabulut & Perez (2013) is a major supporting factor for IRP and it is clear through a review of the literature on the

hemodynamic effects of BFR, that very specific pressures are needed to achieve effectiveness. Increases in circulating lactate help to explain the increases in muscle strength and size, due to the fact that lactate stimulates the release of hormones such as testosterone and growth hormone (Reeves et al., 2006). Changes in MVC and EMG may indicate a greater fatigue response to certain condition and therefore would be ideal variables to include in the proposed research (Cochrane et al., 2014; Cresswell & Ovendal, 2002; Farina et al., 2004; Jones et al., 2013; Rahmani Nia et al., 2013). Because of the inconsistencies of protocols being reported in the literature, it is necessary to evaluate the current method for setting FRP, against the effects of IRP, while examining changes in blood lactate, MVC and EMG.

CHAPTER III

METHODS

Subjects

Thirty-three healthy subjects (20 males and 20 females) between the ages of 18 and 50 were recruited for this study. All subjects were within subject design. The study procedures that were approved by the University of Texas-Brownsville Institutional Review Board for Human Subjects were followed. The length of the study was five 50-minute sessions separated by at least 48 hours in between each session.

Inclusion Criteria

1. Subjects that were within 18-50 years of age.
2. Subjects that did not have hypertension, cardiovascular disease, chronic pain and or joint problems.

Exclusion Criteria

1. Subjects that were taking medication for hypertension.
2. Subjects that were taking medication for cardiovascular disease.
3. Subjects that were suffering from chronic pain.
4. Subjects that were suffering from joint injuries in the lower extremities.

Recruitment

Subjects were recruited from the University of Texas at Brownsville. Fliers (Appendix A) were placed around campus at locations where students frequently gather. The study was also made aware to the student body through word of mouth.

Experimental Protocol

The first day included signing an informed consent form (Appendix B), completing the physical activity readiness questionnaire (PAR-Q) (Appendix C) and health status questionnaire. After completing the paperwork, anthropometric measures that included height, weight, resting heart rate (RHR), resting blood pressure (RBP), thigh circumference at the half waypoint between the greater trochanter and the lateral epicondyle, and one repetition maximum (1RM) for bilateral leg extension were assessed and recorded. 1RM was recorded through multiple RM testing. An extensive explanation of the exercises was given to the subjects before the start of the exercise session for approximately five minutes.

Sessions were randomized into four different bilateral knee extension conditions:

1. Blood flow restriction using thigh circumference for FRP with an IRP of 45-50 mmHg (IRP50) (Control)
2. Blood flow restriction using thigh circumference for FRP with an IRP of 60-65 mmHg (IRP65)
3. Blood flow restriction using thigh circumference for FRP with an added 20 mmHg and an IRP50 (IRP50@FRP+20)
4. Blood flow restriction using thigh circumference for FRP with a subtracted 20 mmHg and an IRP65 (IRP65@FRP-20).

Subjects performed the sessions in a randomized order.

Prior to exercise each of the subjects' legs had the skin prepared by shaving, abrading, and cleansing with alcohol; then fitted with EMG electrodes. Once electrodes

were secured, the subjects were given two warm up sets on the bilateral leg extension machine. Each warm up set consisted of 10 repetitions and 20% 1RM. Subjects were then tested for maximum voluntary contraction (MVC) on the Biodex System 4 Pro Isokinetic Dynamometer (Biodex Medical Systems, Inc., Shirley, NY). Upon completion of MVC testing the subject were fitted with BFR cuffs at the most proximal end of their leg and tightened to the random IRP set for each session. The cuffs were then inflated to the randomized condition and the exercise regimen consisting of four sets of bilateral knee extensions at 20% of their 1RM will begin. The first set consisted of 30 repetitions followed by three sets of 15 repetitions. A 30 second to 1 minute rest period was given between each set. Blood lactate levels were measured before exercise, mid-exercise, immediately post exercise, 5 minutes post-exercise, and 15 minutes post exercise. HR, RPE, and DF were measured before and after each set. BP was recorded pre, immediately post, 5 minutes post-exercise, and 15 minutes post exercise.

EMG Recordings

EMG activity was recorded using the EMG Myomonitor-4 (Delsys Inc., Boston, MA) system consisting of DE-2.3 electrodes. The electrodes consist of two parallel bars spaced 10 mm apart. Prior to electrode placement, the skin was shaved, abraded, and cleansed with alcohol. Electrodes were placed over the vastus lateralis, at one-third the distance from the greater trochanter to the lateral epicondyle, and the rectus femoris, at fifty percent the distance between the greater trochanter and patella. The patella was fitted with a ground electrode. EMG signaling was recorded during MVC and bilateral knee extension testing.

Blood Flow Restriction

Vascular restriction involves the compression of the vasculature proximal to the exercising muscles to reduce blood flow to the limb (Abe et al. 2006). The elastic cuffs are 50 mm in width and were filled with air to create pressure to restrict blood flow. The cuffs were connected to an electronic air pressure control system that monitors the restrictive pressures. The BFR cuffs were placed around the uppermost portion of each thigh, and tightened to the randomized IRP prior to progressive increases in pressure to reach the predetermined FRP. The cuffs were inflated to reach the approximate normal resting systolic blood pressure (120 mmHg) for a healthy adult. The pressure was held at 120 mmHg for 30 s and released for 10 s. The pressure was then increased by 20 mmHg while holding for 30 s at each pressure and releasing for 10 s between increments until the FRP is reached.

Biodex

MVC was tested pre- and post-exercise with the Biodex System. Participants were seated at a back incline of 85˚ and properly secured according to the Biodex User's Guide (Biodex Pro Manual, Applications/Operations. Biodex Medical Systems, Inc., Shirley, NY). The lateral epicondyle of the right knee was used to align the rotational axis of the dynamometer. The subject's lower legs were secured to a lever-arm pad superior to the malleollus Knee joint angle was adjusted to 60°, under the horizontal plane, for all MVC testing.

Blood Lactate

Blood lactate was collected pre-, mid-, post-, 5 minutes post-exercise, and 15 minutes post-exercise. Values were given through the Nova Biomedical Lactate Plus Lactate Meter, which utilizes the Accu-Chek Softclix lancet device to draw blood. Blood samples were collected at the most distal end of the fingertip. Subjects had their lactate levels tested five times per session.

Rate of Perceived Exertion (RPE) (Appendix E)

Borg's 6 to 20 scale was used to assess the subjects' perceived levels of exercise intensity. Subjects were asked to provide a number on the scale that correlated to their level of exertion. If more than one number was given, subjects were asked to specify one specific number.

Visual Analogue Scale (Appendix F)

The visual analogue 0 to 10 scale was used to measure the amount of discomfort/pain the subjects were experiencing due to the cuffs. Subjects were asked to provide a number that corresponded to their level of pain, specifically at the cuff site. If more than one number was given, subjects were asked to specify one specific number. If the subject claimed they felt no pain, they were asked to rate their level of discomfort.

Statistical Analysis

The level of statistical significance was set at $P<0.05$ and data was expressed as means ±SE. Two-way repeated measures ANOVAs was used to test mean differences in the variables tested. Bonferroni post-hoc comparison was used to assess the difference between means when a significant difference is detected.

CHAPTER IV

RESULTS

The purpose of this study was to examine the acute effects of different blood flow restriction setting on 1) the level of blood lactate production, 2) neuromuscular activation (the changes in motor unit activation and median frequency), and 3) fatigue response during bilateral knee extension exercise.

Subject Characteristics

This study consisted of thirty-three male (age= 26.63 (5.8), n=17) and female (age= 22.76 (3.3), n=16) participants. Table 1 displays anthropometric measures of the study population. Subjects were recruited from the University of Texas at Brownsville campus and surrounding community.

Table 1.Anthropometric data of participants.

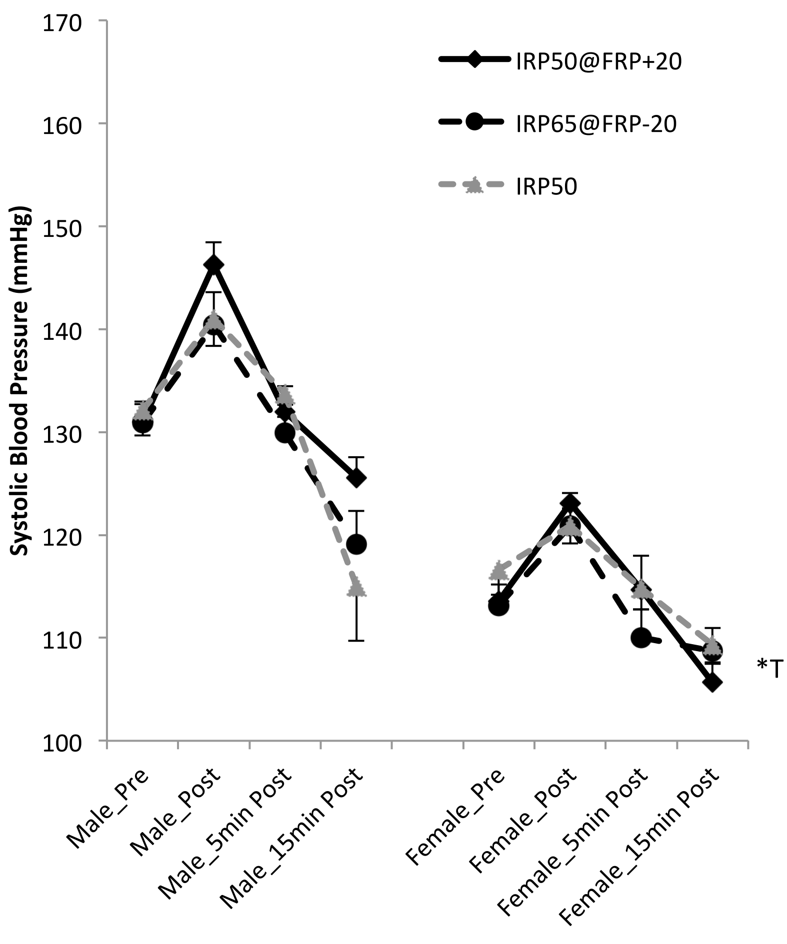
Variables	Male (n=17)	Female (n=16)	Combined Cohort (n=33)
Age (yr)	26.63 (5.8)	22.76 (3.3)	24.64 (5)
Height (cm)	170.35 (5.1)	156.91 (5.9)	163.42 (8.7)
Weight (kg)	90.20 (13.5)	62.87 (18.9)	76.12 (21.4)

Values are reported as means (SD)

Hemodynamic Response

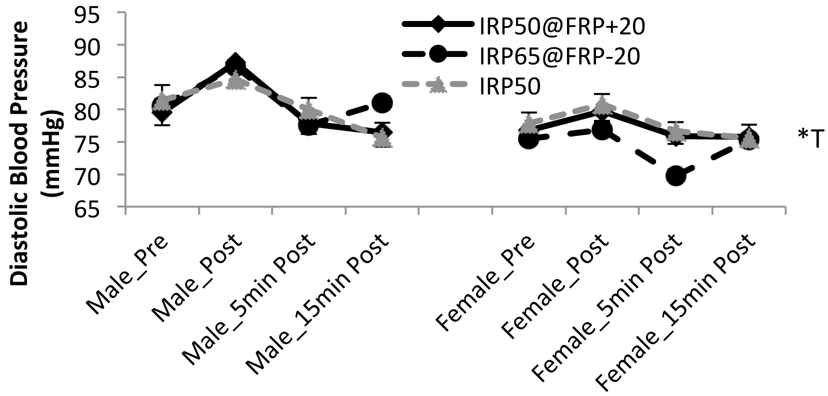
Figures 1-4 display the hemodynamic responses to exercise from rest, through exercise, to 15 minutes post-exercise for all testing conditions. Repeated measures ANOVA revealed a significant time difference in systolic and diastolic blood pressure, and heart rate (p<0.01). Heart rate also revealed a significant condition*gender interaction (p=0.022) with IRP50@FRP+20 significantly affecting males, and a trend for a condition*time interaction (p=0.063) with IRP50@FRP+20.

Figure 1. Changes in Systolic Blood Pressure



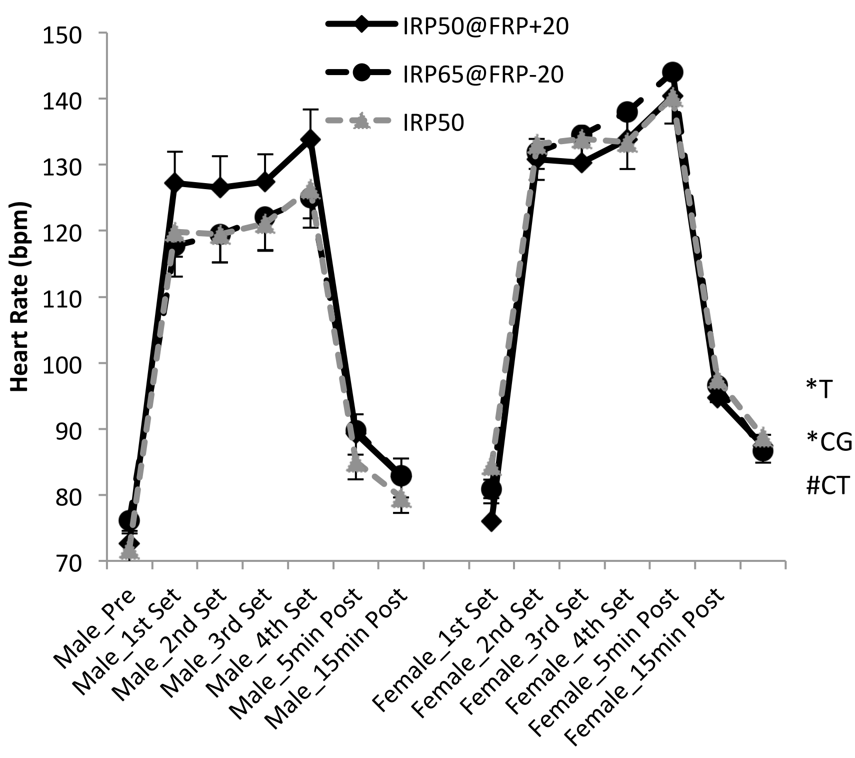
*T Significant time difference ($p < 0.01$). Values reported as mean ± SE. (N=33)

Figure 2. Changes in Diastolic Blood Pressure



*T Significant time difference ($p < 0.01$). Values reported as mean ± SE. (N=33)

Figure 3. Changes in Heart Rate in Males and Females due to Blood Flow Restriction Exercise

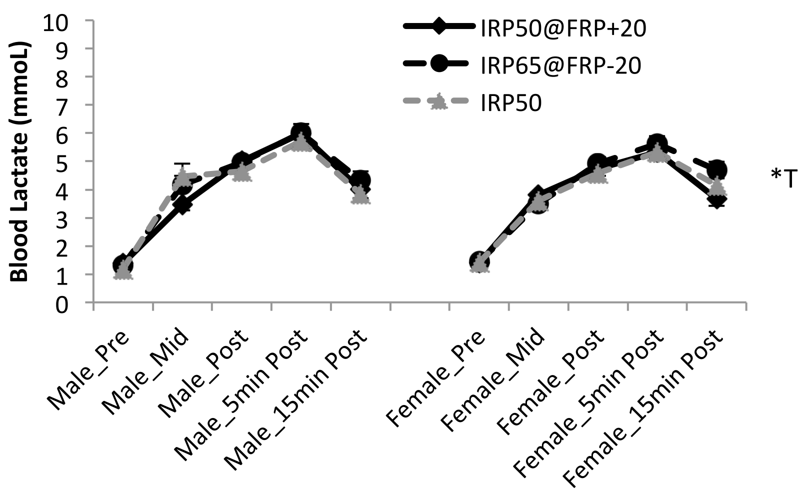


*T Significant time difference ($p < 0.01$). *CG Significant condition*gender interaction ($p=0.022$). #CT Trend for condition*time interaction ($p<0.06$). Values reported as mean ± SE. (N=33)

Blood Lactate Response

No significant difference was found between conditions for blood lactate, however, a significant time difference ($p<0.01$) was discovered.

Figure 4. Blood Lactate Response to Blood Flow Restriction Exercise

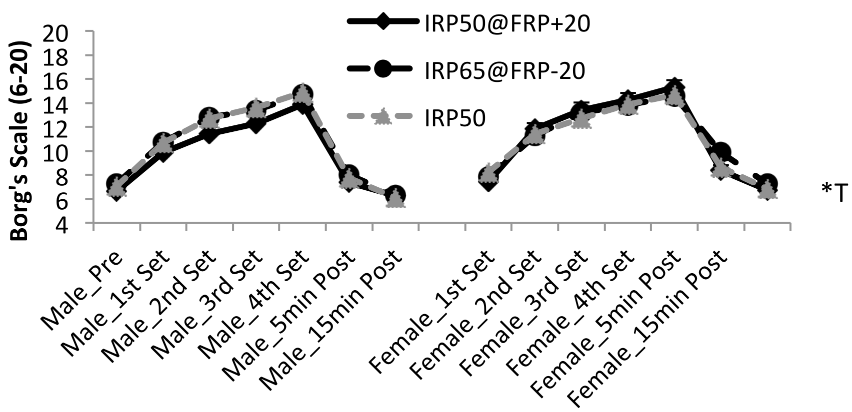


*T Significant time difference ($p < 0.01$). Values reported as mean ± SE. (N=33)

Exertion and Discomfort

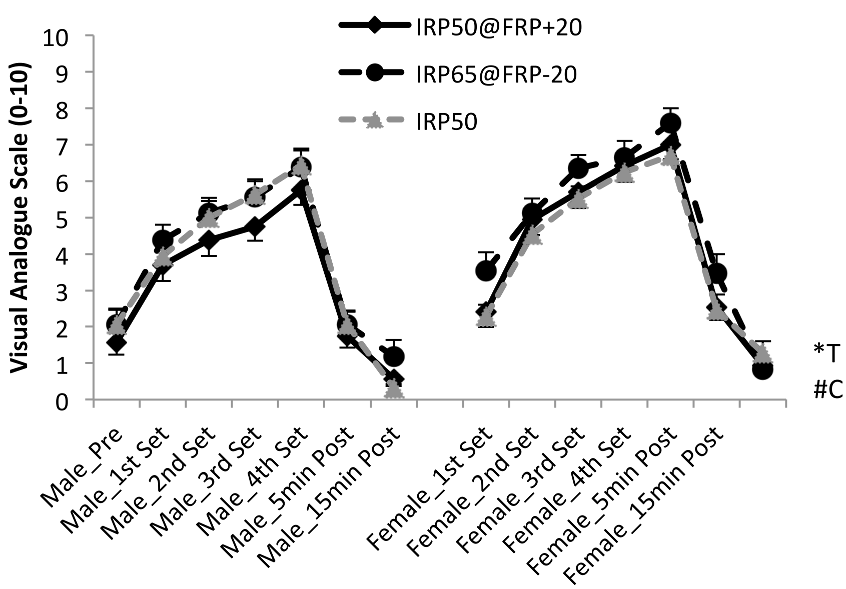
Figure 5 depicts rate of perceived exertion response from pre-exercise to 15 minutes post-exercise. Repeated measures ANOVA revealed a significant ($p<0.01$) time difference. Figure 6 shows discomfort from pre-exercise to 15 minutes post-exercise. Repeated measures ANOVA revealed a trend in condition ($p<0.065$), as well as a significant time difference ($p<0.01$).

Figure 5. Changes in Rate of Perceived Exertion throughout Blood Flow Restriction Exercise



*TSignificant time difference ($p < 0.01$). Values reported as mean ± SE. (N=33)

Figure 6. Reports of Discomfort throughout Blood Flow Restriction Exercise

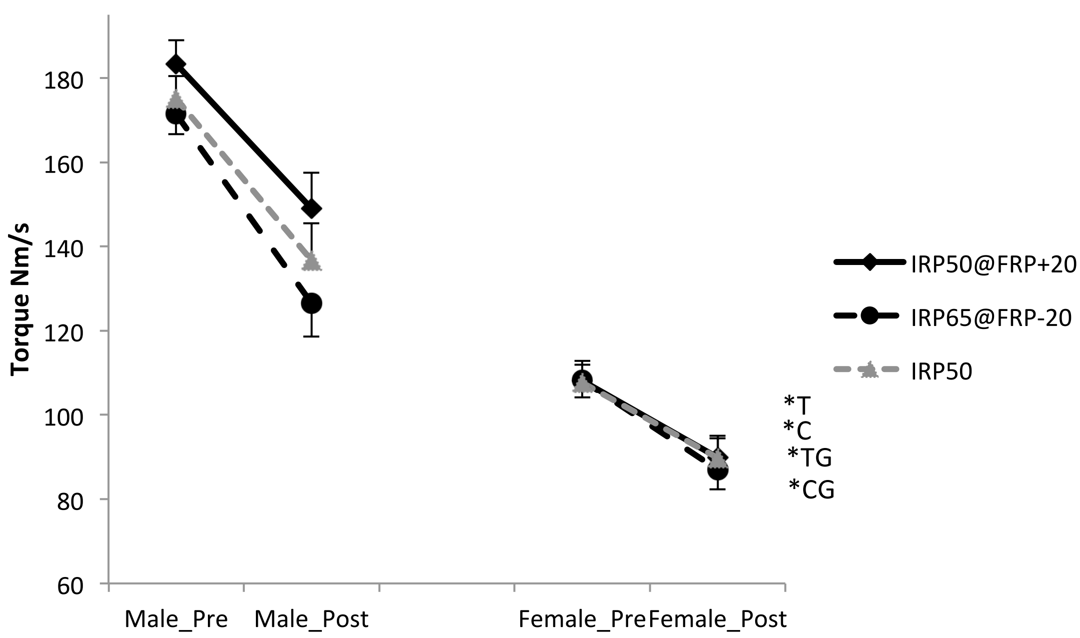


*TSignificant time difference ($p < 0.01$). #CTrend for condition main effect ($p=0.062$). Values reported as mean ± SE. (N=33)

Muscle Strength

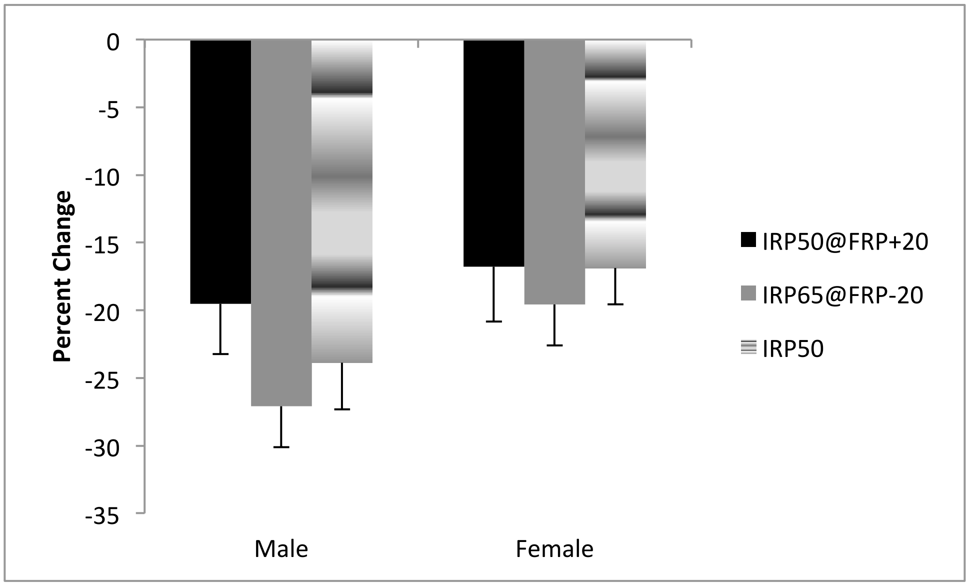
Maximum voluntary contraction was recorded pre and immediately post exercise for all conditions. A significant condition ($p<0.023$) effect, with IRP65@FRP-20, producing the greatest decrease in torque, was revealed through repeated measure ANOVA as shown in Figure 7. A strong trend for condition*gender interaction ($p=0.055$) was also found with males showing greater decreases in pre to post maximum voluntary contraction. Repeated measures ANOVA further revealed a significant time ($p<0.01$) difference and a significant time*gender ($p<0.01$) interaction with males showing greater decreases in pre to post maximum voluntary contraction over time.

Figure 7. Decreases in MVC from Pre to Post Exercise



[*T]Significant time difference ($p < 0.01$). [*C]Significant condition main effect ($p<0.023$). [*TG]Significant time*gender interaction ($p<0.01$). [#CG]Trend for a condition*gender interaction ($p=0.055$). Values reported as mean ± SE. (N=33)

Figure 8. Percent change in torque Pre to Post MVC



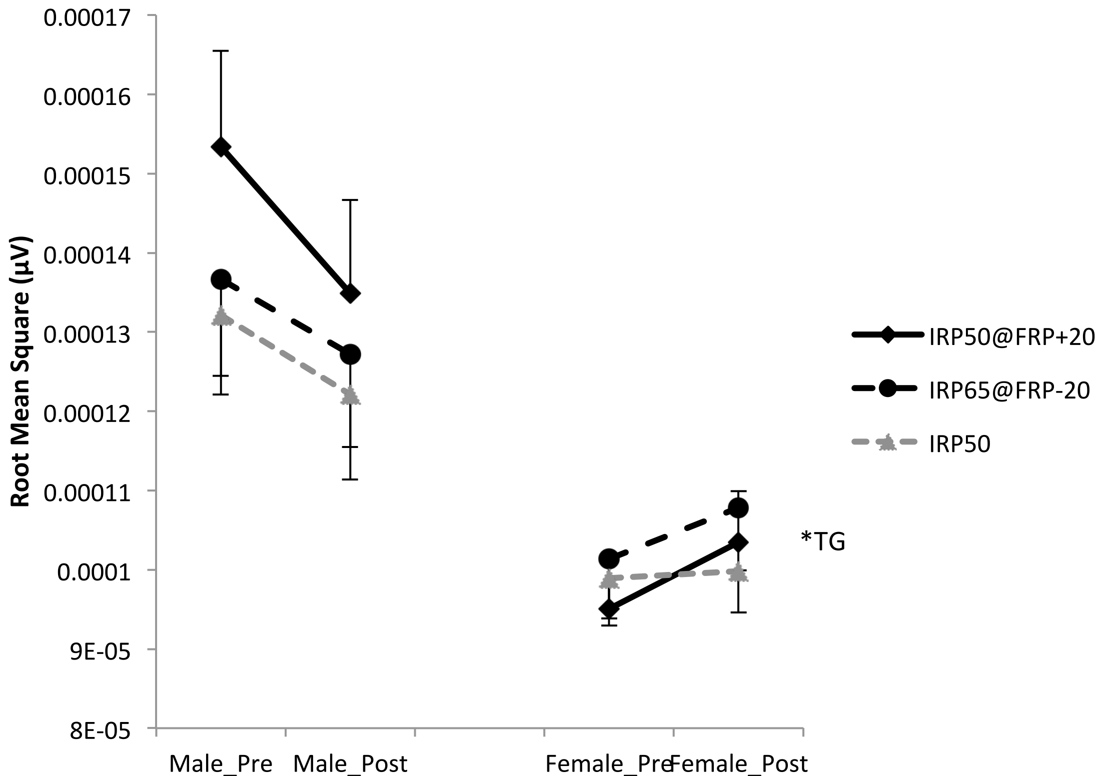
Values reported as mean ± SE. (N=33)

Electromyography

Vastus Lateralis

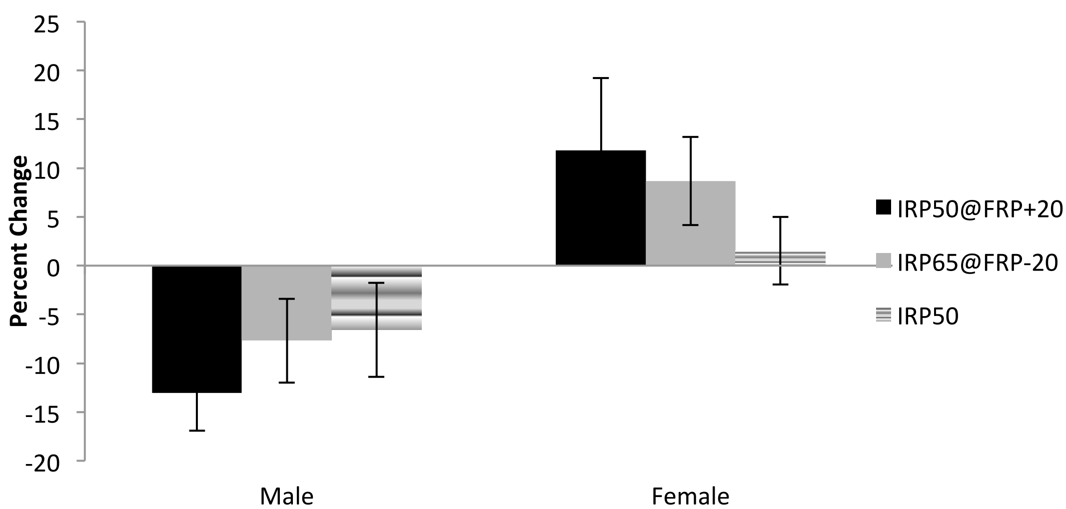
Repeated measures ANOVA revealed a significant time*gender interaction ($p<0.04$) for RMS in pre to post maximum voluntary contraction with males displaying greater decreases in muscle activation. Figure 9 displays pre to post maximum voluntary contraction for all conditions.

Figure 9. Changes in RMS values for MVC from Pre to Post Exercise in the Vastus Lateralis



*TG Significant time*gender interaction ($p=0.033$). Values reported as mean ± SE. (N=33)

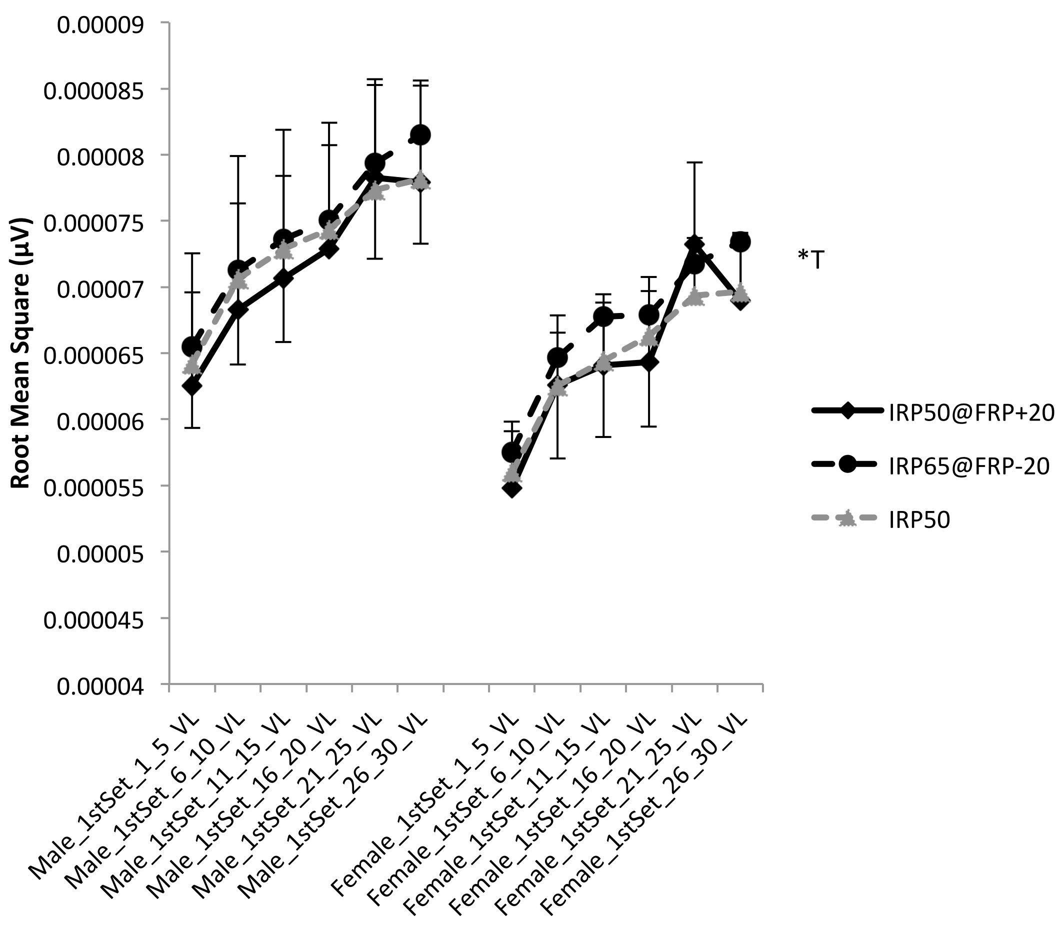
Figure 10. Percent change in RMS for Pre to Post MVC in the Vastus Lateralis



Values reported as mean ± SE. (N=33)

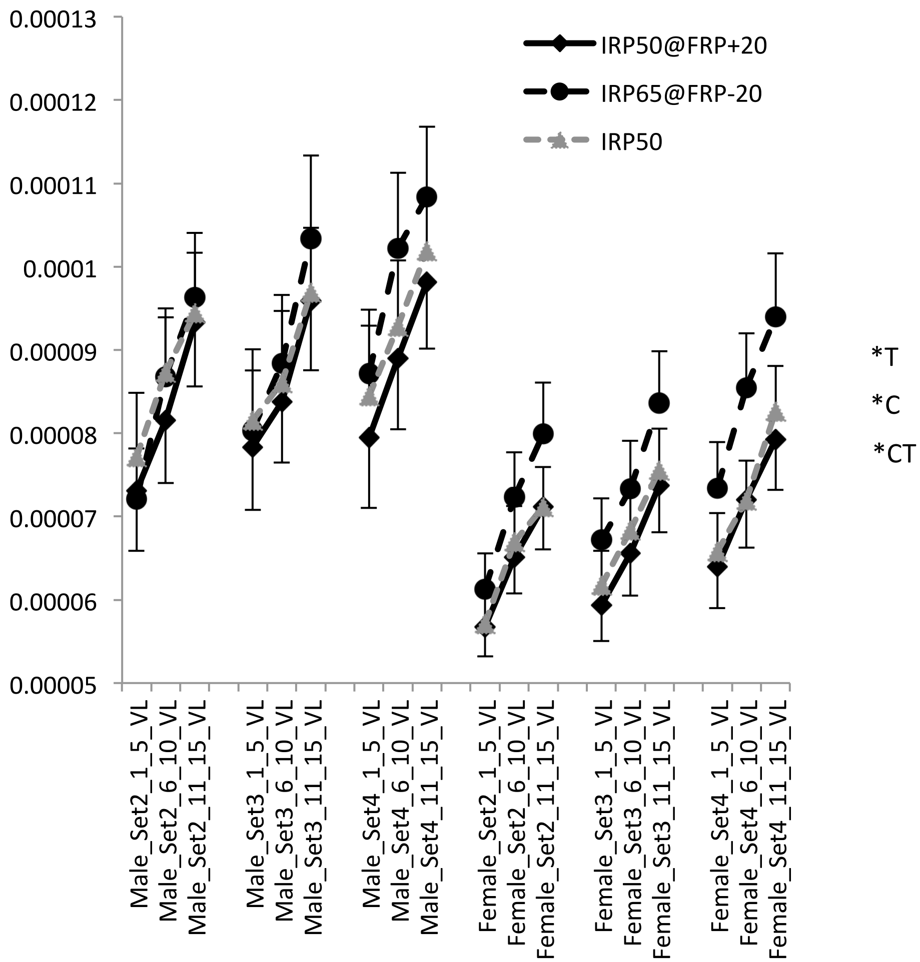
RMS values were recorded for every repetition from sets 1-4. As shown in Figure 11, a significant time ($p<0.01$) difference was found during set 1. Figure 12 displays sets 2-4 showing significance in condition ($p<0.025$) with IRP65@FRP-20 producing greater muscle recruitment, time ($p<0.01$), and a condition*time ($p<0.01$) interaction with IRP65@FRP-20 producing greater increases in muscle recruitment.

Figure 11. Changes in RMS values during Set 1 in the Vastus Lateralis



*T Significant time difference ($p < 0.01$). Values reported as mean ± SE. (N=33)

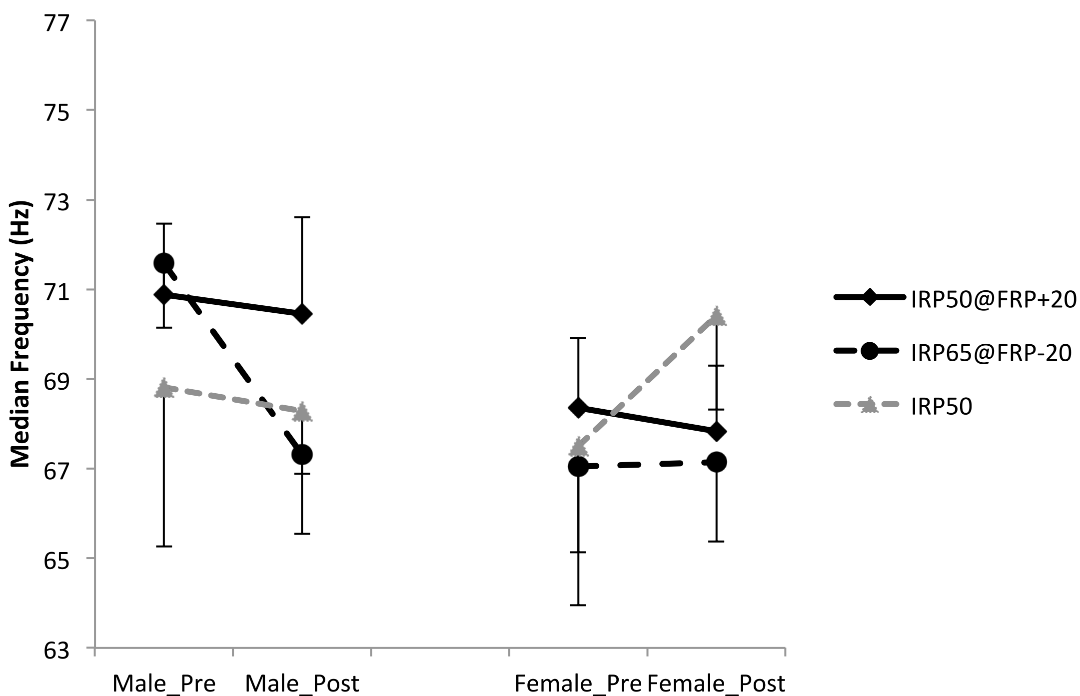
Figure 12. Changes in RMS values during Sets 2, 3 and 4 in the Vastus Lateralis



*T Significant time difference ($p < 0.01$). *C Significant condition main effect ($p=0.024$). *CT Significant condition*time interaction ($p<0.01$). Values reported as mean ± SE. (N=33)

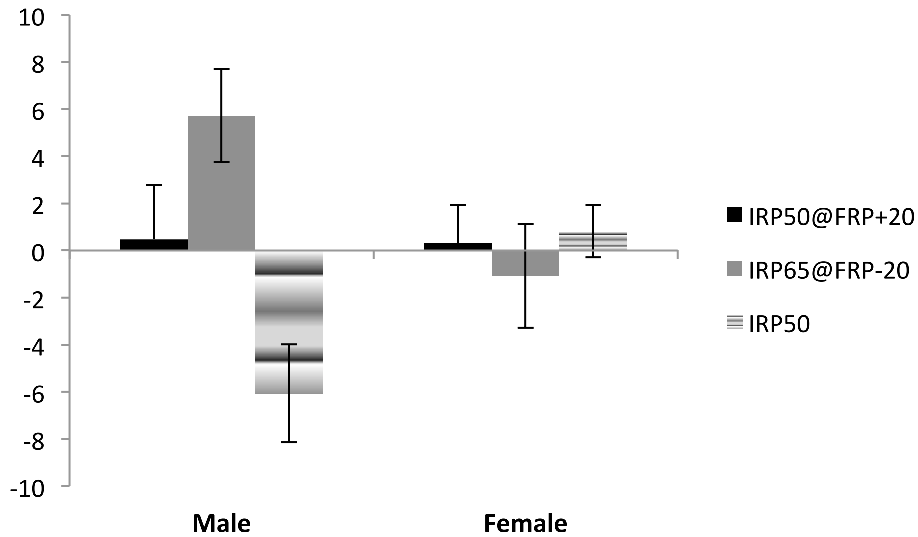
MDF analysis of pre and post maximum voluntary contraction revealed no significance between pre and post values, as shown in Figure 13.

Figure 13. Changes in MDF values for MVC from Pre to Post Exercise in the Vastus Lateralis



Values reported as mean ± SE. (N=33)

Figure 14. Percent change in MDF values for MVC from Pre to Post Exercise in the Vastus Lateralis

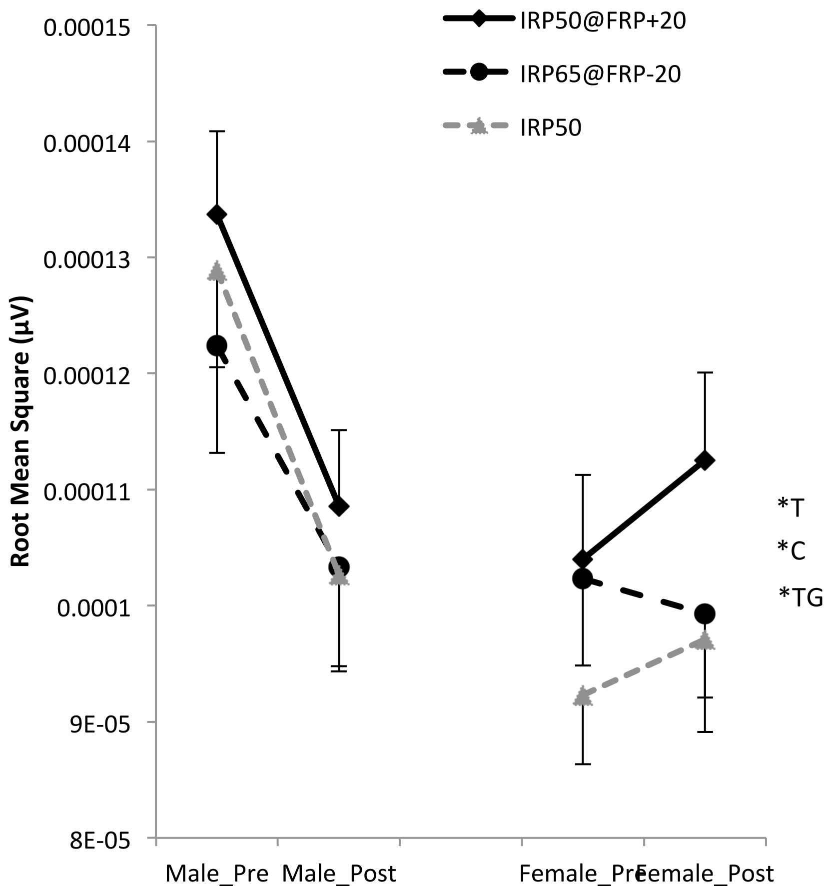


Values reported as mean ± SE. (N=33)

Rectus Femoris

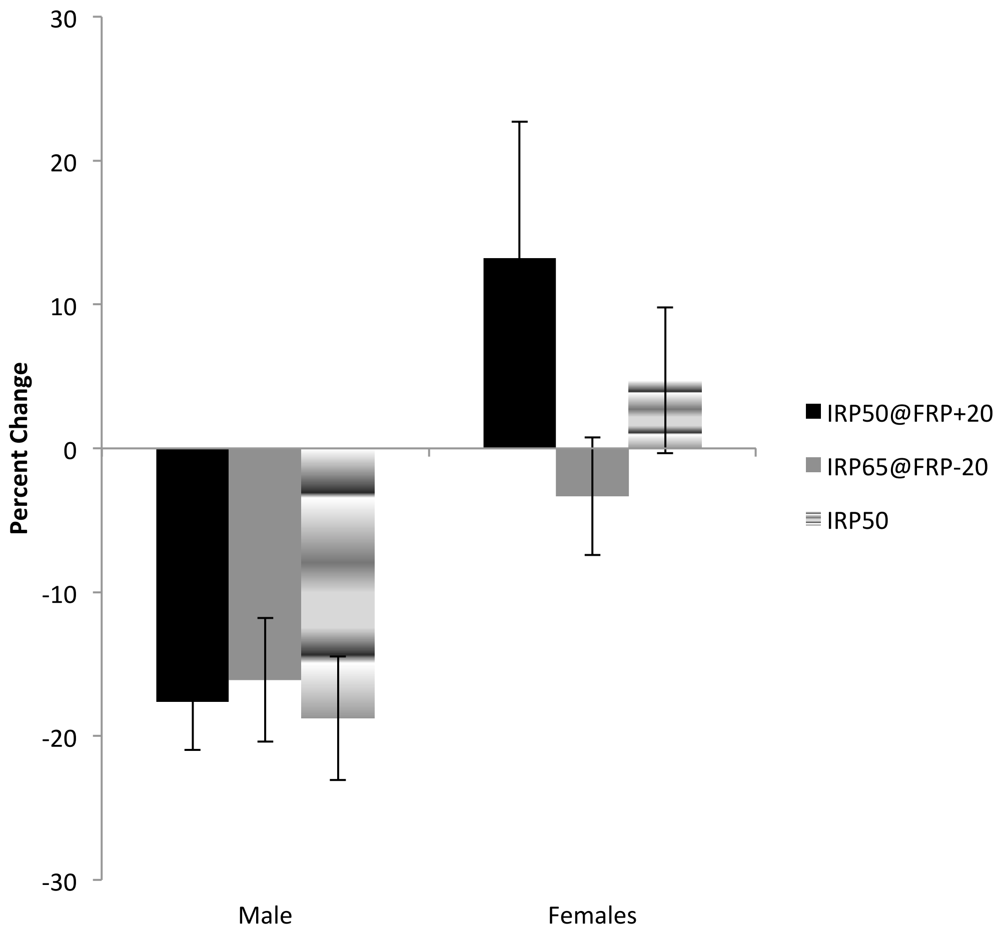
Repeated measures ANOVA revealed a significant time ($p<0.01$) difference and time*gender ($p<0.01$) interaction RMS in pre to post maximum voluntary contraction with males displaying greater decreases in muscle activation. Repeated measure ANVOA also revealed a trend in condition ($p=0.081$) with IRP65@FRP-20 producing the greatest difference in pre to post values. Figure 15 displays pre to post maximum voluntary contraction for all conditions.

Figure 15. Changes in RMS values for MVC from Pre to Post Exercise in the Rectus Femoris



*T Significant time difference ($p < 0.01$). #C Trend for condition main effect ($p=0.087$). *TG Significant time*gender interaction ($p<0.01$). Values reported as mean ± SE. (N=33)

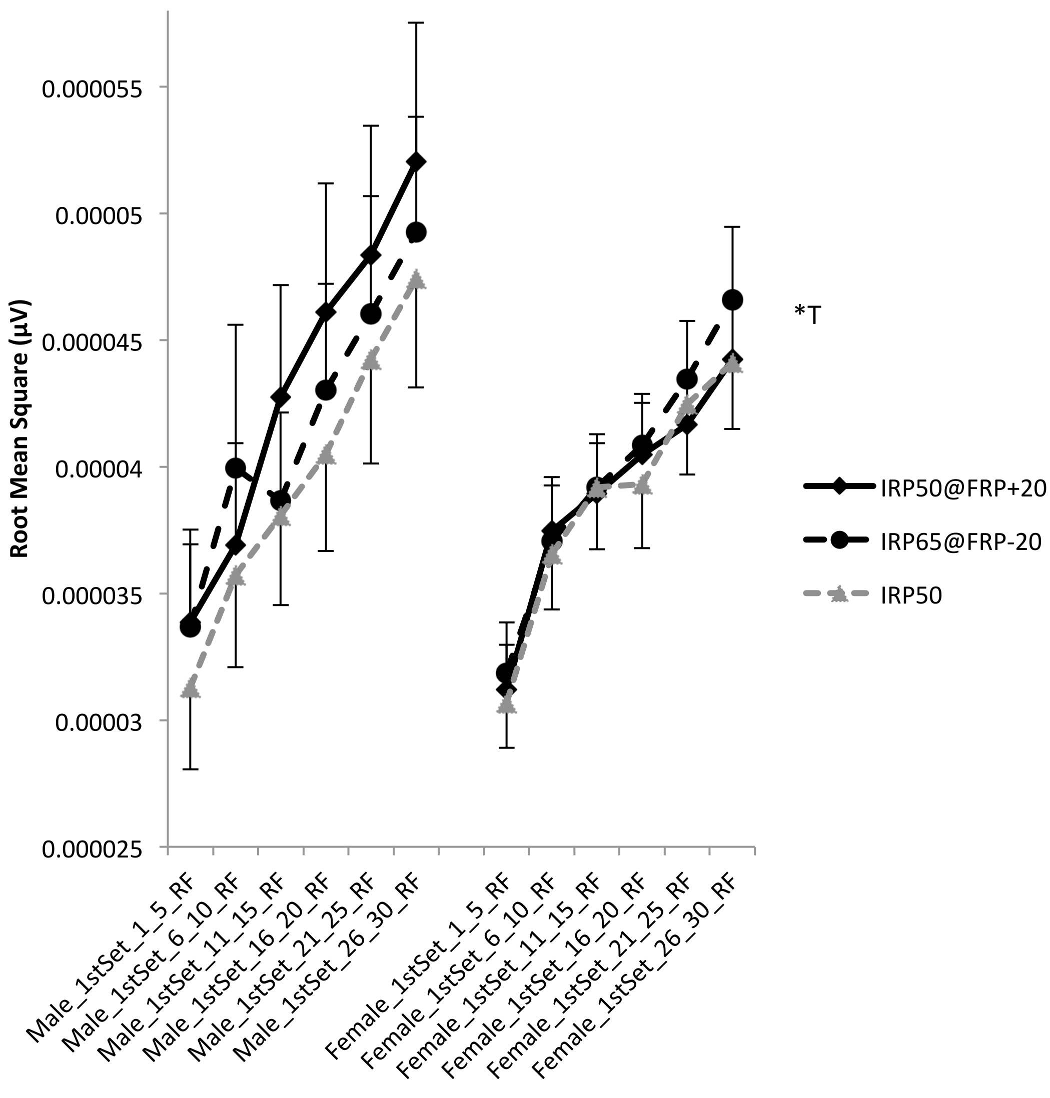
Figure 16. Percent change in RMS for Pre to Post MVC in the Rectus Femoris



Values reported as mean ± SE. (N=33)

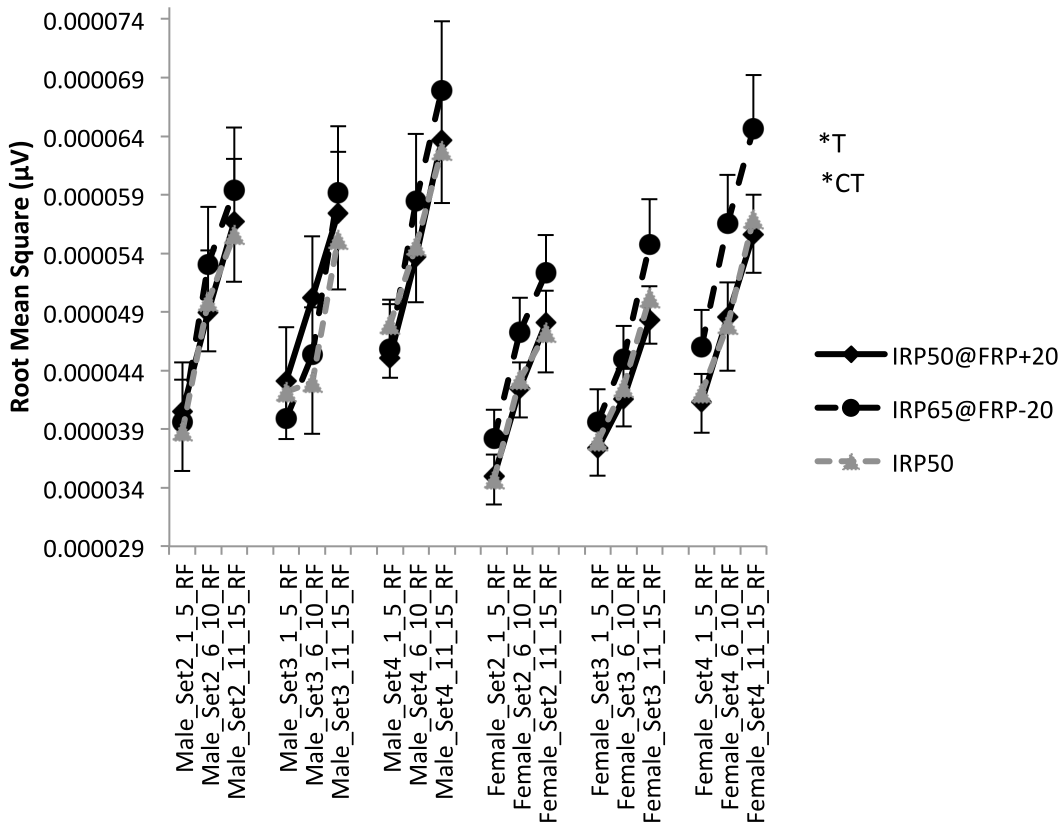
As shown in Figure 17, a significant time (p<0.01) difference was found during set 1. Figure 18 displays sets 2-4 showing significance in time (p<0.01) and a condition*time (p<0.01) interaction with IRP65@FRP-20 producing greater increases in muscle recruitment.

Figure 17. Changes in RMS values during Set 1 in the Rectus Femoris



*T Significant time difference ($p < 0.01$). Values reported as mean ± SE. (N=33)

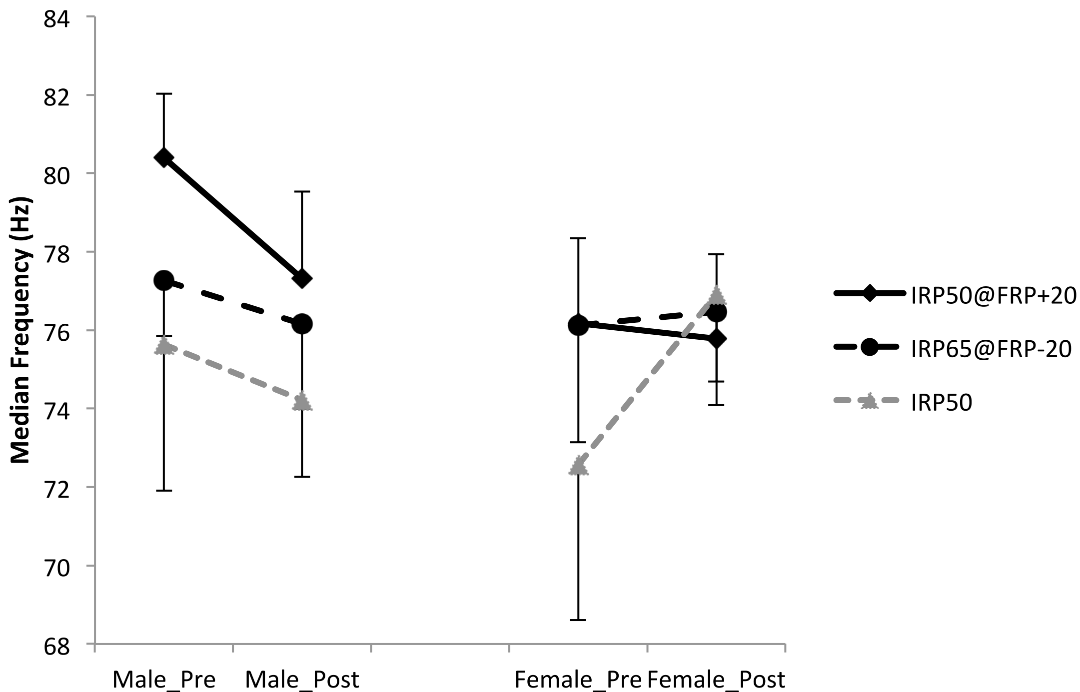
Figure 18. Changes in RMS values during Sets 2, 3 and 4 in the Rectus Femoris



*T Significant time difference ($p < 0.01$). *CT Significant condition*time interaction ($p<0.01$).Values reported as mean ± SE. (N=33)

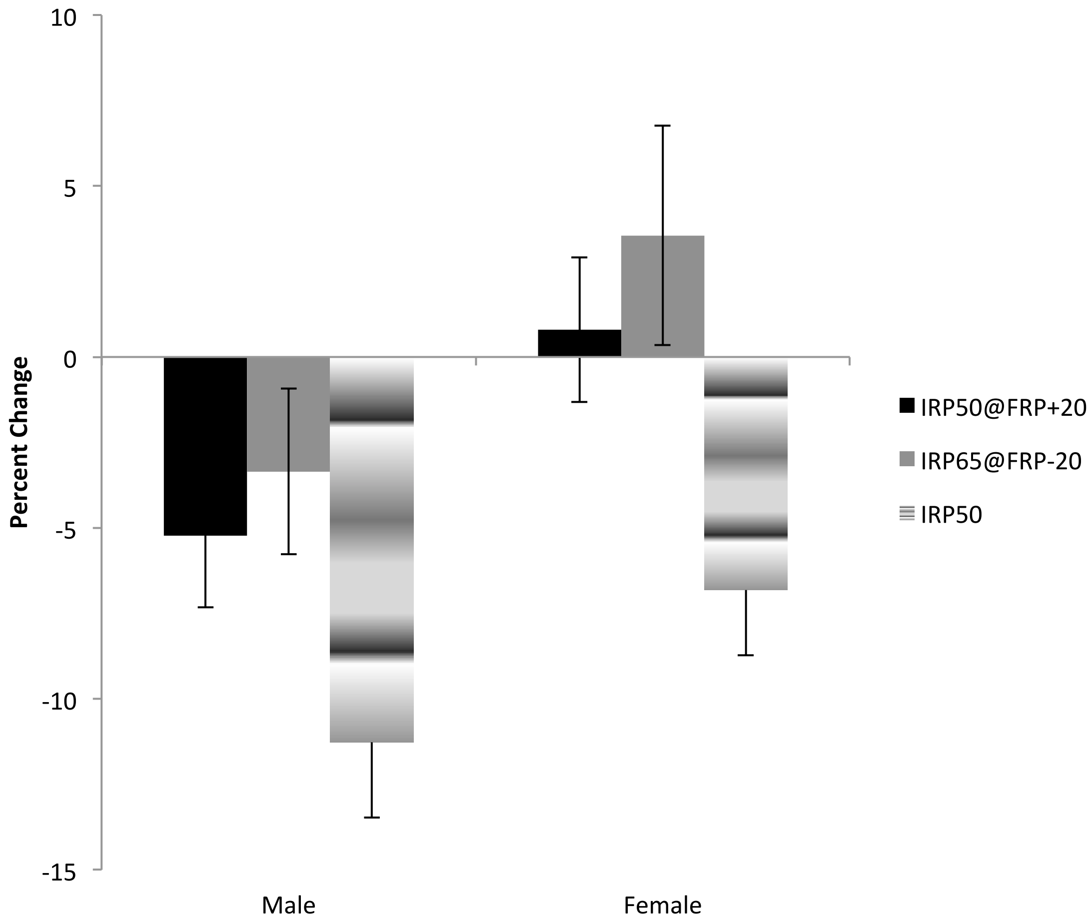
MDF analysis of pre and post maximum voluntary contraction revealed no significance between pre and post values, as shown in Figure 19.

Figure 19. Changes in MDF values for MVC from Pre to Post Exercise in the Rectus Femoris



Values reported as mean ± SE. (N=33)

Figure 20. Percent change in MDF values for MVC from Pre to Post Exercise in the Rectus Femoris



Values reported as mean ± SE. (N=33)

CHAPTER V

DISCUSSION

The present investigation is the first study to examine the effects of IRP against the current BFR protocol. The main finding of the study is that the use of IRP 60-65 mmHg with a subtracted 20 mmHg from the FRP created greater decreases in MVC and greater levels of motor unit recruitment when compared to the control. This determination is significant because it validates the problems presented in chapters 1 and 2, that the current BFR protocol may not be the most valid method of applying BFR considering the fact that the variable IRP was not evaluated in its design.

Hemodynamic Response

This study found no difference between conditions for systolic and diastolic blood pressure as well as blood lactate. Previous research reporting blood pressure adaptations, examined the effects of their treatment on pre and post blood pressure values or against a control that did not include BFR (Kumagai et al., 2012; J. P. Loenneke et al., 2014; J. P. Loenneke, Fahs, et al., 2013; Renzi, Tanaka, & Sugawara, 2010; Vieira, Chiappa, Umpierre, Stein, & Ribeiro, 2013; Williamson, Crandall, Potts, & Raven, 1994). It was not this study's goal to examine the pre to post differences in each condition, rather it aimed to identify whether and how one condition would affect the variables compared to the other condition. The lack of difference between conditions for blood pressure may be due to the fact that each condition experienced BFR with the same exercise intensity. It may be that the total pressure being applied from each condition, while different from one another, was not enough to elicit drastic changes in blood pressure.

The present study discovered males produced a greater heart rate response to IRP50@FRP+20 than the female population. These results may be due to the fact that many of the males experienced an FRP of 230 mmHg during IRP50@FRP+20, while only two females were subjected to that FRP. In previous research it is rare for the FRP to be inflated above 220 mmHg (T. Fujita et al., 2008; Gundermann et al., 2012; Karabulut et al., 2011; Reeves et al., 2006; T. Suga et al., 2010; Tadashi Suga et al., 2009; Takada et al., 2012; Takarada et al., 2000). According to the current protocol's recommendations, subjects with a leg circumference of greater than or equal to 60 centimeters are to have an FRP of 210 mmHg (J. P. Loenneke et al., 2012). Results for heart rate also revealed that the condition IRP50@FRP+20 produced a trend for a greater heart rate response compared to the other conditions.

These findings do not match previous research examining IRP. Karabulut et al. (2011) found that heart rate response increased as IRP increased; however the researchers compared IRP when the cuffs were inflated to the same FRP. Based on Karabulut et al. (2011), it is reasonable to assume that if the present study were to have examined a condition that included an IRP of 60-65 mmHg with an added 20 mmHg to the FRP, the heart rate response would have been greater than the IRP50@FRP+20 condition.

Blood Lactate Response

It is important to note that this study focused on differences between conditions for blood lactate response and not increases from pre to post exercise. The findings for blood lactate match those reported in the literature. Previous studies have shown that comparable conditions producing equivalent physiological demand will result in similar

blood lactate adaptations (Karabulut et al., 2014; J. P. Loenneke, Kearney, Thrower, Collins, & Pujol, 2010; Reeves et al., 2006; T. Suga et al., 2010). Karabulut et al. (2014) observed the effects of different IRP (40-45 mmHg and 60-65 mmHg) on blood lactate while using the method of setting FRP that multiplied arm systolic blood pressure by 1.44. The subjects in this study performed 4 sets of unilateral knee extension exercise. The first set consisted of 30 repetitions, followed by three sets of 15 repetitions. While a significant time difference was found for blood lactate, there was no significant difference between conditions.

Loenneke et al. (2010) examined the effects of BFR with bilateral knee extensions (30% 1RM) on lactate accumulation. Subjects performed four sets of bilateral knee extension with 30 repetitions in the first set and 15 repetitions in the following three sets. Each set was separated by a 150 second rest period. There was no significant difference in the level of lactate between the BFR and non-BFR conditions. The authors speculated that the elastic wraps did not produce enough restrictive pressure to induce BFR effects. While there was pressure being applied by the wraps, the two conditions were very similar. This is very similar to the condition in the present study. While each condition experienced a different IRP and FRP, they only varied slightly from each other.

This study’s findings for blood lactate are once again validated by a study conducted by Reeves et al. (2006). As discussed in the review of literature, these authors compared low intensity resistance exercise with BFR to moderate intensity resistance exercise without BFR. The study found no difference in lactate response between the two conditions and concluded that the two conditions produced a similar metabolic affect.

This may indicate that comparable intensities will results in similar physiological demands, regardless of minor fluctuations, verifying the results of the present study.

Another supporting study was conducted by Suga et al. (2010). This study examined the effects of low intensity resistance exercise on metabolic response. Participants were subjected to unilateral plantar flexion. The exercises consisted of lifts at 20% and 30% of the subject's 1RM with BFR. These conditions were compared to an intensity set at 65% 1RM (high intensity according to the authors). The exercise protocol had subjects lift at a cadence of 30 repetitions per minute for two minutes, with 30 min of rest between conditions. Before the start of the second condition, subjects were tested to see if full recovery had occurred. The results showed that there was no significant difference in blood lactate between low resistance exercise at 30% 1 RM with BFR and high intensity exercise without BFR. The authors conclude by claiming that the low intensity condition produced similar physiological effects as the high intensity condition and therefore lactate levels were not significantly different.

The difference in findings between the present study and previously reported literature may due to the comparisons made in analysis. Other BFR studies that did find significant differences in blood lactate were examining statistical differences from pre to post, BFR to non-BFR, or two different BFR intensities (T. Fujita et al., 2008; Gundermann et al., 2012; T. Suga et al., 2010). Fujita et al. (2008) set out to examine the acute effects of BFR, set at 200 mmHg, combined with unilateral knee extension on lactate response. When analyzing the results of BFR and the non-BFR condition for pre to post lactate values, BFR produced a significant increase in lactate. Gundermann et al. (2012) compared the effects of BFR and sodium nitroprusside (SNP) on blood lactate

during low intensity resistance exercise. Exercise sessions consisted of 4 sets of bilateral knee extension. The first set was 30 repetitions with the following three sets at 15 repetitions. Intensity was set at 20% 1RM. Statistical analysis revealed that the BFR condition resulted in significantly higher lactate levels compared to the SNP condition. Suga et al. (2010), as previously discussed, examined unilateral plantar flexion at 20% and 30% 1RM with BFR, and 65% 1RM without BFR. A significant difference was found between the BFR condition at 20% 1RM and the 65% 1RM condition with the higher intensity condition producing greater increases in blood lactate.

Exertion and Discomfort

The findings of this study for RPE do not coincide with the majority current literature due to the fact that this is the first study examining RPE while comparing multiple BFR condition. There are several published articles comparing BFR to a non-BFR condition in which they do find significantly higher RPE values (Hotta & Ito, 2011; Kumagai et al., 2012; J. P. Loenneke et al., 2010; J. P. Loenneke, Thiebaud, et al., 2013; Tadashi Suga et al., 2009; Sumide, Sakuraba, Sawaki, Ohmura, & Tamura, 2009). One study, however, conducted by Wernbom et al. (2009) compared BFR to non-BFR during leg extension exercises. Subjects performed three sets of unilateral leg extension exercise until volitional fatigue at 30% 1RM. Analysis of the data revealed no significant difference in RPE between conditions. The FRP for the BFR condition was set at 100 mmHg for each subject and no IRP was reported. The authors speculate that 100 mmHg may not have been enough pressure to elicit the desired BFR effects (Wernbom, Jarrebring, Andreasson, & Augustsson, 2009). This suggests that both exercise conditions

were similar to one another, very much like the conditions in the present study. The findings in Wernbom et al. (2009) therefore validate the findings in the present study. Another study performed by Wernbom et al. (2006) found no significant difference between BFR and non-BFR conditions for RPE, even though the BFR condition had an FRP of 200 mmHg. The authors contribute the lack of significance to the fact that both conditions required subjects to perform the exercises with maximal exertion until fatigue was reached (Wernbom, Augustsson, & Thomee, 2006). Maximal effort tends to produce high levels of RPE regardless of the application of BFR. This once again supports the findings of the present study due to the fact that the maximal effort resulted in similar exercise conditions.

IRP65@FRP-20 displayed a tendency to produce greater levels of discomfort/pain in both genders. This finding does not correspond with previous research, which suggests that increased total pressure results in increased levels of discomfort/pain (J. P. Loenneke, Thiebaud, et al., 2013; Sumide et al., 2009; Wernbom et al., 2006). It was the findings of this study that the condition with the lowest total pressure resulted in the greatest reported levels of discomfort/pain. This suggests that IRP has a greater impact on the levels of discomfort/pain reported, rather than FRP. All previous studies failed to evaluate IRP when reporting discomfort/pain. It may be that IRP is a greater predictor of discomfort/pain due to the fact that when the cuffs are initially tightened, greater IRP have a tendency to pinch the skin and/or cluster any fabric that the subjects may be wearing.

Muscle Strength

It has been well established that BFR results in decrements in torque from pre to post MVC (J. P. Loenneke, Thiebaud, et al., 2013; Umbel JD, 2009). Loenneke et al. (2013) examined the effects of unilateral leg extensions with and without BFR on pre and post MVC torque values. Subjects performed exercise at 30% 1RM. Analysis of pre and post MVC torque values revealed that the BFR condition resulted in significantly greater reductions in torque compared to the non-BFR condition. The authors concluded that the decrements in torque were due to increased levels of fatigue caused by the application of BFR. Umbel (2009) performed two separate experiments. In the first experiment the authors had subjects perform three sets of unilateral leg extensions at 35% of their MVC with BFR. The subjects then repeated the protocol on the same leg without BFR. In the second experiment, the subjects performed three sets of concentric unilateral leg extensions at 35% of their MVC with BFR. The other leg performed three sets of eccentric unilateral leg extensions at 35% of their MVC without BFR. In both experiments it was found that the BFR condition produced greater decreases in pre to post MVC torque.

The studies mentioned above demonstrate how pressure applied through BFR can create significant decreases in torque, suggesting that the greater level of pressure applied, the greater the amount of fatigue is to be expected. However, there are variances in the levels of decreased torque in previous studies that used similar exercise intensity. It is possible that the variance reported in the studies is a product of IRP, which has not been mentioned or monitored in most of the previous study protocols. The present study discovered that the condition with the lowest FRP with the greatest IRP created the

greatest levels of fatigue. This may suggest that FRP may not be the greatest indicator of fatigue. A previous study by Karabulut et al. (2014) reported similar findings. The study examined the effects of different IRP during unilateral knee extension exercise consisting of 4 sets at 20% 1RM. The first set required the subjects to perform 30 repetitions while the following three sets had subjects perform 15 repetitions. Pre to post exercise MVC values were analyzed and it was found that the IRP with 60-65 mmHg produced significantly greater decreases in MVC compared to the IRP with 40-45 mmHg, even though each condition experienced the same FRP. Another study by Karabulut and his colleagues (2010) investigated the effects of BFR on neuromuscular fatigue. The study had subjects perform 5 sets of leg extensions on a dynamometer at 20% of each subject's one repetition maximum (1-RM). MVC test was performed pre and post exercise to determine the changes in strength. It was discovered that the BFR condition created greater differences in pre to post MVC compared to the non-BFR condition and that BFR might produce greater instances of either peripheral or central fatigue. The results of the current study may suggest that IRP has a greater effect on fatigue response than FRP alone. Therefore, it may be reasonable to speculate that IRP may contribute to greater levels of peripheral fatigue, central fatigue, or combination of both.

The gender differences discovered for torque in pre to post MVC indicate that males were more affected by the condition IRP65@FRP-20 than females. Previous research conducted by Karabulut et al. (2013) found that not only did a greater IRP of 60-65 mmHg affect both genders more than the lower IRP of 40-45 mmHg when inflating to the same FRP, males were also affected more than the female population. This study concluded that both IRP and FRP must be considered when applying BFR. It is

speculated that body composition differences between males and females, especially in the levels of subcutaneous fat stored in the application site of the BFR cuffs, may be the reason for the gender differences found in the present study (Karabulut & Perez, 2013). It is possible that higher levels of subcutaneous fat between the surface of the skin and the circulatory pathways may buffer the effect of BFR preventing the cuffs from applying the desired restrictive pressure.

Electromyography

The results of this study indicate that the condition IRP65@FRP-20 significantly affected EMG values compared to the other treatments. It was found that IRP65@FRP-20 resulted in greater reductions in motor unit activation from pre to post MVC and greater increases in motor unit activation throughout exercise. Additionally, no change in MDF pre to post MVC was found. These results suggest that the current BFR protocol (Control) may not produce the desired neuromuscular demands.

Reduced motor unit activation from pre to post MVC during IRP65@FRP-20 may be explained by decreased type-1 muscle fiber recruitment. BFR has previously been shown to decrease tissue oxygenation in the working muscles (Ganesan G, 2014). It has further been shown that the use of a higher IRP will result in greater decreases in tissue oxygenation, regardless of FRP (Karabulut et al., 2014). Since type-1 muscle fibers require oxygen for the purposes of oxidative phosphorylation, the reduced levels of tissue oxygenation may have prevented type-1 fibers from being recruited during post-MVC, contributing to the decrease in total motor unit activation.

Yasuda et al. (2008) examined different levels of BFR on muscular activation of the biceps brachii during low intensity resistance exercise. In this study, subjects

performed four sets of elbow flexion exercises, 30 contractions during the first set followed by three sets of 15 contractions, at 20% 1RM. The study included four different FRP settings 0 mmHg, 98 mmHg, 121 mmHg, and 147 mmHg. Integrated electromyography readings were recorded during pre and post MVC as well as throughout the exercise. Analysis revealed that the FRP condition with 147 mmHg produced significantly greater decreases in motor unit activation from pre to post MVC. While Yasuda et al. (2008) does not mention the inclusion of IRP in the experimental design, it can be inferred that IRP was set to similar levels during each condition. If this is in fact the case, it can be concluded that the results do coincide with one another. The present study found that the lowest FRP with the highest IRP produced the greatest decrements in motor unit activation in pre to post MVC, however it has been previously suggested that IRP could be a greater indicator of fatigue response, which may be caused by a change in the levels peripheral and/or central mechanisms of fatigue (Karabulut et al., 2014; Karabulut & Perez, 2013).

Increased levels of motor unit activation in the female population from pre to post MVC were an unexpected outcome. It is possible that the physiological differences in body composition at the application site for BFR produced the recorded results. Greater levels of body fat at the inguinal crease may create a cushioning effect. Even though BFR may have caused production of metabolic byproducts and accumulation of deoxygenated hemoglobin, FRP and IRP may not be high enough to reach the level that can affect the amount of Type I fiber activation caused by the cushioning effect of subcutaneous fat in females. Therefore, the higher values of RMS in post-MVC could be explained by the activity of Type 1 fibers. However, it can be speculated that since Type 1 fibers primarily

depend on oxidative phosphorylation, they may not be fully functional to contribute to maximum force production during the strength test.

Increased motor unit activation throughout exercise is an expected outcome of any exercise that results in any levels of fatigue. As the muscles become fatigued during sub-maximal exercise, many motor units begin to decrease force production causing the working muscle to recruit more motor units in order to maintain activity. The greater levels of motor unit recruitment seen in the condition IRP65@FRP-20 may be a result of increased mechanical stress due to the use of a higher IRP. Karabulut et al. (2013) discovered similar results when examining unilateral leg extension exercises. The authors evaluated the effects of different IRP (40-45 mmHg and 60-65 mmHg) in combination of four sets of unilateral leg extension exercise (20% 1RM) on a dynamometer. EMG was recorded at the vastus lateralis. Analysis revealed that while both conditions resulted in increased motor unit activation, the condition with the greater IRP produced significantly higher values.

Yasuda et al. (2008) discovered that an FRP of 147 mmHg resulted in increases in motor unit activation during four sets (30 repetitions during set one and 15 repetitions in the following three sets) of elbow flexion exercise, however did not significantly differ compared to the control (FRP of 0 mmHg). These findings also coincide with those of the present study that there were increases in motor unit activation during exercise (even though there were no significant differences in the level of activation compared to the control). It should be highlighted that even though the level of motor unit activation during the BFR session was not significantly different from the control, the level of decrease in strength was greater following the BFR session. These results may also

indicate that BFR may affect other mechanisms such as central mechanism of fatigue, causing greater decreases in maximal force production.

The present study failed to find significant differences between the conditions for pre to post MDF during MVC. These results may indicate that the levels of fatigue experienced by the subjects may not have been enough to generate a post-exercise response. Karabulut et al. (2006) examined the effects of isometric contractions with and without the use of blood flow restriction. Study participants were subjected to 5 sets of 20 isometric contractions on two separate occasions. EMG activity was measured at the vastus lateralis. The study revealed a lack of significance between either condition and it was concluded that neither of the conditions produced a great enough fatigue effect.

Conclusion

The purpose of this study was to examine the acute effects of different blood flow restriction setting on 1) the level of blood lactate production, 2) neuromuscular activation (the changes in motor unit activation and median frequency), and 3) fatigue response during bilateral knee extension exercise. This study asked: Will an IRP of 45-50 mmHg (IRP50) or 60-65 mmHg (IRP65) have greater effect on blood lactate, neuromuscular, and fatigue during the different FRP protocols? Will a setting using the recommended FRP with an added 20 mmHg and an IRP50 mmHg have the same effects as the current method? Will a setting using the recommended FRP with a subtracted 20 mmHg and an IRP65 have the same effects as the current method? What changes in heart rate (HR), systolic and diastolic blood pressure (BP), discomfort (DF), and rate of perceived exertion (RPE) will be seen in the four conditions?

Research Hypothesis 1. An increase in blood lactate, differences in EMG activity, and decrease in MVC will be seen in both FRP conditions with an IRP65.

IRP65 showed no significant difference when compared to the Control (IRP50). IRP65@FRP-20 did not significantly differ in blood lactate effect compared to the control, but it did significantly increase EMG activity in both the vastus lateralis and rectus femoris; and it produced significantly greater decreases in pre to post MVC.

Research Hypothesis 2. Using the recommended FRP with an additional 20 mmHg and an IRP of 45-50 will produce similar results as the current protocol.

IRP50@FRP+20 did produce similar results as the Control in all measured variables except HR, where it was shown to significantly affect males more than females.

Research Hypothesis 3. Using the recommended FRP with a subtracted 20 mmHg and an IRP of 60-65 will produce similar results as the current protocol.

IRP65@FRP-20 produced similar results in blood pressure, blood lactate, HR, RPE, motor unit activation during the first set in both the vastus lateralis and rectus femoris, and MDF in during pre to post MVC in both the vastus lateralis and rectus femoris. However, IRP65@FRP-20 showed increased levels of discomfort/pain, it significantly decreased pre to post torque values in MVC, and produced significantly higher motor unit activation during sets 2-4 in both the vastus lateralis and rectus femoris.

Research Hypothesis 4. HR, BP, DF, and RPE will be greater in the condition using the recommended FRP setting with an IRP of 65 mmHg.

No, this hypothesis is not supported by the results of the present study. There were no significant differences between the condition IRP65 and the Control.

This study is novel in that it is the first study to test the current protocol while evaluating various IRP. The most significant findings presented in this study are the greater levels of fatigue produced during the condition IRP65@FRP-20, despite having a lower FRP. These findings indicate that IRP is an important variable affecting the levels of fatigue produced with BFR rather than FRP. This is further supported by the fact that the Control (IRP50) and the condition IRP50@FRP+20 produced similar results despite IRP50@FRP+20 having a greater FRP. The findings of the present study suggest that the current method may not be the most appropriate BFR protocol. Furthermore, the gender differences found in pre to post MVC show that the males experienced greater levels of fatigue in all conditions, including the control. This difference in fatigue suggests that females were not as affected by the conditions as males and therefore the control cannot be considered the most effective method of applying BFR. These findings further highlight the importance of IRP as a variable needed in the design of a valid BFR protocol.

It is this author's recommendation that future research be done in order to 1) develop a protocol that takes thigh circumference, body composition, and IRP into

account so that it can affect all users equally, and 2) create a technique that produces the desired effects of BFR while keeping discomfort/pain to a minimum.

The need for blood flow restriction training technique in clinics, gyms, and homes is evident. The incidences of diabetes, as well as heart and bone disease are increasing every year, and the novelty of an exercise technique that can produce the desired effects of exercise in half the time with less than half of the recommended intensity, may prove to be beneficial to the population as a whole.

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APPENDICES

Appendix A. Recruitment Flyer

UT BROWNSVILLE

PARTICIPANTS NEEDED

LEG EXTENSIONS WITH VASCULAR RESTRICTION RESEARCH

MALES AND FEMALES BETWEEN THE AGES OF 18 AND 50 YEARS

You are invited to participate in a research study at the Health and Human Performance Department at the University of Texas at Brownsville to assess the acute effects of different blood flow restriction setting on blood lactate accumulation, electromyography, and fatigue during bilateral knee extension exercise. Males and females between the ages of 18 and 50 years are encouraged to e-mail Guillermo Perez at Guillermo.Perez40@utb.edu, or Dr. Murat Karabulut at murat.karabulut@utb.edu (956-882-6509) (Total time required for participation in this study will amount to 5 separate days with approximately 45 minutes on each visits.)

PLEASE CONTACT:

Guillermo Perez	Dr. Murat Karabulut
956 882 6509	956 882 6509
E-mail: Guillermo.perez40@utb.edu	Email: murat.karabulut@utb.edu

Appendix B. Informed Consent

University of Texas at Brownsville/TSC
Institutional Review Board
Informed Consent to Participate in a Research Study

Project Title:	The Acute Effects of Different Blood Flow Restriction Settings on Blood Lactate, Electromyography, and Maximum Voluntary Contraction during Bilateral Knee Extensions
Principal Investigator:	Murat Karabulut
Co-Investigator:	Guillermo Perez
Department:	Health and Human Performance

You are invited to participate in a study as part of a research project of testing the acute effects of knee extension exercise, combined with vascular restriction, on blood byproduct, muscle activation, and strength. The research will be conducted by Dr. Murat Karabulut and Guillermo Perez at the research laboratory in the Department of Health and Human Performance.

You were selected as a possible participant because you are a healthy male or female between the ages of 18-50 who is able to perform exercise without risk to health. This study will be conducted on 40 participants (20 males and 20 females). Limitations for participation include high blood pressure, injuries to the lower extremities (e.g. knees and ankles), pregnancy, individuals younger than the age of 18 or older than the age of 50, or any health restrictions that may cause risk to health (determined by PAR-Q).

Purpose of the Research Study

The purpose of this study is to examine the acute effects of different blood flow restriction setting on 1) the level of blood lactate production, 2) neuromuscular activation (the changes in motor unit activation and median frequency), 3) fatigue response during bilateral knee extension exercise, and 4) to further examine whether bilateral knee extensions with blood flow restriction results in any changes in heart rate (HR), blood pressure (BP), discomfort (DF) and rate of perceived exertion (RPE).

Number of Participants

40 males and females, ages 18-50, will participate in this study.

Procedures

If you agree to be in this acute exercise study, you will be asked to do the following:

You will be required to visit the research lab in the Department of Health and Human Performance on 5 separate days for a total time commitment of approximately 5 hrs.

On the first visit, you will be required to read and sign an informed consent, a PAR-Q and health status questionnaire before any testing can occur. Following reading and signing the required paperwork, anthropometric measurements (height, weight), resting heart rate (RHR), and resting blood pressure (RBP) will be assessed and recorded. After completion of baseline testing and questionnaires, you will be tested for one repetition maximum for bilateral knee extension.

On the next 5 visits (separated by at least 48hr), you will perform the designed training. Prior to exercise your legs will be measured for circumference at the half way point between the

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Expiration Date 10/29/2014

greater trochanter and the lateral epicondyle. Your skin will be prepared and then fitted with EMG electrodes. Once electrodes have been secured you will be given a two minute warm up period on the treadmill at a walking pace you deem comfortable. You will then be tested for maximum voluntary contraction (MVC) on the Biodex dynamometer. Upon completion of MVC testing, you will be fitted with blood flow restriction (BFR) cuffs at the most proximal end of your legs and the cuff will be tightened to the random initial ristrictive pressure (IRP) set for each session. The cuff will then be inflated to the randomized final restricitve pressure (FRP). You will then begin the exercise regimen consisting of four sets of bilateral knee extensions at 20% of their 1RM. The first set will be for 30 repetitions followed by three sets of 15 repetitions. Blood lactate levels will be measured before exercise, mid-exercise, after the post-exercise MVC, 5 minutes post exercise, and 15 minutes post exercise. HR, RPE, discomfort, and BP will be measured before and after each set.

Length of Participation
You will be required to visit the research lab in the Department of Health and Human Performance on 5 separate days (approximately 50 minutes each session) for a total time commitment of approximately 5 hrs.

This study has the following risks:

You understand there are minimal risks to heathy individuals when performing any of the requirements for this project. However, even though these standard protocols have been approved at numerous other institutions and will be performed by qualified and trained personnel, you should be aware of the following:

<u>Physical Risks</u>

a) You may experience slight, temporary discomfort from the inflation of the BFR cuffs around the upper most portion of your extremities. During the session, the cuffs will be inflated for about 20-30 minuntes. A properly trained researcher will set the pressure and continuosly monitor you while the cuffs are inflated.
b) There is a possibility of temporary muscle soreness occurring 24-48 hours after the first visit which could be the result of beginning a new exercise protocol.
c) You may experience small amounts of pain during lactate collection. A lancet is used to make a small in puncure the skin in order to draw blood. Mild soreness may be present for several minutes following the puncure.

Benefits of being in the study are

There may be no direct benefit for participation; however participation in a strength training program may increase strength and muscle mass, and data will help researchers understand the effects of bilateral knee extension exercise combined with blood flow restriction on blood lactate, neuromuscular activation, and strength.

Injury
In case of injury or illness resulting from this study, emergency medical services will be contacted. However, you or your insurance company may be expected to pay the usual charge from this treatment. The University of Texas at Brownsville/TSC has set no funds to compensate you in the event of injury.

Confidentiality
In published reports, there will be no information included that will make it possible to identify you without your permission. Research records will be stored securely for 3 years after completion of the study and only approved researchers will have access to the records. There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include Murat Karabulut and the UTB Institutional Review Board.

Costs
There is no cost for participation.

Compensation
You will not be reimbursed for you time and participation in this study.

Rights
Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You can discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled.

Voluntary Nature of the Study
Participation in this study is voluntary. If you decline to participate, you will not be penalized or lose benefits or services unrelated to the study. If you decide to participate, you may decline to answer any question and may choose to withdraw at any time.

Waivers of Elements of Confidentiality
Your name will not be linked with your responses unless you specifically agree to be identified. Please select one of the following options

_____ I consent to being quoted directly.

_____ I do not consent to being quoted directly.

Contacts and Questions
You should feel free to ask questions now or at the any time during the study. If you have any questions, you can contact (Give your name and phone number and the name and phone number of any co-investigator. For research studies involving more than minimal risk, the home/night phone number(s) of the investigator(s) must be provided. For research studies conducted by students, the name and office telephone number of the student's advisor must be provided.) If you have any questions about the right of research subjects, contact the Chair of the UTB IRB-Human Subjects at (956) 882-8888 (Dr. Matthew Johnson) or the Research Integrity and Compliance Office at (956) 882-7731 (Lynne Depeault).

You are voluntarily making a decision whether or not to participate. Your signature indicates that, having read and understood the information provided above, you have decided to participate. You will be given a copy of this information to keep for your records. If you are not given a copy of this consent form, please request one.

Statement of Consent
I have read the above information. I have asked questions and have received satisfactory answers. I consent to participate in the study.

Signature Date

Appendix C. PAR-Q

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME ____

SIGNATURE ________________________________ DATE ____________________

SIGNATURE OF PARENT ________________________ WITNESS ____________________
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

 Supported by: Health Canada Santé Canada

continued on other side...

Appendix D. IRB Approval Letter

Research Integrity and Compliance

The University of Texas at Brownsville

Matthew Johnson, Ph.D.
IRB Chair

October 30, 2013

Dr. Murat Karabulut
Health and Human Performance
The University of Texas at Brownsville
One West University Blvd.
Brownsville, Texas 78520

Approval Type:
- ☐ Full Board Review
- ☒ Designated Member Review
- ☐ Continuing Review
- ☐ Change request/Modification/Amendment
- ☐ Exempt Category
- ☒ Expedited Category **2**

RE: IRB-HS Approval

Study Title: "The Acute Effects of Different Blood Flow Restriction Settings on Blood Lactate, Electromyography and Leg Strength during Bilateral Knee Extensions"

Approval Period:

Start Date: October 30, 2013

End Date: October 29, 2014

Protocol #: 2013-075-IRB

Dear Dr. Karabulut,

In accordance with Federal Regulations for review of research protocols, the Institutional Review Board – Human Subjects of The University of Texas at Brownsville has reviewed your study as requested.

The IRB-HS grants its approval for this project contingent on compliance with the following items. You may make as many copies of the stamped consent form as are necessary for your activity. All consent forms MUST bear the UTB IRB stamp indicating approval.

Responsibilities of the Principal Investigator also include:

- **Inform the IRB-HS in writing immediately of any emergent problems or proposed changes.**
- **Do not proceed with the research until any problems have been resolved and the IRB-HS have reviewed and approved any changes.**
- **Report any significant findings that become known in the course of the research that might affect the willingness of the subjects to take part.**
- **Protect the confidentiality of all personally identifiable information collected.**
- **Submit for review and approval by the IRB-HS all modifications to the protocol or consent form(s) prior to implementation of any change(s).**
- **Submit an activity/progress report regarding research activities to the IRB-HS on no less than an annual basis or as directed by the IRB-HS through the Continuing Review Form.**
- **Notify the IRB-HS when study has been completed through submission of a Project Completion Report.**

Should you have any questions or need any further information concerning this document please feel free to contact me at (956) 882-8888 or via email at Matthew.Johnson@utb.edu .

Sincerely yours,

Matthew Johnson, Ph.D.

Matthew Johnson, Ph.D.
IRB – Chair

80 Fort Brown • BRHP 2.210 • Brownsville, Texas 78520 • 956-882-7731 • research.compliance@utb.edu

Appendix E. RPE Borg's Scale

Borg Rating of Perceived Exertion

6 No exertion at all

7

Extremely light

8

9 Very light

10

11 Light

12

13 Somewhat hard

14

15 Hard (heavy)

16

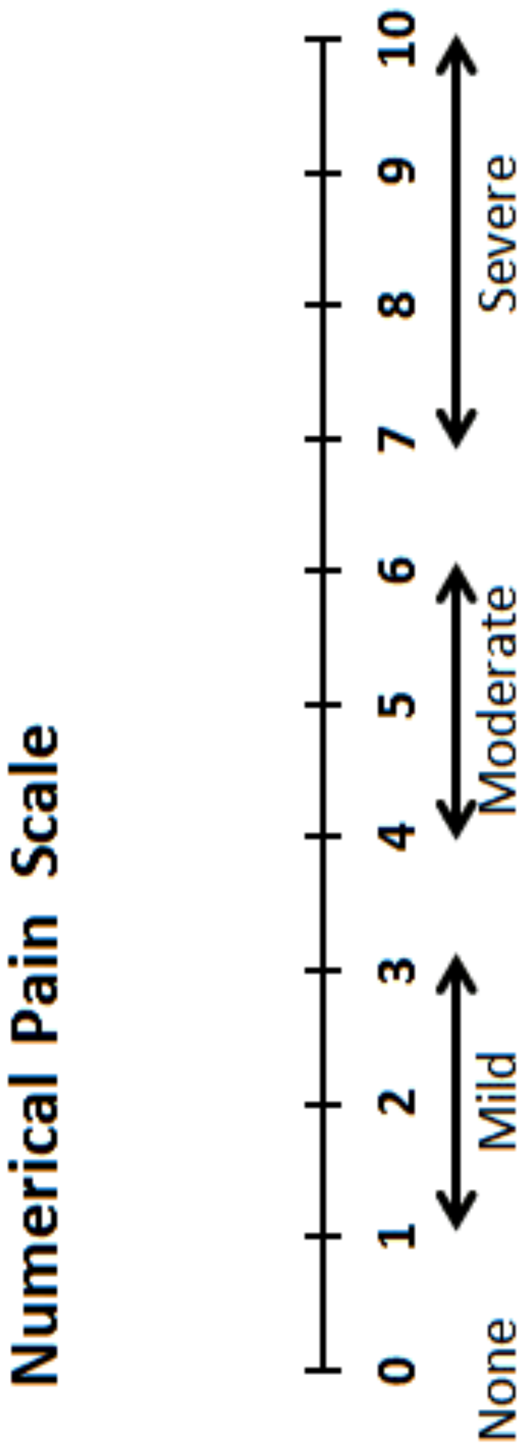
17 Very hard

18

19 Extremely hard

20 Maximal exertion

Appendix F. Visual Analogue Scale



Name ______________________ Age ________ FRP ________

Height ________ Weight ________ Phone ________________

1RM _________ 20% 1RM ________ Email ________________

Right Thigh Circumference ________ Right Femur Length ________

Left Thigh Circumference ________ Left Femur Length ________

Right VL Placement ________ Left VL Placement ________

Right RF Placement ________ Left RF Placement ________

45-50=120 51-55=150 56-59=180 >60=210

Leg Extension Machine Settings

Back Adjustment

Knee Angle Adjustment

Lower Limb Adjustment

BioDex Adjustments Subject ID ________

Back Adjustment

Seat Height

Knee Adjustment

Dynamometer Adjustment

Shin Pad Adjustment

Condition IRP50 IRP65 BFR+20 BFR-20 Date ________

Resting HR ______ Resting BP ______

MVC 1 ______ Pre MVC ________

MVC 2 ______

MVC 3 ______ Post MVC __________

Pre Exercise Values

Lactate	HR	BP	RPE	Pain

1st Set

HR	RPE	Pain

2nd Set

Lactate	HR	BP	RPE	Pain

3rd Set

HR	RPE	Pain

4th Set

Lactate	HR	BP	RPE	Pain

5 Minutes Post

Lactate	HR	BP	RPE	Pain

15 Minutes Post

Lactate	HR	BP	RPE	Pain