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AN EVALUATION OF THE INFLAMMATORY TIME COURSE RESPONSE FOLLOWING TRADITIONAL AND BLOOD FLOW RESTRICTION RESISTANCE EXERCISE MEASURED BY PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY

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AN EVALUATION OF THE INFLAMMATORY TIME COURSE RESPONSE FOLLOWING TRADITIONAL AND BLOOD FLOW RESTRICTION RESISTANCE EXERCISE MEASURED BY PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

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ABSTRACT

Acute muscle swelling following resistance exercise can increase mCSA when assessed by pQCT. This swelling does not reflect muscle hypertrophy, but rather a fluid shift in and around the exercised musculature that may stimulate protein synthesis. This creates a need for determining the inflammatory time course response following a bout of resistance exercise to pinpoint the earliest a pQCT scan can be performed to predict mCSA with minimal error. Furthermore, the degree of muscle swelling following traditional resistance exercise and blood flow restriction resistance exercise has yet to be compared. **PURPOSE:** The purpose of this investigation was to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout. **METHODS:** Ten men, aged 18-30 years, completed three experimental conditions in random order separated by at least one week: traditional resistance exercise [TRE], blood flow restriction resistance exercise [BFR], and a non-exercise control [CON]. For TRE subjects completed three sets of 8-10 repetitions on leg press, leg extension, and leg curl machines at an intensity of 75%-80% 1RM with two minutes of rest allowed between sets and exercises. For BFR, subjects wore five cm wide electronically controlled elastic pressure cuffs around their upper thighs during the exercise bout at a restrictive pressure of 160 mmHg. The same three exercises were completed during BFR but at an intensity of 20% 1RM. Subjects completed 30 repetitions for their first set, followed by three sets of 15 thereafter. Rest intervals were set at 30 seconds. For TRE, subjects remained in resting state, seated for approximately 20 minutes. Prior to exercise and 15 minutes, 75 minutes, 24h, 48h, 72h, and 96h after

exercise in TRE and BFR protocols, subjects underwent a pQCT scan and thigh circumference measurement. Additionally, blood samples were collected via finger prick prior to, immediately after, and 1h after exercise to assess plasma volume. Muscle thickness of the quadriceps and hamstring were determined prior to exercise and immediately, 30 min, and 1h after exercise via ultrasound. **RESULTS:** MTQ for BFR was significantly greater immediately post-exercise ($p < 0.001$) and 30 minutes post-exercise ($p = 0.001$) when compared to pre-exercise. MTQ for TRE was significantly greater immediately post-exercise ($p = 0.010$), 30 minutes post-exercise ($p = 0.007$), and 60 minutes post-exercise ($p = 0.019$) when compared to pre-exercise. MTQ for BFR was significantly greater than TRE immediately post-exercise ($p = 0.016$). MTH for BFR was significantly greater immediately post-exercise ($p = 0.036$) when compared to pre-exercise. PV%Δ significantly decreased from pre- to immediately post-exercise in both BFR ($p < 0.001$) and TRE ($p < 0.001$) conditions. In BFR, mCSA was significantly greater at 15 minutes post-exercise ($p < 0.001$) and 75 minutes post-exercise when compared to pre-exercise mCSA. In TRE, mCSA was significantly greater at 15 minutes post-exercise compared to pre-exercise mCSA. Thigh circumference was significantly greater at 15 minutes post-exercise in BFR ($p < 0.001$), TRE ($p = 0.002$), and CON ($p = 0.016$) compared to their respective pre-exercise thigh circumference values. Additionally, thigh circumference was significantly greater at 75 minutes post-exercise in BFR ($p = 0.032$) and TRE ($p = 0.007$) compared to their respective pre-exercise thigh circumference values. **CONCLUSION:** Muscle swelling returns to pre-exercise levels within 24 hours after completing a moderate to high

volume heavy-resistance exercise bout and a low-intensity coupled with blood flow restriction resistance exercise bout.

CHAPTER I

INTRODUCTION

Sarcopenia, meaning loss of flesh, is characterized by a loss of skeletal muscle mass and muscular strength that accompanies the ageing process. Physiological changes associated with a decline in muscle mass and strength include: 1) a decrease in type II muscle fibers (66), 2) a decrease in type II motor units (70), 3) impairments in protein synthesis (104), increased skeletal muscle proteolysis of contractile proteins (105), and a decrease in the production of the anabolic hormones testosterone (57), insulin-like growth factor 1 (IGF-1) (57), and growth hormone (GH) (89) . Additionally, exogenous factors such as an insufficient dietary protein intake (16) and a lack of or decrease in daily physical activity/exercise (40) can influence the rate of sacropenia. Collectively, the aforementioned events contribute to and reflect the 20% - 30% loss in skeletal muscle mass observed between the third and eighth decades of life (64). In the year 2000, health care costs directly related to sarcopenia were estimated at $18.5 billion, and a 10% reduction in the prevalence of sarcopenia would save approximately $1 billion annually (51). As a result, there is a need for the development of therapeutic and/or exercise interventions intended to reduce and possibly reverse the progression of sarcopenia not only improve the quality of life with ageing, but to diminish the astronomical health care costs associated with this condition.

Resistance exercise of adequate intensity (60% - 90% of the one-repetition maximum [1RM]) has been demonstrated to induce phenotypic outcomes that can improve muscle quality and function during biological ageing. For example, Yarasheski and colleagues (110) showed a discrepancy in resting fractional rates of muscle protein synthesis in older men and women (63-66 yrs) when compared to younger (24 yrs) counterparts. After two weeks of 5 d/wk moderate to high intensity resistance exercise consisting of exercises that load both the upper and lower body musculature, fractional muscle protein synthesis rates were similar amongst young and older subjects (110), suggesting that chronic resistance exercise may increase resting rates of muscle protein synthesis in older individuals. Pyka et al. (86) implemented a one year resistance training program comprised of 12 exercises at an intensity of 75% 1RM to 25 older (68 ± 1 yrs) men and women. Muscular strength increases ranged between 30% (hip extensors) and 90% (hip flexors), depending on the specific muscle group, and type I and type II muscle fiber cross-sectional area increased by 59% and 67%, respectively, after 30 weeks of training (86). These results indicate that older, healthy adults can participate in moderate to high-intensity resistance exercise and experience increases in muscular strength and hypertrophy.

Over the last two decades, a novel form of resistance exercise intended to reduce blood flow to the exercising muscles has gained popularity among the research community as a complementary exercise modality. Blood flow restriction (BFR) exercise, also known as KAATSU training, uses electronically controlled,

pneumatic air pressure cuffs (similar to blood pressure cuffs) placed around the most proximal segment of an exercising limb to reduce arterial blood flow and occlude venous return, resulting in venous pooling around the working muscles. BFR resistance exercise utilizes low to moderate exercise intensities (20% - 50% 1RM), and has been demonstrated to elicit muscular hypertrophic adaptations once thought to occur exclusively through the implementation of high-intensity resistance exercise (72). Consequently, BFR exercise has the potential to provide health-related benefits to certain populations that are unable to place heavy external loads on the body's musculature. Although incompletely understood at this time, several mechanisms contributing to the effectiveness of BFR training have been proposed: 1) acute (83, 87) and chronic (2) increases in anabolic hormone secretion, 2) increased motor unit recruitment/muscle activation during exercise (111), and 3) cell swelling (11), all of which have the potential to influence skeletal muscle protein synthesis and related signaling pathways (35). Gaining a better understanding of these mechanisms will result in more efficacious BFR exercise prescription. Of interest to the proposed investigation is the significance of cell swelling (muscle swelling) and the role it may serve in the remodeling/hypertrophic response to exercise.

Resistance exercise is also a widely accepted and utilized training modality for athletes, and it is often the focal point of many strength and conditioning programs. The evolution of research in the strength and conditioning field over the last 20 years has led to the development of year round resistance exercise practices.

Periodized resistance exercise programs are those that modify the acute training variables (exercise load, number of sets, number of repetitions, length of rest periods, and training frequency) throughout the year based on the current sport season (off-season, pre-season, in-season). By combining the principles of periodization with a year round resistance exercise program, athletes are able maximize the benefits of resistance exercise while reducing the chance of experiencing a reversal in training adaptations (58).

In terms of sport performance, resistance exercise has a profound effect on several key variables including muscular strength, muscular power/speed, muscle hypertrophy, and muscular endurance (54). The importance of muscle hypertrophy to athletic success cannot be understated, as muscle hypertrophy is closely related to other variables associated with athletic success, such as maximal strength (71) and muscular power during anaerobic (82) and aerobic (50) modes of exercise. Because of this, many athletes participate in resistance exercise programs to increase muscle mass with the intent of enhancing sport performance.

Since increasing muscle mass has positive implications on health, quality of life, and athletic performance, it is critical that scientists can accurately access muscle mass as well as track changes throughout ageing or across an exercise program. Some of the commonly used field based methods for tracking changes in muscle mass are not sensitive enough to detect small changes in muscle morphology, thus warranting the use of technologically advanced body composition equipment. Peripheral quantitative computed tomography (pQCT) has

recently become prevalent in the scientific community for analyzing the health and bone and soft tissues. In relation to muscle mass assessment, the pQCT scanner demonstrated to be a valid and reliable assessment of mCSA when compared with magnetic resonance imaging (MRI) (23), the current gold standard in body composition assessment. A potential limiting factor however, is that the pQCT scanner is unable to distinguish between an increase in muscle tissue and an increase intramuscular fluid when measuring muscle cross-sectional area (mCSA). Therefore, an increase in intramuscular fluid following a resistance exercise bout could artificially inflate/increase mCSA as determined by pQCT (supported by unpublished data), which would decrease the validity of the pQCT for predicting mCSA after one or more bouts of resistance exercise. This creates a need for determining the inflammatory time course response following a bout of resistance exercise to pinpoint the earliest a pQCT scan can be performed to predict mCSA with minimal error.

Purpose

The purpose of this investigation was to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout. Specifically, it was our objective to decipher the post-exercise time point at which increased intramuscular fluid, as a result of inflammation from resistance exercise, is returned to baseline (resting) levels.

Research Question

1. How long will muscle swelling remain above baseline levels after performing a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout?

Hypothesis

1. Muscle swelling will return to baseline levels within 96 hours after performing the traditional high-intensity resistance exercise bout and low-intensity combined with blood flow restriction resistance exercise bout.

Subquestions

1. Will there be a difference in the degree of muscle swelling between a traditional high-intensity resistance exercise bout and low-intensity with blood flow restriction resistance exercise bout?
2. Will there be differences in the degree of muscle thickness changes in response to a traditional high-intensity resistance exercise bout compared to a low-intensity with blood flow restriction resistance exercise bout?

Subhypotheses

1. Muscle swelling will be greater in response to the low-intensity with blood flow restriction resistance exercise bout compared to the traditional high-intensity resistance exercise bout.
2. Muscle thickness changes in response to the low-intensity with blood flow restriction resistance exercise bout will be greater than the muscle thickness

changes experienced after performing the traditional high-intensity resistance exercise bout.

Significance

By determining the inflammatory (muscle swelling) time course in response to a traditional bout of resistance exercise and low-intensity with blood flow restriction resistance exercise bout via pQCT, researchers interested in examining mCSA at multiple time points over the duration of a resistance exercise training study may know the earliest a pQCT measurement can be obtained following exercise to most accurately predict mCSA. Specifically, the pQCT scanner detects muscle swelling as an increase in mCSA (i.e., muscle hypertrophy), which decreases the validity of this body composition assessment technique when tracking muscle mass changes over time. Therefore, knowing when muscle swelling returns to baseline levels after resistance exercise will provide researchers a time frame when the pQCT can be utilized to assess mCSA with minimal error.

Several mechanisms have been proposed regarding the musculoskeletal adaptations that occur with blood flow restriction training, one of which is cell swelling. By examining the degree and duration of muscle swelling after performing a bout of resistance exercise with blood flow restriction as well as a traditional bout of resistance exercise, further insight concerning the effects of cell swelling on the hypertrophic response to exercise may be provided.

Assumptions

1. Subjects gave 100% effort during1-RM testing and during each of the exercise bouts.
2. Subjects did not participate in any exercise or physical activity within 72 hours prior to baseline testing.
3. Subjects did not participate in any exercise outside of what the study entailed while they participated in this investigation.
4. Subjects answered and filled out questionnaires and paperwork truthfully.

Delimitations

1. The findings of this study are only applicable to young men between the ages of 18-30 not participating in a structured resistance or aerobic exercise program that were from the Norman, Oklahoma and surrounding areas. However, it can be assumed that the findings of this investigation are applicable to individuals currently participating in a resistance exercise program, since the inflammatory and muscle damage response from exercise lessens with more training experience.
2. Subjects were free of any physical or medical conditions that could prevent them from exercising.

Limitations

1. Exercise outside of the study protocol was not strictly monitored. However, all subjects were asked to refrain from participating in any additional exercise.

2. Dietary intake may affect the inflammatory response from exercise. Therefore, 3-day food logs were collected from all subjects across each experimental condition.
3. A potential for the "repeated bout effect" existed. To limit this possibility, subjects performed each condition in random order.
4. Since normal fluctuation in bodily fluid shifts may affect the measurement precision of dependent variables, a control condition was used to account for any such fluctuation in fluid shifts.

Operational Definitions

Blood Flow Restriction training (BFR) - Exercise involving the use of electronically controlled pneumatic air pressure cuff placed around the most proximal portion of an extremity and inflated during exercise, also called KAATSU training.

Diastolic Blood Pressure (DBP) – The brachial diastolic blood pressure or the pressure blood exerts on the brachial arterial walls during diastole.

Dual Energy X-ray Absorptiometry (DXA) – Bone and soft tissue assessment till that uses two x-ray beams to generate a two-dimensional replica of the skeleton and surrounding tissues. DXA calculates the attenuation values of photons that travel from the x-ray tube through the measurement site.

Lancet Device – A hand-held device containing a lancet that is used collect a droplet of capillary blood via finger prick.

Mammalian Target of Rapamycin (mTOR) Pathway - Thought to be the critical pathway necessary to be activated in order for skeletal muscle protein synthesis to occur.

Mitogen Activated Protein Kinases (MAPK's) – A group of protein kinases that are activated by different cellular stimuli that play a role in skeletal muscle growth.

Muscle Cross-Sectional Area (mCSA) – The area of a cross section of muscle that is perpendicular to its longitudinal fiber arrangement.

Muscle Thickness – As measured by ultrasound, the distance from the adipose tissue-muscle interface to the muscle-bone interface.

One-Repetition Maximum (1-RM) – The maximal amount of weight that can be lifted through a full range of motion with proper form.

Peripheral Quantitative Computed Tomography (pQCT) – Bone and soft tissue measurement tool that generates a three-dimensional representation of a measurement site. pQCT measures the amount of radiation attenuated as it passes from the source through the measurement site, and it classifies tissue based on a density measurement.

Sarcopenia – The age related loss of muscle mass and strength.

Systolic Blood Pressure (SBP) – The brachial systolic blood pressure or the pressure blood exerts on the brachial arterial walls during systole.

Ultrasound – A medical imaging technique that uses ultrasonic waves to capture images of underlying tissues in the body.

Upper Leg Circumference – The distance between the lateral epicondyle and the greater trochanter is measured, and a mark will be made on the leg halfway between the two landmarks to serve as the circumference measurement site. A tape measure is then wrapped around the leg at this location to obtain a measurement.

CHAPTER II

LITERATURE REVIEW

Introduction

Resistance exercise provides many health and performance-related benefits to individuals that regularly participate in this form of exercise. Favorably altering body composition (i.e., increasing lean tissue mass and decreasing fat mass) is one such desired outcome that can positively influence quality of life as well as athletic performance. Thus, the ability to accurately assess body composition has become of upmost importance in the scientific community to not only determine an individual's body composition with precision, but to enable physiologists to properly prescribe diet and exercise and other therapeutic interventions that will improve athletic performance and promote a healthy lifestyle.

It is well established that biological ageing is associated with, or potentiates an array of physiological changes that adversely affect risk factors for chronic diseases/conditions, physical activity levels, and functionality during normal, everyday activities. One such physiological change is the age associated loss of skeletal muscle mass and muscular strength, known as sarcopenia, which can manifest itself as early as the third decade of life in some individuals. The development and progression of this condition during biological ageing is amplified if a sedentary lifestyle is pursed and certain lifestyle changes are not employed.

As one ages, the participation in weight bearing physical activity that places mechanical strain to the skeletal musculature becomes vital for maintaining muscle mass, muscle quality, and overall functionality (19). The American College of Sports Medicine and the American Heart Association recommend older adults to participate in resistance exercise of moderate (5-6) to vigorous (7-8) intensity on a 10 point scale (79). This level of exercise intensity may be feasible for many older adults. However, some older individuals may be physically unable to partake in heavy resistance exercise due to frailty, previous musculoskeletal injuries, and other health-related issues. Therefore, the need for an alternative mode of exercise that results in similar skeletal muscle adaptations would be of extreme value. BFR training may be a possible exercise substitute that can be performed by individuals who are unable to lift heavy loads while still placing a sufficient, anabolic stimulus to the working muscles. Despite the musculoskeletal benefits observed with BFR training, the mechanisms responsible for generating these phenotypic changes are unclear at this point in time. Gaining a better understanding of these mechanisms will result in more efficacious BFR exercise prescription.

This literature review discusses the structure, physiology and function of muscle, factors initiating and exercise prescription for muscle hypertrophy, blood flow restriction training, measurement techniques for assessing body composition, and current ways for determining inflammation in response to exercise.

Muscle Structure, Physiology, and Function

Skeletal muscle is the bodily tissue that facilitates biomechanical movement of the skeletal components. It is very unique in nature by possessing the ability to adapt to a wide variety of stimuli, repair itself when trauma or damage occurs, and serve as an energy reservoir for the body in extreme circumstances. Each muscle in the body contains a vascular network that provides the surrounding fibers with oxygen and nutrients to maintain a constant cellular environment during rest and times of stress, such as exercise.

Muscle is composed of a network of proteins organized into primary, secondary, tertiary, and quaternary structures. Muscle proteins are constructed by the linking of amino acids by polypeptide bonds. From an entire muscle belly to the skeletal muscle microstructure, layers of connective tissue surround and protect the various layers of muscle tissue. Each muscle cell (or muscle fiber) possesses both structural and functional units. The cytoskeleton maintains the shape and integrity of the muscle fiber during muscle contraction and relaxation. Protein complexes (i.e., dystrophin complex) anchored by the sarcolemma attach to the z-discs of the contractile elements and organelles within the cytosol of the muscle fiber to ensure their stability. The smallest functional unit of a muscle fiber is the sarcomere, which contains the two myofibrils responsible for muscle contraction, actin and myosin. Actin is a thin protein arranged in a double helix that is located on both ends of a sarcomere. The actin filament contains two additional proteins, troponin and tropomyosin, that play integral parts during muscle contraction.

Myosin is the thicker of the two myofilaments and it is distinguished by the presence of two globular heads. In a resting state, the actin and myosin filaments partially overlap with one another and are not connected in any way. During contraction, the myosin globular heads attach to the actin filaments and pull them toward the center of the sarcomere, thereby shortening the distance between the z-discs located on either of the sarcomere. Sarcomeres are aligned in adjacent series throughout a muscle belly, and as the contraction process takes place, force is transmitted from the sarcomeres through the structural proteins and layers of connective tissue within the muscle to the myotendinous junction at the ends of the muscle belly where the bone is attached and movement is generated.

The myosin heavy chain protein can exist as one of two primary isoforms, each of which is differentiated by structural, metabolic, and contractile properties. Thus, it is the myosin proteins contained within a muscle fiber that dictates the fiber's properties and overall function. Type I muscle fibers are characterized by small fiber diameters, high mitochondrial and capillary densities, high resistance to fatigue during muscular work, and low force production capabilities. Type II muscle fibers have larger fiber diameters, lower mitochondrial and capillary densities, low resistance to fatigue, and high force production capabilities. Muscle fibers are therefore recruited to perform muscular work based on the demands of the activity. During light to moderate intensity exercise requiring aerobic metabolism, type I muscle fibers are primarily utilized, whereas high intensity exercise relying on the phosphocreatine and/or glycolytic energy systems utilizes

type II muscle fibers in addition to type I fibers. The architecture and functional capabilities of muscle fibers can be manipulated by external stimuli such as exercise or dieting habits. In particular, aerobic exercise can improve the oxygen consumption and utilization capacity of muscle fibers (46), and resistance exercise can increase the force production capabilities and overall size (i.e., muscle hypertrophy) of muscle fibers (86). These phenotypic changes are beneficial for athletic success at recreational and elite levels alike and the maintenance of functionality during biological ageing.

Factors Initiating Skeletal Muscle Hypertrophy

Mechanical tension, that is the result of force generation and induced stretch, is considered a primary contributor of skeletal muscle growth. The buildup of tension that accompanies resistance exercise disturbs the structural integrity of muscle fibers causing a cascade of events initiated by mechanosensors within the affected muscle tissue. The resulting signals are mediated through the Akt/mTOR protein synthesis pathway via direct or indirect activation (47). The involvement of the Akt/mTOR (mammalian target of rapamycin) pathway in muscle growth and cell cycle regulation is well-studied, but its role in modulating the effects of resistance exercise (i.e. mechanical tension) has more recently been documented. Barr and Esser (6) were the first to demonstrate that the extent of p706K (a downstream protein kinase of mTOR) phosphorylation after an initial bout of resistance exercise is highly correlated with the amount of muscle hypertrophy in rodents after six weeks of resistance exercise training. Similarly, Terzis et al. (101)

saw the same correlation in human subjects after 14 weeks of resistance training. Further support for the Akt/mTOR pathway in having a primary function in anabolic signaling in response to mechanical tension comes from a series of studies by Kubica and colleagues (62, 63). In these investigations, rats performed a bout of lower-body resistance exercise, and it was determined that protein translation (as measured by monosomal to polysomal RNA changes) was increased in the gastrocnemius 16 hours following the bout. When rapamycin, an inhibitor of mTOR, was administered to the rats two hours prior to exercise, protein translation measured after the exercise bout was inhibited. This collection of data shows that mTOR phosphorlyation, and downstream signaling leading to protein translation, are highly responsive to mechanical tension, and mTOR inhibition results in a decrease in protein synthesis.

Exercise training can produce localized trauma and damage to skeletal muscle that is hypothesized to influence tissue remodeling and hypertrophy (30). Muscle damage, specifically microtears in the membranes of myofibers, is suggested to commence the cascade of inflammatory events necessary for restoring a homeostatic environment. The perception of damage by the body causes neutrophils to migrate to the injured area. Macrophages and lymphocytes are then signaled to the area to clear debris and produce cytokines that activate immune cells which produce growth factors, including IGF-1, involved with the repair of damaged muscle tissue (69). IGF-1 is a very diverse hormone that exerts its anabolic effects in several distinct ways. Mechano growth factor (MGF), a splice

variant of IGF-1, activates satellite cells to undergo proliferation and differentiation (45), and another splice variant, IGF-1Ea, is thought to aid the fusion of satellite cells with mature muscle fibers (103). The myonuclear domain theory states that a nucleus within a mature myofiber controls mRNA production for a fixed sarcoplasmic volume, and any increase in fiber size necessitates additional myonuclei to help manage the increase in cell volume. Thus, satellite cells, through IGF-1 stimulation, donate themselves to the myogenic lineage to aid in tissue repair and hypertrophy (103). IGF-1 can also directly stimulate muscle protein synthesis (9), and its anabolic effects have been observed for up to 72 hours following a bout of damaging resistance exercise in humans (75).

A large body of evidence supports exercise-induced metabolic stress as a regulator of muscle hypertrophy. This stress occurs as the result of exercise utilizing the glycolytic energy system to derive adenosine triphosphate (ATP). An exercise stimulus (moderate to heavy resistance) such as this causes a local buildup of lactate, inorganic phosphates, hydrogen ions, free creatine, and other metabolites (102). Performing exercise under ischemic conditions with light exercise loads also induces the accumulation of metabolic byproducts (98). Metabolite buildup caused by exercise is proposed to alter anabolic hormonal concentrations, cell swelling, free radical production, and the recruitment patterns of motor units, all of which may trigger the inflammatory cascade previously discussed and hypertrophy of skeletal muscle (38, 72).

Exercise Prescription for Muscle Hypertrophy

Resistance exercise program design, specifically the manipulation of acute training variables (intensity, volume, rest intervals), exercise selection, and program length, dictate the acute inflammatory response and chronic adaptations observed within skeletal muscle.

Exercise intensity (i.e. load) may very well be the primary variable for stimulating muscle hypertrophy. It is generally believed that the use of light loads does not provide enough of a stimulus the working muscles to initiate muscle growth, whereas the use of moderate (6-12 1-RM loads) and heavy (1-5 1-RM loads) loads have demonstrated elicit considerable gains in muscle size (17). With the exception of blood flow restriction training, exercising with loads less than 65% of 1-RM minimally affect the physiological processes that drive hypertrophy (74). It is currently believed that moderate loads optimize the hypertrophic response to resistance exercise, as such loads have shown to increase metabolic byproducts (102) that influence anabolic processes leading to muscle growth (59).

Training volume, which is the product of total repetitions, sets, and load performed during a training session, is another contributing factor regulating muscle hypertrophy. Resistance exercise that incorporates high volume, multiple set regimens are consistently shown to increase hypertrophy to a greater extent than single set protocols (60). Anabolic hormones (testosterone and growth hormone) thought to play a role in muscle hypertrophy are elevated more so during multiple set resistance exercise protocols when compared to single set protocols (22, 56).

Rest intervals, or the time taken between working sets, can also influence the hypertrophic response to resistance exercise, and can be categorized as short (30 seconds or less), moderate (60-90 seconds), or long (3 minutes or more). Short rest intervals can maximize the metabolic response to resistance exercise (37), but muscular strength decrements are observed over multiple sets which can offset the positive effects of minimal rest. Long rest intervals maximize the mechanical tension experienced during a training session due to the ability to complete more repetitions across multiple sets at moderate to heavy exercise loads (26). However, the hypertrophic benefits of metabolic stress resulting from short rest intervals are compromised when rest periods are extended to several minutes, thus leading to the belief that long rest periods are not favorable for increasing muscle size.

Therefore, moderate rest intervals seem to be an effective solution for obtaining the unique benefits of both short and long rest periods. Moderate rest periods allow individuals to regain the majority of their strength capacity between sets (97) as well as maintain a higher percentage of the 1-RM over the course of a training session (55).

While manipulation of acute resistance exercise training variables is important for regulating the outcomes of a training program, exercise selection must also be considered. It is advantageous to include both multi- and single-joint exercises into a training program to maximize the hypertrophic response. Multi-joint exercise recruit two or more muscle groups during mechanical work, which results in the recruitment of more motor units and subsequently additional muscle

fibers during multi-joint exercises when compared to single-joint exercises. Contrarily, single-joint exercises allow for enhanced focus toward a particular muscle group that may be utilized to a lesser extent when recruited during a multi-joint exercise. In this instance, single-joint exercise may be used to elicit different motor unit recruitment patterns (5) in an attempt to amplify the overall hypertrophic response.

The cohort of variables mentioned up to this point can singularly or collectively impact the amount of time necessary for muscle hypertrophy to occur. It is currently thought and preached that a sufficient number of exercise bouts over the course of several weeks to months must be completed before muscle hypertrophy is observed (76, 96). This belief is predicated on the notion that neural changes are thought to precede hypertrophic changes during the course of a resistance exercise program. However, there is no evidence to suggest that muscle hypertrophy is not simultaneously occurring with neurological changes. In fact, recent evidence suggests that muscle hypertrophy can occur earlier than once thought utilizing both traditional resistance exercise (28) and a novel form of resistance exercise coupled with blood flow restriction (2).

Blood Flow Restriction Training

Over the last two decades, a novel form of resistance exercise intended to reduce blood flow to the exercising muscles has gained popularity among the research community as a complementary exercise modality. Blood flow restriction (BFR) exercise, also known as KAATSU training, uses electronically controlled,

pneumatic air pressure cuffs (similar to blood pressure cuffs) placed around the most proximal segment of an exercising limb to reduce arterial blood flow and occlude venous return, resulting in venous pooling around the working muscles. BFR resistance exercise utilizes low to moderate exercise intensities (20% - 50% 1RM), and has been demonstrated to elicit muscular hypertrophic adaptations once thought to occur exclusively through the implementation of high-intensity resistance exercise (72). Consequently, BFR exercise has the potential to provide health-related benefits to certain populations that are unable to place heavy external loads on the body's musculature. Although incompletely understood at this time, several mechanisms contributing to the effectiveness of BFR training have been proposed: 1) acute (83, 87) and chronic (2) increases in anabolic hormone secretion, 2) increased motor unit recruitment/muscle activation during exercise (111), and 3) cell swelling (11), all of which have the potential to influence skeletal muscle protein synthesis and related signaling pathways (35). These mechanisms are described in detail below.

The proposed mechanism currently with the most supportive evidence is the endocrine response to BFR training. Takarada and colleagues (100) showed nearly a 300-fold increase in growth hormone (GH) concentrations 15 minutes after completing five sets of bilateral leg extension to failure at 20% 1-RM coupled with thigh compression (214 mmHg). Likewise, Pierce et al. (83) saw a marked increase in GH levels at 20 minutes and up to 50 minutes following leg extension exercise at approximately 20% maximal voluntary contraction with BFR at a mean

pressure of 280 mmHg. Furthermore, BFR exercise resulted in significantly greater GH levels than the ischemia without BFR condition (5 minutes inflate and 3 minutes off, 5 total sets) starting at 30 minutes post-exercise and lasting until 80 minutes post-exercise. Reeves et al. (87) compared moderate intensity (70% 1-RM) resistance exercise (MR) versus low-intensity resistance exercise coupled with BFR to determine the anabolic hormone response. Subjects completed 3 sets of single-arm bicep curls and single-leg calf extensions with and without BFR on separate occasions. Growth hormone concentrations in the BFR condition measured immediately following exercise were significantly higher than respective pre-exercise levels and post-exercise GH levels in MR. These results indicate that BFR coupled with resistance exercise has the potential to increase the anabolic hormone response to an exercise bout that is traditionally thought to have no effect on hormone concentrations due to the small amount of muscle involvement. Together, these studies, and others (49, 99) reveal that BFR exercise can acutely increase GH, which is thought to play a significant role in muscle protein synthesis (15, 59).

Other researchers have analyzed the effects of BFR training on acute and chronic insulin-like growth factor-1 (IGF-1) changes. Takano et al. (99) observed a 12% post-exercise increase in IGF-1 levels after completing 4 sets of bilateral leg extensions at 20% 1-RM with BFR, while others (35) have reported no such increase in IGF-1 after a similar BFR exercise protocol. Abe and colleagues (2) employed a two week BFR exercise program consisting of twice daily squat and

leg curl exercise (3 sets at 20% 1-RM) performed by young men. From baseline to post-training, resting serum IGF-1 levels increased 24%, which is a similar increase seen in IGF-1 levels after high-intensity resistance exercise programs (13, 73). These findings suggest that low-intensity BFR resistance exercise may acutely and chronically increase circulating IGF-1 levels, which has positive implications on protein synthesis pathways and satellite cell activity (95).

Other research has focused on investigating muscle activation/recruitment during BFR resistance exercise. Yasuda et al. (111) examined muscle fiber activation during 4 sets of bicep curls at 20% 1-RM on four separate occasions, each using a different occlusive pressure. Results displayed increased muscle activation with each subsequent set, regardless of occlusive pressure, and greater muscle activation during the overall exercise bout as occlusive pressure was increased (0 mmHg vs. 98 mmHg vs. 121 mmHg vs. 147 mmHg). In another study, Krustrup et al. (61) determined the amount of ATP and creatine phosphate (CP) depletion in type I and type II muscle fibers in response to low-intensity, low-intensity with BFR, and high-intensity resistance exercise using the knee extensors. The authors found comparable decreases in ATP and CP concentrations in both muscle fiber types in the low-intensity BFR and high-intensity conditions. No such changes were seen in the low-intensity resistance exercise condition. Because the extent of ATP and CP depletion during exercise resembles the involvement of anaerobic metabolism and type II muscle fiber recruitment, it can be concluded that low-intensity BFR exercise activates a similar proportion of anaerobic muscle

fibers as high-intensity resistance exercise. Results from other studies (61, 112) showing increases in metabolic byproducts of anaerobic metabolism after performing BFR exercise support the above findings illustrating a shift from aerobic to anaerobic metabolism when occlusion is added to low-intensity exercise.

The last proposed mechanism relevant to the hypertrophic response to BFR exercise is muscle or cell swelling. It is well established that exercise, particularly forms that contain eccentric muscle action, causes swelling within the utilized muscle tissue (18, 81, 85) due to alterations in extra- and intracellular water concentration (93). Cell swelling seems to be potentiated by exercise regimens that tax the glycolytic energy system, as lactate accumulation has been suggested to regulate fluid shifts within skeletal muscle (32, 94). Interestingly, Haussinger and colleagues (41) showed a close relationship between intracellular water content and the extent of proteolysis in cells of isolated perfused rat liver. Specifically, they noticed as intracellular water increased, proteolysis within liver cells decreased, thereby resembling an anti-catabolic effect. Furthermore, when cellular dehydration was induced, muscle proteolysis increased. A more recent study (11) analyzed whole body protein turnover in humans under hypo- and hyperosmolar conditions previously shown to cause cell swelling and cell shrinkage, respectively (25). Leucine release from endogenous proteins (representing protein breakdown) and leucine oxidation (representing irreversible catabolism) were diminished in the hypoosmolar condition even though markers of protein synthesis were not affected. The authors concluded that overall protein balance was improved during the

hypoosmolar state. The findings from these studies suggest that cell swelling may have an anti-catabolic effect within skeletal muscle. Because low-intensity BFR exercise acutely increases lactate (35) and muscle swelling for 24 to 48 hours (106), the anti-catabolic effect of cell swelling described above may play a role in the hypertrophic response seen in BFR resistance (2) and aerobic (3) exercise programs. However, it has yet to be determined if BFR resistance exercise induces greater muscle swelling than a traditional resistance exercise bout.

Measurement Techniques for Assessing Body Composition

Several laboratory measurement techniques/devices, such as underwater weighing, dual energy x-ray absorptiometry (DXA), bioelectric impedance spectroscopy (BIS), air displacement plethysmography, and skin fold measurements are commonly used and relied upon to assess body composition for research purposes. When tracking changes in body composition where repeated measurements over time are required, some of these techniques may be less sensitive to change (78) than the current technology, especially since recent evidence demonstrates that muscle hypertrophy can occur much earlier during the course of a resistance exercise program than once thought (2, 76).

pQCT has become a popular alternative to the DXA for measuring clinical markers related to bone health and osteoporosis. Computed tomography is based on the attenuation of x-ray beams as it passes through an object, and the resulting images denote volumetric tissue slice with which measurements are taken from. pQCT is exclusively used to measure appendicular bone characteristics (7, 29, 39,

109) whereby cortical and trabecular bone can be independently evaluated. While pQCT is conventionally used for examining bone health indices, researchers have begun to use its technology for assessing muscle cross-sectional area of the upper (80) and lower extremeties (65). When compared with the magnetic resonance imaging (MRI) scanner, Cramer and colleagues (23) demonstrated that the pQCT offers a valid and reliable assessment of mCSA. Sherk et al. (91) provides further supportive evidence, as this research group showed that pQCT scan images at the mid-thigh displayed a 3.1% difference than mid-thigh MRI images when a strong measurement filter was used. Thus, the pQCT may be a highly sensitive technique that can be utilized for tracking changes in bone and muscle tissue parameters alike.

A potential limiting factor of the pQCT is that it is unable to distinguish between an increase in muscle tissue and an increase intramuscular fluid when measuring muscle cross-sectional area (mCSA). Therefore, an increase in intramuscular fluid following a resistance exercise bout could artificially inflate/increase mCSA as determined by pQCT (supported by unpublished data), which would decrease the validity of the pQCT for predicting mCSA after one or more bouts of resistance exercise. This creates a need for determining the inflammatory time course response following a bout of resistance exercise to pinpoint the earliest a pQCT scan can be performed to predict mCSA with minimal error.

Assessing Inflammation in Response to Exercise

Over the last two decades, numerous studies have been conducted examining the inflammatory/damage response following various modes of exercise. Because eccentric muscle action exacerbates the amount of muscle damage occurring from exercise, researchers will typically use an exercise bout focusing on eccentric action if markers of muscle damage are of interest (20). Such exercise bouts may involve isolated muscle groups (i.e., elbow flexors or knee extensors), a high volume of work without rest (50+ eccentric contractions), performing negatives with greater than 100% 1-RM, or downhill walking. Little research assessing muscle damage has used an exercise bout that is practical in nature , specifically one that a recreational weightlifter would perform during a resistance exercise session to induce muscle hypertrophy (31).

Direct assessment of muscle damage is a difficult task due to the limited number and accessibility of techniques, for instance, analyzing tissue from a muscle biopsy sample. However, a small muscle sample may not be an accurate representation of the damage within an entire muscle belly. Therefore, the majority of researchers interested in quantifying muscle damage do so via indirect measures. Warren and colleagues (108) determined that the three most commonly used indices of muscle damage are subjective muscle soreness scales, analyzing proteins in the blood indicative of muscle damage, and by evaluating maximal voluntary contractions (MVC) before and after a damaging exercise bout. Measuring the amount of swelling within the exercised muscle(s) is another commonly used

method for ascertaining damage or inflammation. Using a pQCT scanner, our research group detected an increase in muscle cross-sectional area (mCSA) after performing a high volume lower-body resistance exercise bout and after wearing pneumatic pressure cuffs around the most proximal portion of each leg for 10 minutes without performing exercise (unpublished data). Both of these pilot tests imply that the pQCT scanner is sensitive enough to detect muscle swelling acutely after resistance exercise or following blood flow occlusion without exercise. As a result, the pQCT scanner may be a feasible tool to accurately measure inflammation/muscle swelling following exercise.

Summary

From the body of literature, it appears that few studies have investigated the inflammatory time course response following a traditional bout of resistance exercise via pQCT. Furthermore, the underlying mechanisms causing skeletal muscle hypertrophy in response to BFR training are incompletely understood. The potential for cell swelling to augment muscle hypertrophy still exists, and it has yet to be determined if the amount or duration of muscle/cell swelling in response to BFR training is different from that of a traditional resistance exercise bout.

CHAPTER III

METHODOLOGY

The purpose of this investigation was to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout. Specifically, it was our objective to decipher the post-exercise time point at which increased intramuscular fluid, as a result of inflammation from resistance exercise, is returned to baseline (resting) levels.

Subjects

Ten recreationally active, but non-resistance trained men between the ages of 18-30 from the University of Oklahoma and city of Norman, OK and its surrounding area were recruited to participate in this study. Recruiting was carried out via posting university approved fliers in appropriate posting areas on the University of Oklahoma Norman campus and visiting classrooms within the Department of Health and Exercise Science and other academic departments on the Norman campus.

Inclusion Criteria

1. Male between the ages of 18-30 years.
2. Not currently participating in a structured resistance exercise program (within the last 3 months).
3. Not currently participating in moderate to high intensity aerobic exercise more than 2 days per week within the last 3 months.

4. Free of orthopedic problems/injuries limiting exercise ability.

Exclusion Criteria

1. Outside the age range of 18-30 years.
2. Female.
3. Regular use of tobacco products (cigarettes, cigars, chew/snuff etc.).
4. Structured resistance exercise within the past 3 months or moderate to high intensity aerobic exercise more than 2 days per week within the last 3 months.
5. Taking medications known to affect bone metabolism such as heparin, thyroid, cyclosporine, or glucocorticoids.
6. Taking any prescription medication.
7. Having a history of cardiovascular disease or thromboembolic disease.
8. Are currently students in Dr. Michael Bemben's class(es).
9. Are identified as a moderate-to-high risk individual as described by the American College of Sports Medicine:
    a. At least two of the following: Father or brother, or mother or sister that has had a sudden death before 55 or 65 years of age, respectively; Is a current cigarette smoker or has quit smoking within the previous 6 months; Is on hypertensive medication or has a confirmed systolic or diastolic blood pressure $\geq$ 140 or 90 mmHg, respectively; Is on lipid lowering medication or has a total

cholesterol level ≥200 mg/dL; Has a confirmed fasting blood glucose of ≥100 mg/dL; is clinically obese.

10. Having more than one risk factor for thromboembolisms:
    a. Classified as Obese based on a Body Mass Index of $\geq$ 30 (kg/m^2).
    b. Diagnosed Crohn's or Inflammatory Bowel Disease.
    c. Past fracture of a hip, pelvis, or femur.
    d. Major Surgery within the last 6 months.
    e. Varicose veins.
    f. Family history of Deep Vein Thrombosis or Pulmonary Embolism.
11. Having a Body Mass Index of $\geq$ 30 (kg/m^2).

Research Design

The current study was a crossover design that was conducted at the University of Oklahoma's Neuromuscular and Bone Density laboratories located in the Department of Health and Exercise Science. The total duration of the study was approximately four months. However, each subject was only required to visit the laboratory 16 times over the course of four weeks. Ten men aged 18-30 years from the University of Oklahoma and city of Norman, OK and its surrounding area were screened (questionnaires) and consented prior to participation. Subjects were scheduled for a second visit to undergo height and weight assessments, a DXA scan, and strength testing on exercise equipment. Approximately one week after strength testing, subjects were randomly assigned to complete one of the experimental conditions (traditional resistance exercise [TRE], blood flow restriction resistance exercise [BFR], or control [CON]). Subjects then, in random

order, completed the additional two study conditions under investigation during weeks three and four.

Exercise Protocols

For TRE, subjects warmed-up for five minutes on a stationary bike prior to performing resistance exercise. Subjects then completed three sets of 8-10 repetitions on a supine leg press machine at an intensity of 75%-80% 1RM with two minutes of rest allowed between sets and exercises. If subjects were able to complete more than 10 repetitions, or cannot complete a minimum of eight repetitions for a given set, the weight was adjusted accordingly so that the subject would reach muscular failure (point at which no more repetitions can be completed through a full range of motion with proper form) between 8-10 repetitions. Subjects completed the same routine for the leg extension and leg curl exercises as described above. For BFR, subjects warmed-up for five minutes on a stationary bike prior to performing resistance exercise. Before the session began, subjects were seated and 5 cm, elastic pressure cuffs (Kaatsu-Mini, Tokyo, Japan) were placed on the upper most portions of their legs and inflated to 120 mmHg for 30 seconds and then deflated. This process was repeated by adding 10 mmHg of pressure until the target exercise pressure was reached (160 mmHg). This process of slowly reaching the exercise pressure took approximately five minutes. Subjects then completed one set of 30 repetitions, and three sets of 15 repetitions at an exercise intensity of 20% 1RM on the supine leg press, leg extension, and leg curl exercise machines while wearing the inflated cuffs. Thirty (30) seconds of rest was

allowed between sets and exercises. If subjects were unable to complete the desired number of repetitions for a given set, the weight was not changed and subjects were instructed to complete as many repetitions as possible with proper form for subsequent sets. The cuffs were deflated and removed after obtaining immediate post-exercise ultrasound measurements. During the CON trial, subjects did not complete any exercise and remained in a resting state (seated position). Prior to exercise and 15 minutes, 75 minutes, 24h, 48h, 72h, and 96h after exercise in TRE and BFR protocols, subjects underwent a pQCT scan and thigh circumference measurement. Additionally, blood samples were collected via finger prick prior to, immediately after, and 1h after exercise to assess plasma volume. Muscle thickness of the quadriceps and hamstring were determined prior to exercise and immediately, 30 min, and 1h after exercise via ultrasound. During the control condition, subjects completed all tests on the exact time course as the exercise conditions. Subjects recorded all nutrient intake the day prior to, the day of, and the day after each experimental condition.

Questionnaires

All potential subjects first completed an informed consent form. Once consent had been granted, each potential subject filled out a health status questionnaire, Physical Activity Readiness Questionnaire (PAR-Q), and a research privacy form. The health status questionnaire and PAR-Q were used to determine any additional exclusion criteria, and the research privacy form was used to inform subjects about data confidentiality.

Body Composition

1. Dual Energy X-Ray Absorptiometry (DXA)

Subjects completed a total body DXA (GE Medical Systems, Lunar Prodigy encore software version 10.50.086, Madison, WI) scan to evaluate percent body fat. Quality assurance testing (QA) was performed each day prior to testing to ensure the DXA was functioning properly. The QA for the DXA involved scanning a calibration block of known density along with a series of mechanical functioning tests, which the DXA software performed automatically. All tests had to pass in order for the overall QA to pass.

Prior to each DXA scan, subjects were instructed to refrain from wearing metal-containing clothing and jewelry. Subjects were asked to lie supine on the DXA table centered within the scanning area with hands placed at the side of the hips/legs in a prone position. Velcro straps were wrapped around the ankles and knees so that the subject did not have to hold his/her feet together for the duration of the scan. Scan speed for the total body scan was determined by the thickness of the subject at the naval (Thick = > 25 cm; Standard = 13-25 cm, and Thin = < 13 cm). All scans were performed by a single, trained technician. One scan was performed on each subject during their initial visit.

2. peripheral Quantitative Computed Tomography (pQCT)

Subjects had mCSA at 50% site of the femur assessed via a peripheral quantitative computed tomography scanner, XCT 3000 with software version 6.00 (Stratec Medizintechnik GmbH, Pforzheim, Germany) by a trained pQCT

technician. Quality assurances assessments were completed each day prior to testing which consisted of performing a scout view scan followed by additional scans on a phantom (cone and cortical) of known densities. The densities must have been within 99% accuracy in order for the quality assurance test to pass. Scans were performed on each subject with a voxel size of 0.4 mm, slice thickness of 2.2 mm, and a scan speed of 20 mm/sec. Subjects were positioned with their right upper leg centered in the gantry of the pQCT machine. A scout scan was then performed to visualize and mark a reference line at the distal end of the femur. A tomographic slice at the 50% femur site was then taken in accordance to the reference line (femur length was assessed prior to the scan as the distance from the lateral epicondyle to the greater trochanter). Analysis of CSA slices was executed by drawing a region of interest around the total CSA scan, then using custom macros created within the XCT software and the median smoothing filter F01F06U01. Analysis thresholds were selected to separate 1) fat from bone and muscle and 2) bone (cortical and marrow) from muscle. Thresholds used for mCSA analysis at the 50% femur site are Contmode 31, Peelmode 2, Threshold1 40, Threshold2 40, Cortmode 4, Threshcrt1 710, and Threshcrt2 40. All scans were performed by a single, trained technician, and in vivo precision for determining mCSA in the Bone Density Research Laboratory is 0.9%. Scans were performed prior to each condition and 15 minutes, 75 minutes, 24h, 48h, 72h, and 96h after each condition.

3. Thigh circumference

Each subject had their right, upper leg circumference assessed using a tape measure at the 50% femur site. The distance between the lateral epicondyle and the greater was measured prior to the initial pQCT scan, and a mark was made on the leg halfway between the two landmarks as determined by the pQCT machine to serve as the circumference measurement site. Thigh circumference was measured to the nearest tenth of a centimeter and, two measurements within two millimeters of each other were obtained at each time point and averaged. This assessment was completed immediately before/after each pQCT scan. In vivo precision for determining thigh circumference in the Neuromuscular Laboratory is 0.28% at the 50% femur site.

4. Ultrasound

Subjects were also measured both anteriorly and posteriorly for muscle tissue thickness at the 50% femur site of the right leg using a Fukuda Denshii 4500 ultrasound machine. A 5-MHz scanning head was covered with transmission gel and subsequently placed over the sites of measurement (perpendicular to the tissue interface) to create acoustic contact without causing indentation of the dermal surface. Once an appropriate image was obtained, it was printed for later analysis. Muscle thickness was defined as the distance from the adipose tissue-muscle interface to the muscle-bone interface. The quadriceps and hamstring muscle groups were assessed for overall muscle thickness. Ultrasound measurements were performed before each condition and immediately, 30 minutes, and 1h after each condition, and all measurements were performed by a single, trained technician. In

vitro precision for determining muscle thickness in the Neuromuscular Laboratory is 2.71% for the quadriceps and 3.01% for the hamsrings.

Systolic and Diastolic Blood Pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined using an automated blood pressure cuff (Omron, Model HEM-773). Blood pressure was taken in duplicate in attempt to obtain two SBP values within 5 mmHg. A third measurement was taken if the initial measurements were not within the desired range. The closest two blood pressure values were averaged for analysis.

Strength Testing

Subjects performed one-repetition maximum (1-RM) tests at baseline to determine lower-body muscular strength using the leg press, leg extension, and leg curl weight machines. Subjects first warmed-up for five minutes on a stationary exercise bike. Subjects then performed two warm up sets of 8-10 repetitions and 3-4 repetitions at submaximal loads (estimated 50% 1-RM and 80% 1-RM, respectively). Following the warm-up, subjects completed one repetition of a given load through the full range of motion. The load was increased until the participant was unable to complete the subsequent repetition, or the load was decreased if the 1-RM was overestimated. The greatest load lifted through the full range of motion was considered the 1-RM. Two minutes of recovery was allowed between attempts and exercises.

Blood Sampling

Each subject's index finger was first sterilized with an alcohol pad. The subject's finger was then pricked with a Microlet lancet device. A drop of blood was then collected into a heparinized plastic micro-hematocrit tube and subsequently centrifuged to separate hematocrit from plasma. A digital hematocrit reader was used to determine the percent of hematocrit to plasma in each sample. Percent change in plasma volume (PV%Δ) was determined by the following equation described (107) and utilized (92) previously by our research group:

$$PV\%\Delta = (100/(100 - Hct\ Pre) \times 100\ ((Hct\ Pre - Hct\ Post)/Hct\ Post)$$

This procedure was performed in all three experimental conditions before, immediately after, and 1h after exercise/control (precision is 2.13%).

Dietary Monitoring

To determine habitual nutritional habits, subjects completed a 3-day nutritional log, consisting of the day prior, the day of, and the day after each experimental condition; nutritional intake during the course of this study was determined from this nutritional log. This log was used to ensure dietary habits remained constant across the study duration by assessing total kilocalorie and individual macronutrient intakes.

Data Analyses

Data were analyzed using PASW Statistics 18 for Windows (Chicago, IL). The Kolmogorov Smirnov test was performed on all variables to determine normality distribution. Condition (TRE, BFR, CON) x time (pre-exercise and post-

exercise measurement points) repeated measures analysis of variance (ANOVA) were utilized to determine significant changes in quadriceps and hamstring muscle thickness, percent hematocrit/plasma volume, mCSA, and thigh circumference. In the event that a significant condition x time interaction occurred for any dependent variable, the statistical model was decomposed by examining the simple main effects with separate one-way repeated measures ANOVAs with Bonferroni correction factors for each condition and time point. Additional repeated measure ANOVAs were used to determine if any differences existed in total caloric or macronutrient intakes across conditions. For all statistical methods, an alpha-level of 0.05 was used to ascertain significant differences between group means. Data are reported as mean ± SD for all dependent variables.

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this investigation was to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout. Specifically, it was our objective to decipher the post-exercise time point at which increased intramuscular fluid, as a result of inflammation from resistance exercise, is returned to baseline (resting) levels.

Subject Characteristics

A total of 10 subjects originally qualified for the study and were randomly assigned to the order in which they completed each of the three experimental conditions. Seven of the 10 subjects completed all facets of the study protocol. Of the three that failed to do so, one subject failed to complete a three-day food log, one subject did not return to the laboratory at his designated time to complete a pQCT scan and thigh circumference assessment, and the other did not complete a pQCT scan and thigh circumference assessment due to experiencing lightheadedness from one of the exercise bouts. However, these three subjects were still included in the analysis. Baseline demographic characteristics and strength measures are presented in Table 1.

Table 1. Baseline Subject Characteristics (n = 10)

Demographics:	
Age (yrs)	22.1 ± 3.0
Height (cm)	180.7 ± 5.7
Weight (kg)	80.2 ± 15.9
Body Fat (%)	23.2 ± 5.9
Muscular Strength:	
Leg Press 1RM (lbs)	374.0 ± 87.5
Leg Extension 1RM (lbs)	221.3 ± 44.1
Leg Curl 1RM (lbs)	207.5 ± 44.6

Values are mean ± SD. 1RM: One-repetition maximum. lbs: pounds

Total Caloric and Macronutrient Intakes

Three-day average total caloric intake, macronutrient intake, and macronutrient percentages of total caloric intake for each condition are presented in Table 2. A significant condition difference was found for carbohydrate intake ($p = 0.024$). However, follow-up comparisons revealed no such group difference ($p > 0.05$). No significant condition differences were detected for total caloric intake ($p = 0.111$), protein intake ($p = 0.280$), or fat intake ($p = 0.293$).

Table 2. Three day average total caloric intake, macronutrient intake, and percentages of total daily caloric intake across each experimental condition.

	Condition		
	BFR (n = 9)	TRE (n = 9)	CON (n = 9)
3-Day Avg:			
Total CI (kcal)	2110.9 ± 452.4	1870.6 ± 625.7	1689.4 ± 836.7
CHO Intake (g)	279.4 ± 104.8	204.0 ± 72.6	200.0 ± 98.3
Protein Intake (g)	87.6 ± 16.0	101.0 ± 32.5	84.6 ± 39.6
Fat Intake (g)	71.5 ± 19.9	75.8 ± 34.4	58.8 ± 35.7
% TDCI:			
Carbohydrate	51.5 ± 10.6	43.6 ± 8.2	47.5 ± 7.0
Protein	17.6 ± 6.8	21.6 ± 12.4	20.3 ± 6.0
Fat	31.1 ± 7.9	36.5 ± 8.8	31.6 ± 7.1

Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. CI: Caloric Intake. CHO: Carbohydrate. TDCI: Total Daily Caloric Intake.

Muscle Thickness

Figure 1 illustrates quadriceps muscle thickness (MTQ) values for each of the three experimental conditions (mean values for each condition are presented in Table 3 on p. 112 in Appendix G). A significant condition x time interaction ($p < 0.001$), main effect for time ($p < 0.001$), and main effect for condition ($p < 0.001$) were detected for MTQ. Follow-up analyses showed that MTQ for BFR was significantly greater immediately post-exercise ($p < 0.001$) and 30 minutes post-exercise ($p = 0.001$) when compared to pre-exercise. MTQ for TRE was significantly greater immediately post-exercise ($p = 0.010$), 30 minutes post-exercise ($p = 0.007$), and 60 minutes post-exercise ($p = 0.019$) when compared to pre-exercise. However, the mean value at 60 min post-exercise fell within the minimal difference (0.42 cm) needed to be considered a real change. MTQ did not significantly change ($p > 0.05$) in response to CON. No significant group

differences ($p > 0.05$) in MTQ were seen at pre-exercise. MTQ for BFR and TRE were significantly greater than CON immediately post-exercise (BFR: $p < 0.001$, TRE: $p = 0.021$) and 30 minutes post-exercise (BFR: $p = 0.003$, TRE: $p = 0.009$). Additionally, MTQ for BFR was significantly greater than TRE immediately post-exercise ($p = 0.016$).

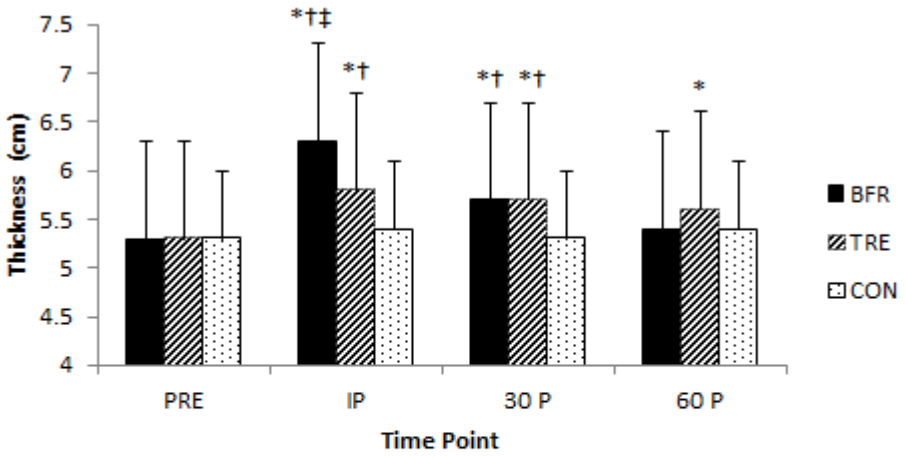

Figure 1. MTQ values before and after exercise across conditions. Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. MTQ: Muscle Thickness Quadriceps. *Significant increase from Pre ($p < 0.05$). †Significantly greater than CON at respective time point ($p < 0.05$). ‡Significantly greater than TRE at respective time point ($p < 0.05$).

Figure 2 illustrates hamstring muscle thickness (MTQ) values for each of the three experimental conditions (mean values for each condition are presented in Table 3 on p. 112 in Appendix G). A significant condition x time interaction ($p = 0.034$) and main effect for time ($p < 0.001$), were detected for MTH. Follow-up analyses showed that MTH for BFR was significantly greater immediately post-exercise ($p = 0.036$) when compared to pre-exercise. No significant changes over time were observed in CON for MTH ($p > 0.05$). Furthermore, no significant

differences in MTH ($p > 0.05$) were seen across conditions at any measured time point.

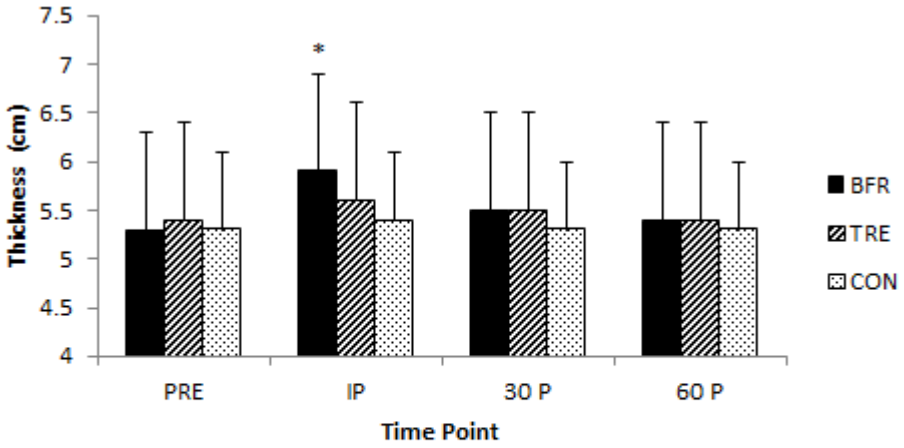

Figure 2. MTH values before and after exercise across conditions. Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. MTH: Muscle Thickness Hamstring. *Significant increase from Pre ($p < 0.05$).

Hematocrit and Plasma Volume

Table 4 presents the percent of blood volume consisting of red blood cells across all three experimental conditions (also presented in figure 3 located on p. 113 in Appendix G). A significant condition x time interaction ($p = 0.003$), main effect for time ($p < 0.001$), and main effect for condition ($p = 0.026$) were observed for hematocrit. Follow-up analyses revealed that hematocrit was significantly greater immediately post-exercise in both BFR ($p < 0.001$) and TRE ($p = 0.001$) conditions. No pre-exercise differences across conditions were seen for hematocrit ($p > 0.05$). Contrarily, hematocrit was significantly greater in BFR ($p = 0.002$) and TRE ($p = 0.003$) when compared to CON immediately post-exercise.

Table 4. Hematocrit values expressed as percent of blood volume and plasma volume percent changes expressed relative to baseline values.

	Condition		
	BFR (n = 10)	TRE (n = 10)	CON (n = 10)
Hematocrit (%):			
Pre-Exercise	41.4 ± 3.7	42.5 ± 3.6	41.4 ± 3.2
Im Post-Ex	44.7 ± 3.5*†	45.5 ± 3.2*†	41.9 ± 2.7
60 min Post-Ex	41.9 ± 3.8	43.0 ± 3.5	41.3 ± 2.6
PV %Δ:			
Pre-Exercise	N/A	N/A	N/A
Im Post-Ex	-12.3 ± 5.7*†	-11.6 ± 5.9*†	-2.1 ± 5.8
60 min Post-Ex	-1.7 ± 8.0	-3.1 ± 5.8	0.5 ± 5.3

Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. PV %Δ: Plasma Volume percent change. *Significant change from Pre ($p < 0.05$). †Significantly different from CON at respective time point ($p < 0.05$).

Table 4 and figure 4 present the PV%Δ seen across each experimental condition. A significant condition x time interaction ($p = 0.002$), main effect for time ($p < 0.001$), and main effect for condition ($p = 0.026$) were noted for PV%Δ. Follow-up analyses showed that PV%Δ significantly decreased from pre- to immediately post-exercise in both BFR ($p < 0.001$) and TRE ($p < 0.001$) conditions. Furthermore, PV%Δ immediately post-exercise was significantly greater in BFR ($p = 0.002$) and TRE ($p = 0.003$) when compared to CON.

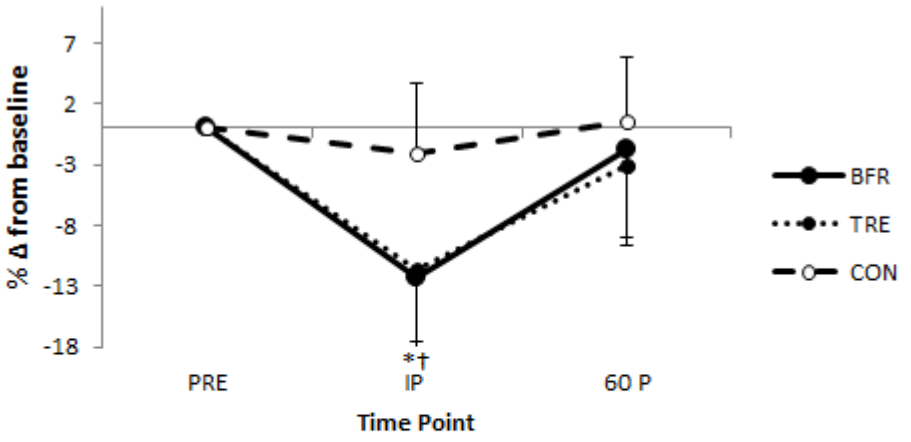

Figure 4. Plasma volume percent changes espressed relative to baseline values. Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. %Δ: Plasma Volume percent change. *Significant change from Pre ($p < 0.05$). †Significantly different from CON at respective time point ($p < 0.05$).

Thigh Muscle Cross-Sectional Area

Figure 5 presents thigh mCSA at 50% femur length across conditions (also presented in Table 5 on page 114 in Appendix G). A significant condition x time interaction ($p < 0.001$), main effect for time ($p < 0.001$), and main effect for condition ($p = 0.031$) were found for mCSA. No baseline differences in mCSA were seen across conditions ($p > 0.05$). In BFR, mCSA was significantly greater at 15 minutes post-exercise ($p < 0.001$) and 75 minutes post-exercise when compared to pre-exercise mCSA. However, the mCSA at 75 minutes post-exercise is within the minimal difference (MD = 407.9 mm^2) considered to be a real change. From 24 hours to 96 hours post-exercise in BFR, mCSA was not significantly different from pre-exercise mCSA ($p > 0.05$). In TRE, mCSA was significantly greater at 15 minutes post-exercise compared to pre-exercise mCSA, but returned and remained similar to pre-exercise mCSA from 75 minutes to 96 hours post-exercise ($p > 0.05$).

No significant changes in mCSA were observed in CON ($p > 0.05$). There were no significant differences in mCSA at baseline across conditions ($p > 0.05$). At 15 minutes post-exercise, mCSA was significantly greater in BFR ($p = 0.001$) and TRE ($p = 0.002$) when compared to CON. Likewise, mCSA was significantly greater in TRE ($p = 0.019$) compared to CON at 48 hours post-exercise. No other within or between condition significant differences were seen for mCSA.

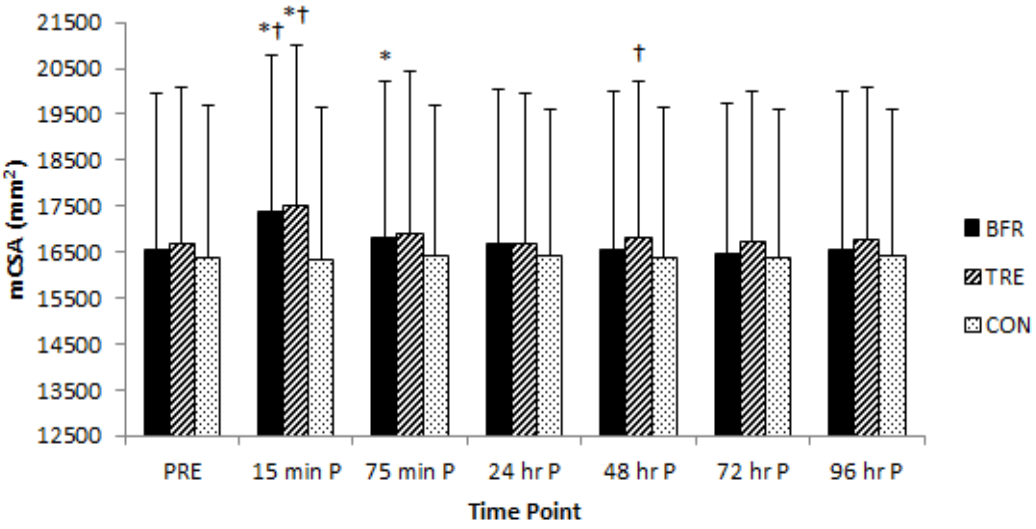

Figure 5. Muscle cross-sectional area. Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. mCSA: Muscle Cross-Sectional Area. *Significant increase from Pre ($p < 0.05$). †Significantly greater than CON at respective time point ($p < 0.05$).

Thigh Circumference

Figure 5 presents thigh circumference at 50% femur length across conditions (also presented in Table 5 on page 114 in Appendix G). A significant condition x time interaction ($p < 0.001$) and main effect for time ($p < 0.001$) were observed for thigh circumference. No baseline differences in thigh circumference were seen across conditions ($p > 0.05$). Follow-up analyses displayed that thigh

circumference was significantly greater at 15 minutes post-exercise in BFR ($p < 0.001$), TRE ($p = 0.002$), and CON ($p = 0.016$) compared to their respective pre-exercise thigh circumference values. Additionally, thigh circumference was significantly greater at 75 minutes post-exercise in BFR ($p = 0.032$) and TRE ($p = 0.007$) compared to their respective pre-exercise thigh circumference values. Thigh circumference was also significantly greater in BFR ($p = 0.022$) and TRE ($p = 0.003$) at 15 minutes post-exercise when compared to CON. No other within or between condition significant differences were observed for thigh circumference.

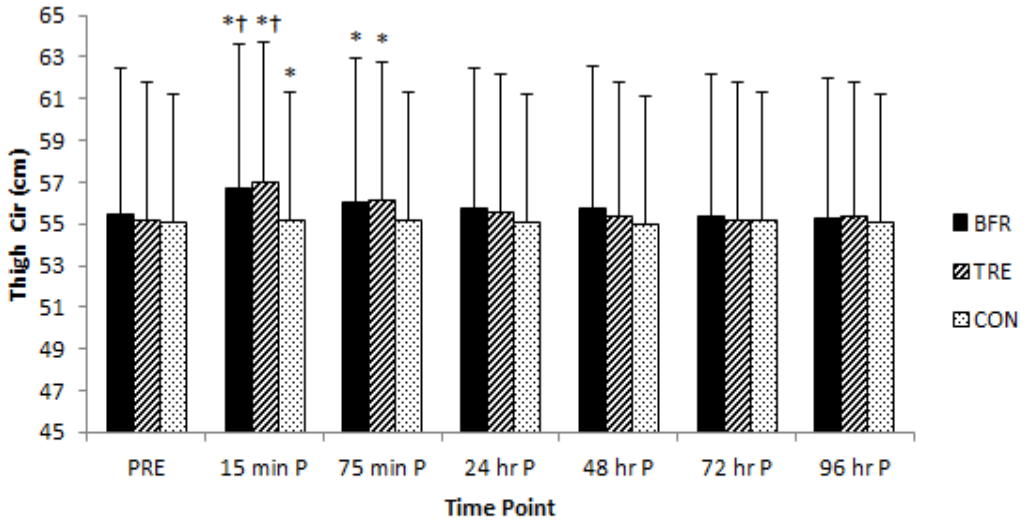

Figure 6. Thigh Circumference. Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. Thigh Cir: Thigh Circumference. *Significant increase from Pre ($p < 0.05$). †Significantly greater than CON at respective time point ($p < 0.05$).

DISCUSSION

The primary objective of this investigation was to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout. Specifically, it was our objective to decipher the post-exercise time point at which increased intramuscular fluid, as a result of inflammation from resistance exercise, is returned to baseline (resting) levels. Secondary objectives were to analyze the response of quadriceps and hamstring muscle thickness, plasma volume, and thigh circumference to a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout. The time course of skeletal muscle hypertrophy is well documented in the literature, and recent evidence (2, 28) suggests that this physiological adaptation can occur much sooner than once thought. Technological advancements have improved the precision of body composition analytical devices, which may partially explain the recent findings indicating an earlier time course of muscle hypertrophy. The pQCT scanner used in the current study is one such technological advancement in body composition analysis, and it has been demonstrated to accurately predict mCSA when compared to the gold standard, MRI (91). However, the pQCT cannot distinguish between acute muscle swelling that can occur from exercise and the actual addition of contractile proteins (i.e., muscle hypertrophy). Therefore, it is imperative to determine the time course of muscle swelling from a traditional bout of resistance exercise via pQCT to pinpoint

the earliest a pQCT scan can be performed following exercise to predict mCSA with minimal error. To our knowledge, the current investigation was the first to do so. Muscle swelling, or cell swelling, is one of the proposed mechanisms attempting to explain the effectiveness of BFR training, which has the potential to provide health-related benefits to certain populations that are unable to place heavy external loads on the body's musculature. Evidence supporting the anabolic potential of muscle swelling is promising, especially since some of the original theories explaining the hypertrophic adaptations observed with BFR exercise have recently been questioned. Therefore, the current investigation sought to determine the degree and time course of muscle swelling in response to a bout of low-intensity BFR resistance exercise, and how this muscle swelling response compared to a bout of traditional, high intensity resistance exercise. To our knowledge, this study was the first to investigate this comparison.

Muscle Cross-Sectional Area

mCSA is a widely utilized measure indicative of skeletal muscle size, and it is considered the gold standard in regards to assessing skeletal muscle mass changes in exercise intervention and unloading studies alike. mCSA primarily increases when there is an addition of sarcoplasmic proteins, or contractile proteins, within a muscle cell (36). However, an acute bout of exercise has been demonstrated to decrease plasma volume while subsequently increasing mCSA of actively involved muscles (84). Such an increase in mCSA does not accurately reflect a true increase in contractile protein content, but rather a fluid shift from the

vascular space to the exercised musculature. This fluid shift induced muscle swelling was the principal variable of interest in the current investigation. mCSA was analyzed prior to, and 15 minutes, 75 minutes, 24, 48, 72, and 96 hours following each experimental condition. Significant changes over time were detected for mCSA at 50% femur length in both exercise conditions. Specifically, mCSA significantly increased following BFR at 15 minutes post-exercise and remained elevated through 75 minutes post-exercise (even though it was not considered to be a real change at the 75 min time point). mCSA significantly increased following TRE at 15 minutes post-exercise only, and no significant differences in mCSA between exercise conditions were noticed. Therefore, these results indicate that mCSA increased similarly following BFR and TRE measured at 15 minutes post-exercise, and returned to baseline in a similar fashion. To answer our original research question of at what time point does mCSA return to baseline or a non-significant level of change after a TRE and BFR exercise bout, it does so within 24 hours. Both the BFR and TRE exercise protocols were of moderate to high volume, and the subjects in the current study that completed them were not currently participating in a resistance exercise program. Hence, the higher the exercise volume or muscle involvement (84) and the less resistance exercise experience an individual has, the greater the inflammatory and muscle swelling response would be expected to accrue. It would then be plausible to assume that individuals with resistance training experience beyond that of the subjects in the current study, and exercise protocols that consist of comparable or less volume

would also experience similar or less muscle swelling following exercise as measured by pQCT.

In terms of BFR exercise, Loenneke and colleagues (68) established that the restriction pressure and extent of arterial occlusion is largely related to thigh circumference and the composition of the underlying tissue. On this notion, subjects in the current study whose thigh circumferences were larger than others may not have restricted blood flow to the same degree at 160 mmHg of pressure and blunted the overall muscle swelling response. A higher restriction pressure used by these subjects may have produced a similar degree of blood flow restriction to the exercising muscles as 160 mmHg did for the subjects with smaller thigh circumferences. Further insight into regulating exercise restriction pressure based on the subject's limb size and composition is necessary in order to more closely compare the muscle swelling response between BFR and TRE exercise protocols. Since the restriction pressure utilized can affect the actual amount of blood flow restriction within the exercising musculature, it is reasonable to believe that a greater restriction pressure may produce a greater degree of muscle swelling and subsequent anabolic potential. As mentioned previously, cell swelling can inhibit protein catabolism triggering a protein sparing effect, which fluxes the overall protein balance toward anabolism (11, 42). The mechanisms responsible for stimulating the anabolic response from cell swelling via BFR are inconclusive at this time, but rational theories have been proposed by our research group (67). Briefly, BFR may alter the intra- to extracellular pressure gradient thereby driving

water into cells, and the reestablishment of blood flow following BFR may also provide the force necessary to influx water into cells due to a shift in the pressure gradient. In addition, a membrane channel known as aquaporin 4 found in anaerobic muscle fibers seems to balance osmotic gradients caused by intense activity (33). Aquaporin 4 is thought to transfer water into muscle cells that have accumulated metabolic by products, thus causing an increase in muscle cell volume. This increase in volume is then intrinsically sensed (based on Haussinger's hypothetical cell swelling model (43)), thereby activating a G-protein and tyrosine kinase, which facilitates the activation of mTOR and mitogen-activated protein-kinase (MAPK) pathways. Both of these pathways are thought to play integral roles during protein synthesis and skeletal muscle growth (12, 88) and have both shown to be upregulated in response to BFR exercise (34). Thus, the degree of muscle swelling may be an important factor driving protein synthesis and muscle growth. Since muscle swelling increased similarly in response to both BFR and TRE and acute responses to exercise lead to long-term training adaptations, it is possible that BFR can stimulate muscle hypertrophy to comparable levels as TRE in young males that are currently not participating in resistance exercise. However, post-exercise muscle swelling from BFR exercise may be affected by restriction pressure, exercise intensity (percent of 1-RM), and time under restriction, all of which could influence the hypertrophic potential of BFR.

Muscle Thickness

Muscle thickness, as measured by ultrasound, is an index of mCSA and is generally used to assess muscle mass changes in exercise training (1) and disuse/unloading (53) studies. Furthermore, muscle thickness has been measured acutely following a single exercise bout to evaluate muscle swelling and indirectly to assess fluid shifts (8, 31, 77), as was the case in the current study. Specifically, MTQ and MTH were measured at baseline and immediately, 30 minutes, and 60 minutes following each experimental condition. Significant changes in MTQ were observed in response to both TRE and BFR exercise protocols, while MTH experienced significant change after BFR only. Distinctively, MTQ was significantly increased from pre-exercise at immediately post-exercise and at 30 minutes post-exercise in BFR and immediately post-exercise through 60 minutes post-exercise in TRE (60 min post-exercise mean was within the minimal difference). MTQ was significantly greater in BFR compared to TRE measured immediately post-exercise. Collectively, these results display that BFR caused a greater initial increase in MTQ post-exercise, while MTQ remained above pre-exercise levels similarly in both conditions. It is assumed that since the immediate post-exercise muscle thickness assessments were completed with the cuffs still inflated in the BFR condition, venous blood pooling contributed some degree to the greater increase in MTQ when compared to TRE. Regarding MTH, BFR experienced a significant increase immediately post-exercise, again with the cuffs still inflated, while no changes were found in TRE. It is likely that the quadriceps

experienced a greater response in muscle thickness compared to the hamstrings, because the exercises employed in our protocol placed a greater stress and recruited the quadriceps more so than the hamstrings. This was also in agreement with the subjects' perception of which muscle groups, the quadriceps, were most fatigued following both exercise bouts (unpublished data).

The majority of studies analyzing the acute inflammatory or muscle swelling response after resistance exercise have used exercise protocols comprised of extremely high volume or primarily eccentric muscle actions which cause excessive muscle damage. However, two recent studies have assessed muscle swelling via ultrasound using resistance exercise bouts that are more practical in nature. Ahtiainen and colleagues (4) found a significant increase in vastus lateralis muscle thickness in resistance-trained men at 24 and 48 hours post-exercise after completing five sets of leg press and four sets of squats, each with a 10RM load. Umbel et al. (106) had subjects complete three sets of unilateral leg extensions at 35% MVC to failure under restriction (30% above brachial systolic pressure), without restriction, under restriction performing only eccentric muscle actions, and under restriction performing only concentric muscle actions. The authors found a significant main effect for time ($p = 0.02$) when collapsed across exercise conditions for vastus lateralis CSA at 24 and 48 hours post exercise. Both of the above studies are in agreement with the current study in the fact that they also experienced increases in quadriceps muscle swelling in response to heavy resistance exercise (4) and low-intensity with blood flow restriction resistance

exercise (106). Unlike the current investigation which only saw quadriceps muscle thickness increases up to 30 minutes post exercise, the Ahtiainen and Umbel studies saw quadriceps muscle swelling last up to 48 hours post exercise in the vastus lateralis. The exercise protocol in the Ahtiainen study (4) used a similar exercise intensity as the protocol for the TRE condition in the current study, but performed two additional sets of exercise stressing the quadriceps musculature, which may partially explain the difference in results. Furthermore, our ultrasound measurements represented the entire quadriceps musculature, rather than just the vastus lateralis. Therefore, it is possible that the anterior position of our ultrasound measurement did not include the entire or part of the vastus lateralis muscle, which may have been stressed more than the other quadriceps muscles and was not reflected in our results. The participants in the Umbel study (106) used 6 cm Hokanson cuffs to restrict blood flow, and a relative restriction pressure (30% above systolic pressure) for each subject, whereas the present study used 5 cm Kaatsu-Mini cuffs to restrict pressure and a standard restriction pressure (160 mmHg). Loenneke and colleagues (68) showed that cuff width, thigh circumference, and underlying tissue composition all can influence the degree of blood flow restriction at a given restrictive pressure. It is unknown whether the thigh circumferences of our subjects differed significantly from Umbel's subjects, but nonetheless, it is an influential variable that should be addressed. Also, the Hokanson cuffs are not elastic and do not give or stretch to any degree when surrounding a contracting muscle like the Kaatsu-Mini cuffs used in the current

study do. In theory, the Hokanson cuffs may have restricted more blood flow than the Kaatsu-Mini cuffs during exercise simply by their non-elastic properties. In summary, the summation of these factors may have influenced the muscle swelling response and therefore could explain the muscle swelling differences observed between our study and the Umbel investigation.

Hematocrit and Plasma Volume

The alteration of the hematocrit to plasma volume ratio or simply plasma volume shifts can occur in response to various stimuli, two being a change in hydration status and exercise (52). In the present study, the percent of hematocrit and plasma volume in the blood were measured prior to, immediately following, and one hour after each experimental condition. Precisely, the percent of hematocrit in the blood significantly increased in BFR and TRE immediately post-exercise, but returned to non-statistically significant level within one hour of both exercise bouts. Likewise, the PV%Δ from pre-exercise to immediately post-exercise was statistically significant (decrease in plasma volume) in both BFR and TRE, while the PV%Δ from pre-exercise to one hour post-exercise in both exercise conditions was non-significant (no change in plasma volume).

Previous studies have analyzed the effect of resistance exercise on plasma volume changes. Collins et al. (21) measured plasma volume change before, and over a one hour recovery period after completing three sets to failure at 70% 1RM of arm curl, bench press, bent-arm row, and squat exercises. The authors found a 14.3% decrease in plasma volume ($p < 0.05$) immediately following the exercise

protocol, but it had returned to baseline levels 30 minutes into the recovery period. Ploutz-Snyder and colleagues (84) observed a 22% change in plasma volume immediately after subjects completed six sets to failure of 10 RM barbell squats. The percent change in plasma volume had returned to a non-significant change by 30 minutes post-exercise and entirely back to pre-exercise levels within one hour after the exercise bout. The current investigation, like the Collins and Ploutz-Snyder studies, noted a significant decrease in PV%Δ in response to resistance exercise which returned to non-significant levels within one hour of each of the exercise bouts. However, the current study detected a smaller PV%Δ, than the above two mentioned studies. The exercise protocol employed by Collins et al. (21) stressed muscle groups in both the upper and lower body, where the current study only exercised muscles in the lower body. Therefore, it is plausible to assume that more plasma would leave the blood in a case where more skeletal muscle tissue is activated. The Ploutz-Snyder (84) exercise protocol, which consisted of barbell squats only, elicited a greater PV%Δ than the exercise protocols used in the present study as well as the Collins study. Even though the barbell squat is considered a primarily lower-body exercise, additional muscle groups are activated to stabilize the weight as it rests on the trapezius/upper back muscles during the entire range of motion. These supplementary muscle groups may cause a further decrease in plasma volume, which is supported by Ploutz-Snyder and colleagues' findings. In conclusion, the BFR and TRE exercise protocols in the current study were able to prompt significant plasma volume shifts

that are comparable to those in previously conducted studies. Moreover, low-intensity BFR resistance exercise was as effective at inducing a decrease in plasma volume as TRE, which suggests that BFR may stimulate any anabolic response similar to that of heavy resistance exercise.

Thigh Circumference

Circumference is a widely used, inexpensive field measurement tool that has previously been utilized as an indirect assessment of mCSA changes over the course of a resistance exercise program (24). More recently, circumference measures, in conjunction with other laboratory techniques to form prediction equations, have demonstrated to be a reliable and accurate estimation of mCSA (10, 27). In the current investigation, thigh circumference was used as an indirect measurement of muscle swelling. Thigh circumference at the 50% femur site was analyzed prior to, and 15 minutes, 75 minutes, 24, 48, 72, and 96 hours following each experimental condition. Results indicated that thigh circumference significantly increased from pre-exercise to 15 minutes post-exercise in all experimental conditions, and up to 75 minutes post-exercise in BFR and TRE. The significant increase observed in CON is not considered real, as the minimal difference score must be $\geq$ 0.4 cm.

As a measure of muscle swelling, thigh circumference changes followed a similar trend to the changes in mCSA in the current study. For both mCSA and thigh circumference measures, significant increases occurred from pre-exercise to 15 minutes post-exercise in BFR and TRE. However, thigh circumference was

significantly greater at 75 minutes post-exercise in BFR and TRE when compared to pre-exercise circumference, which was not the case for mCSA analyses in BFR and TRE for the same respective time course. Hayashi et al. (44) found that thigh circumference peaked approximately five minutes following isokinetic knee exercise and returned to pre-exercise levels within 40 minutes. These authors also went on to show that muscle swelling as measured by thigh circumference closely correlated with the MR images obtained at the rectus femoris ($r = 0.930$, $p < 0.01$) and gracilis ($r = 0.946$, $p < 0.01$) over the same time course. The results of this study along with the similar pattern of thigh circumference and mCSA changes noticed in the present study suggest that thigh circumference may be a valid and reliable measurement tool used to assess acute muscle swelling following exercise when expensive laboratory methodologies are an unavailable resource. However, further insight is warranted to support this notion. Finally, the thigh circumference findings of the present study also suggest that low-intensity BFR resistance exercise may be as effective as heavy resistance exercise at inducing muscle swelling and its potential anabolic effects.

Dietary Intake

Dietary intake, specifically macro- and micronutrients, are capable of alleviating markers of muscle damage arising from intense and strenuous forms of exercise (48). Micronutrients such as vitamins C and E are often required to be supplemented with over a period of several days to weeks prior to and following exercise before beneficial effects are observable (14, 90). Therefore, in the current

investigation, only macronutrient intakes were analyzed the day before, the day of, and the day following each experimental condition, since nutrient timing of macronutrients around a bout of exercise have been shown to reduce markers of inflammation and muscle damage during recovery (48). The macronutrient intakes across the three recorded days for BFR, TRE, and CON were averaged and compared across conditions. No significant within or between condition differences for carbohydrate, protein, and fat intake were detected, implying that the subjects' dietary intakes remained similar across each condition and did not influence the outcome of any dependent variables.

CHAPTER V

CONCLUSIONS

The purpose of this investigation was to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout. Specifically, it was our objective to decipher the post-exercise time point at which increased intramuscular fluid, as a result of inflammation from resistance exercise, is returned to baseline (resting) levels. The following research questions addressed: 1) How long will muscle swelling remain above baseline levels after performing a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow restriction resistance exercise bout? 2) Will there be a difference in the degree of muscle swelling between a traditional high-intensity resistance exercise bout and low-intensity with blood flow restriction resistance exercise bout? 3) Will there be differences in the degree of muscle thickness changes in response to a traditional high-intensity resistance exercise bout compared to a low-intensity with blood flow restriction resistance exercise bout?

Research Hypothesis 1. Muscle swelling will return to baseline levels within 96 hours after performing the traditional high-intensity resistance exercise bout and low-intensity combined with blood flow restriction resistance exercise bout.

Yes, the results of the study support this hypothesis. Muscle swelling returned to resting levels within 24 hours as measured by pQCT and thigh circumference after performing traditional high-intensity resistance exercise and low-intensity combined with blood flow restriction resistance exercise.

Subhypothesis 1. Muscle swelling will be greater in response to the low-intensity with blood flow restriction resistance exercise bout compared to the traditional high-intensity resistance exercise bout.

No, the results of the current study do not support this hypothesis. Muscle swelling as measured by pQCT increased similarly from pre-exercise to 15 minutes post-exercise after TRE and BFR with no between condition differences and returned to baseline values in a similar time frame. Muscle swelling measured by thigh circumferences increased similarly from pre-exercise to 75 minutes post exercise in BFR and TRE, with no between condition differences.

Subhypothesis 2. Muscle thickness changes in response to the low-intensity with blood flow restriction resistance exercise bout will be greater than the muscle thickness changes experienced after performing the traditional high-intensity resistance exercise bout.

The results of the current study somewhat support this hypothesis. MTQ was increased to a greater extent immediately post-exercise in BFR, but MTQ remained elevated similarly after TRE and BFR when compared to pre-exercise MTQ. MTH was significantly increased immediately post-exercise in BFR, while no significantly changes were observed in TRE.

Significance of the Study

The results of the current study suggest that muscle swelling returns to pre-exercise levels within 24 hours after completing a moderate to high volume heavy-resistance exercise bout and a low-intensity coupled with blood flow restriction resistance exercise bout. Therefore, it may be possible to measure mCSA via pQCT 24 hours after completing a resistance exercise bout without swelling contributing to the measurement error. This would allow researchers to frequently track mCSA changes via pQCT over the course of a training program without having to account for long rest intervals between measurements. The present study was the first to demonstrate the time course of muscle swelling from exercise as measured by pQCT, and future studies are warranted to build upon these results. Furthermore, the findings of the current investigation suggest that low-intensity resistance exercise with blood flow restriction may result in a similar muscle swelling response to traditional, heavy resistance exercise of similar volume. If in fact cell swelling initiates an anabolic environment by reducing muscle protein breakdown, increasing muscle protein synthesis, or a combination of the two, low intensity resistance exercise coupled with blood flow restriction may be an efficacious alternative for stimulating protein synthesis, and possibly muscle hypertrophy, for individuals who are unable to apply heavy external loads to the body's tissues. However, there is not enough evidence at this time to support the cell swelling theory.

Future Research

Future studies aimed at expanding on our findings should take into account hydration status when measuring muscle swelling. Water accounts for a large percentage of muscle tissue, and therefore could provide a source of measurement error if not taken into account. Also, researchers could experiment with acute training variables (intensity, number of sets, number of repetitions per set, and rest intervals between sets) to determine their influence on muscle swelling. If muscle swelling is a variable of interest, researchers should closely monitor the rest intervals between exercise bouts, if more than one exercise bout is employed, to account for any effect that rest time may have on the subsequent exercise recovery response. In the current study, not all rest intervals were the same across conditions for each subject. Moreover, researchers should explore the ability of the pQCT to determine muscle density. If this were possible, swelling due to water, as well as contractile protein content, could be indirectly measured. In regards to BFR exercise, exercise restriction pressures must be considered. It is probable that prescribed restrictive pressures need to be relative to the individual's limb composition, rather than utilizing a universal pressure, or one relative to systolic blood pressure. Therefore, more consistent comparisons could be made between heavy resistance exercise protocols and BFR exercise, along with comparing the effects of BFR exercise to other studies using BFR exercise protocols. Lastly, the cell swelling theory should be further analyzed to determine its mechanistic role, if any, in stimulating muscle anabolism.

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APPENDICES

Appendix A

Recruitment Materials

Male Participants Needed

The Neuromuscular Laboratory is looking for males between 18-30 yrs who currently <u>do not</u> strength train to participate in some ongoing research looking at the effects of two types of resistance exercise on inflammation. Participation will last approximately 4 weeks. *IRB# 16052*

Benefits Include:

- ***Muscular Strength Analysis***
- ***Body Composition Analysis***
- ***Dietary Analysis***

Contact Chris Poole at cpoole@ou.edu or call (405) 325-5211

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Verbal Recruitment Script

Hello, my name is ----------, and I am a graduate student in the Department of Health and Exercise Science at the University of Oklahoma. I am inviting you to participate in a research study we are conducting. The title of the study is "An evaluation of the inflammatory time course response following traditional and blood flow reduction resistance exercise measured by peripheral quantitative computed tomography." We are specifically looking for men between the ages of 18-30 years who are not currently weight training or participating in moderate to high intensity aerobic exercise more than 2 days per week. We are performing this research study to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow reduction resistance exercise bout. Blood flow reduction during exercise is a new training technique using a specially designed cuff (50 mm width) placed around the most upper portion of the leg to reduce blood flow during exercise.

If you decide to participate, you will complete 3 experimental conditions: traditional high-intensity resistance exercise, low-intensity resistance exercise with blood flow reduction, and a non-exercise control condition.

The total time commitment for this study is 4 weeks. The two exercise conditions consist of completing resistance exercise using 3 lower-body exercise machines. During the control condition, you will remain sedentary. During pre and post testing, we will measure your height and weight, bone density, lower-body strength, lean and fat body mass, muscle cross-sectional area, muscle thickness, thigh circumference, dietary habits, and you will have your blood sampled.

The pre and post testing sessions will involve exposure to low-dose radiation (DXA and peripheral quantitative computerized tomography) to obtain measures of muscle mass, fat, bone density, and thigh muscle cross-sectional area.

There is a possibility of mild soreness because of the testing and exercise, but any discomfort should be gone within a couple of days. Additionally, there may be some discomfort and mild subcutaneous bruising associated with the blood draws that will be performed by a trained research assistant.

No financial compensation will be provided if you decide to participate. I would be happy to answer any questions that you may have about the study.

Appendix B

IRB Approval Letter

The University of Oklahoma®
Health Sciences Center
INSTITUTIONAL REVIEW BOARD

IRB Number: 16052
Meeting Date: August 15, 2011
Approval Date: September 16, 2011

September 23, 2011

Michael Bemben, Ph.D.
Univ of Oklahoma, Dept of Health & Exercise Sci
1401 Asp Avenue
Norman, OK 73019

RE: An Evaluation of the Inflammatory Time Course Response Following Traditional and Blood Flow Reduction Resistance Exercise Measured by Peripheral Quantitative Computed Tomography

Dear Dr. Bemben:

The University of Oklahoma Health Sciences Center's Institutional Review Board (IRB) reviewed the above-referenced research protocol at its regularly scheduled meeting on August 15, 2011. It is the IRB's judgement that the rights and welfare of the individuals who may be asked to participate in this study will be respected; that the proposed research, including the process of obtaining informed consent, will be conducted in a manner consistent with the requirements of 45 CFR 46 or 21 CFR 50 & 56, as amended; and that the potential benefits to participants and to others warrant the risks participants may choose to incur.

On behalf of the IRB, I have verified that the specific changes requested by the convened IRB have been made. Therefore, on behalf of the Board, I have granted final approval for this study.

This letter documents approval to conduct the research as described:

IRB Application Dated: August 01, 2011
Radiation Safety Application Dated: August 01, 2011
Priv - Research Auth 1 Dated: January 06, 2005
Phone Script - Recruitment Dated: August 01, 2011
Advertisement - Email Dated: August 01, 2011
Recruitment flyer Dated: August 01, 2011
Other Dated: August 01, 2011 Health Status Questionnaire
Other Dated: August 01, 2011 PAR-Q and YOU
Other Dated: August 01, 2011 Lower Body 1RM Testing/Training Session Log
Other Dated: August 01, 2011 3-Day Dietary Log
Protocol Dated: September 13, 2011
Radiation Safety CA Ltr Dated: September 12, 2011
Consent form - Subject Dated: September 08, 2011

As principal investigator of this protocol, it is your responsibility to make sure that this study is conducted as approved by the IRB. Any modifications to the protocol or consent form, initiated by you or by the sponsor, will require prior approval, which you may request by completing a protocol modification form.

It is a condition of this approval that you report promptly to the IRB any serious, unanticipated adverse events experienced by participants in the course of this research, whether or not they are directly related to the study protocol. These adverse events include, but may not be limited to, any experience that is fatal or immediately life-threatening, is permanently disabling, requires (or prolongs) inpatient hospitalization, or is a congenital anomaly, cancer or overdose. For multi-site protocols, the IRB must be informed of serious adverse events at all sites.

The approval granted expires on July 31, 2012. Should you wish to maintain this protocol in an active status beyond that date, you will need to provide the IRB with an IRB Application for Continuing Review (Progress Report) summarizing study results to date. The IRB will request a progress report from you approximately three months before the anniversary date of your current approval.

Post Office Box 26901 • 1000 S.L. Young Blvd., Room 176
Oklahoma City, Oklahoma 73126-0901 • (405) 271-2045 • FAX: (405) 271-1677

If you have questions about these procedures, or need any additional assistance from the IRB, please call the IRB office at (405) 271-2045 or send an email to irb@ouhsc.edu. Finally, please review your professional liability insurance to make sure your coverage includes the activities in this study.

Sincerely yours,

Karen J. Beckman, M.D.
Chair, Institutional Review Board

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Appendix C

Informed Consent and Authorization to use or Disclose Protected Health Information

701-A

Version 09/08/10 *IRB No: 16052*

Consent Form
University of Oklahoma Health Sciences Center (OUHSC)
University of Oklahoma – Norman Campus

An evaluation of the inflammatory time course response following traditional and blood flow reduction resistance exercise measured by peripheral quantitative computed tomography

Sponsor: Department of Health & Exercise Science
University of Oklahoma
Norman, OK 73019

Principal Investigator: **Michael Bemben, PhD**
University of Oklahoma
405-325-2717

This is a research study. Research studies involve only individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?
You are being asked to take part in this trial/study because you meet the inclusion criteria of a male between the ages of 18-30 years who is/has not participated in a structured resistance exercise program in the past 3 months or has not participated in moderate to high intensity aerobic exercise more than twice per week for the last 3 months.

Why Is This Study Being Done?
The purpose of this study is to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow reduction resistance exercise bout.

What is the Status of the Devices or Procedures involved in this study?
No experimental devices or procedures will be used in this research study.

How Many People Will Take Part In The Study?
About 10 young men will take part in this, all at this location.

What Is Involved In The Study?
If you take part in this study, you will have the following tests and procedures:

Consent Form and Questionnaires
You will be asked to thoroughly review, and then sign and date an informed consent document (this document) and additional screening forms (health history questionnaire and physical activity readiness to determine your eligibility to participate in this study.

You may be excluded from the study if any of your questionnaire responses indicate that you have an exclusion criterion for the study. (30 minutes or as long as needed)

During the first week, you will have a total of two visits. The following three weeks will consist of performing each of the experimental conditions (two exercise protocols and one control protocols).

Height and Weight
Your height and body weight will be measured. (2 minutes)

Peripheral Quantitative Computed Tomography (pQCT) scans:
You will have 21 pQCT scans of the right thigh to measure the muscle. The pQCT scan is a simple, non-invasive, type of x ray procedure where you will sit as still as possible in a chair with your leg in a positioning brace for approximately 15 minutes. Scans will be administered prior to each exercise bout and control condition, and immediately after, 1 hour, 4 hours, 24 hours, 48 hours and 72 hours after each exercise bout and control condition.

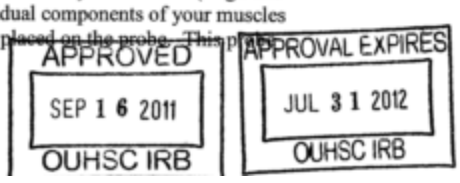
Dual Energy X-ray Absorptiometry (DXA) Testing:
You will complete a full body DXA scan, a type of x ray, to determine body composition (fat mass, lean tissue mass) and bone density. The scan will take about 10 minutes. You will have this scan at the beginning of the study.

One repetition maximum (1RM) testing:
You will perform 1RM testing to determine muscular strength of the muscle that flex and extend the upper and lower legs using the leg press, leg extension, and leg curl weight machines in the Neuromuscular Laboratory. Prior to the test, you will perform two warm-up sets. Following warm-up, you will complete one repetition of a given weight through the full range of motion. The weight is increased until you cannot complete the repetition with proper form, and this will be achieved within 5 attempts. This test will be done at the beginning of the study. (20-25minutes)

Blood Sampling:
You will be asked to give a blood sample from your finger (1 to 2 drops by pricking your finger). You will be asked to give the sample prior to each exercise bout and control condition and immediately after, 30 minutes after, and 1 hour after each exercise bout and control condition. After your blood is analyzed for plasma volume, it will be discarded. This procedure will take about 5 minutes.

Thigh Circumference:
You will be asked to have your thigh circumference measured with a tape measure. This procedure will be done prior to each pQCT scan.

Ultrasound of the thigh and hamstring musculature:
An ultrasound machine will be used to look at the thickness of your muscles (thigh and hamstring in the upper leg) and the angle of the individual components of your muscles (called muscle fibers). A small amount of gel will be placed on the probe. This p



will then be positioned over your muscles where measurements will be taken. When an appropriate image is obtained, we will save this image on the ultrasound machine and print it for later analysis. This analysis will be performed prior to each exercise and control protocol, and immediately after (before removing the cuffs), 30 min, and 1h after each protocol. Each ultrasound measurement will take 15-20 minutes.

Experimental Conditions
The order in which you do each protocol will be random (like rolling dice).

1. Traditional resistance exercise protocol:
You will warm-up for 5 minutes on a stationary bike prior to performing resistance exercise. You will then complete 3 sets of 8-10 repetitions on a supine leg press machine at a high intensity. You will complete the same routine for the leg extension and leg curl exercises. This exercise session will take approximately 25-30 minutes.

2. Blood flow reduction resistance exercise protocol:
You will warm-up for 5 minutes on a stationary bike prior to performing resistance exercise. Before the session begins, you will be seated and the pressure cuffs will be placed on the upper most portions of your legs and inflated to a low level for 30 seconds and then deflated. This process will be repeated increasing the pressure until the target exercise pressure is reached. This process of slowly reaching the exercise pressures will take approximately 3-5 min. You will then complete 1 set of 30 repetitions, and 3 sets of 15 repetitions at a low intensity while wearing the inflated cuffs. If you are unable to complete the desired number of repetitions for a given set, the weight will not change and you will complete as many repetitions as possible with proper form for subsequent sets.

3. Control protocol:
During this session, you will sit in a chair for a duration similar to that of the exercise sessions (25 minutes). All other tests will be performed identically as they would be in the exercise conditions.

Nutrition logs:
You will complete a 3-day nutritional log consisting of the day prior, the day of, and the day after each experimental condition; nutritional intake during the course of this study will be determined from this nutritional log. This log will be checked to ensure your dietary habits remained constant across the study duration.

How Long Will I Be In The Study?
Your participation is anticipated to last approximately four (4) weeks.

There may be anticipated circumstances under which your participation may be terminated by the investigator without regard to your consent, for reasons such as not adhering to all study guidelines, for health concerns observed by the investigators, or if the study is terminated by the Investigators.

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher.

What Are The Risks of The Study?
While in the study, you are at risk for these side effects. There may also be risks that are currently unforeseeable. You should discuss these with the researcher prior to providing your consent to participate.

Risks and side effects related to blood flow restriction:
- Feeling faint, fatigued, lightheaded and possibility of passing out.
- Bruising and discomfort caused by the strap.
- There is a theoretical risk that restricting blood flow in the leg could increase the risk of developing a blood clot in one of the veins but this has not been observed to date.

Risks and side effects related to exercise and exercise testing:
- Feeling faint, fatigued, lightheaded and possibility of passing out due to physical exertion.
- Muscle soreness and/or stiffness beginning within 24 hours post-exercise and lasting for several days.
- Muscle fatigue, shortness of breath, and elevated heart rate during and trouble walking immediately following maximal exercise tests.

Risks and side effects related to having a Blood Draw:
- Bleeding at the sight of puncture
- Pain at the sight of puncture
- Bruising to the surrounding area for a couple of days
- Feeling lightheaded or faint
- A slight possibility of infection which can occur anytime the skin is broken (rare)

Risks and side effects related to having a pQCT and DXA scan:
- This research study involves exposure to radiation from 1 DXA scan and 21 pQCT scans, which are types of x-ray procedures. This radiation is not necessary for medical care and is for research purposes only. You will receive radiation exposure of less than 2 mrem from each DXA scan and less than 1 mrem from each pQCT scan for a total dose of 23 mrem for all scans, which is less than the radiation received in 25 days from natural background radiation (~ 300 mrem/yr), such as naturally occurring radioactivity in soil. It is important for you to be aware that the risk from radiation exposure is cumulative over your life time.

Are There Benefits to Taking Part in The Study?
You will receive pertinent health information regarding muscular strength and body composition. Additionally, you will receive a copy of your DXA scan upon completion of the study if requested.

What Other Options Are There?
There are no alternative procedures for this investigation; your alternative is to not participate.

What About Confidentiality?
Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We

cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the faculty members and graduate students appointed to this protocol from the Department of Health & Exercise Science at the University of Oklahoma, and the OUHSC Institutional Review Board.

What Are the Costs?
There is no cost to you for participating in this study.

Will I Be Paid For Participating in This Study?
No, you will not be paid.

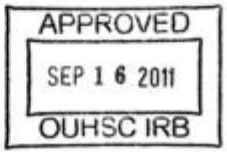
What if I am Injured or Become Ill While Participating in this Study?
In the case of injury or illness resulting from this study, emergency medical treatment will be available. If injury occurs as a result of participation, you should consult with your personal physician to obtain treatment. However, you or your insurance company will be responsible for the costs of this treatment. No funds have been set aside by The University of Oklahoma Health Sciences Center or the Department of Health & Exercise Science to compensate you or pay for the costs associated with treatment in the event of injury.

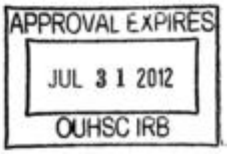
What Are My Rights As a Participant?
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, please be sure to discuss leaving the study with the principal investigator. You may discontinue your participation at any time without penalty or loss of benefits, to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare or willingness to continue your participation in this study.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?
If you have questions, concerns, or complaints about the study or have a research-related injury, contact Michael Bemben, PhD at 405-325-2717 or Chris Poole at 254-493-1547.





If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

For questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

Signature:
By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18)	Printed Name	Date
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

IRB Office Version Date: 07/07/2009

AUTHORIZATION TO USE or DISCLOSE
PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required.
Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **An evaluation of the inflammatory time course response following traditional and blood flow reduction resistance exercise measured by peripheral quantitative computed tomography**

Leader of Research Team: **Michael Bemben**

Address: **1401 Asp Avenue HHC #104, Norman, OK 73019**

Phone Number: **405-325-2717**

If you decide to join this research project, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private Information To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to determine the time course of increased intramuscular fluid following a traditional high-intensity resistance exercise bout and a low-intensity combined with blood flow reduction resistance exercise bout. Specifically, it is our objective to decipher the post-exercise time point at which increased intramuscular fluid, as a result of inflammation from resistance exercise, is returned to baseline (resting) levels.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research sponsor, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS). The researchers

may also share your private information with other researchers in the Health and Exercise Science Department.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

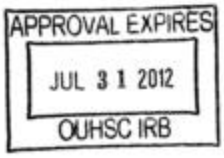
Voluntary Choice. The choice to give OUHSC researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care from OUHSC.

Revoking Permission. If you give the OUHSC researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OUHSC researchers to use or share your private information for their research will never end. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma Health Sciences Center
PO Box 26901, Oklahoma City, OK 73190
If you have questions call: (405) 271-2511



Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by Michael Bemben, permission to share your private information for the research project called An evaluation of the inflammatory time course response following traditional and blood flow reduction resistance exercise measured by peripheral quantitative computed tomography.

Patient/Subject Name: _____

_____ _____
Signature of Patient-Subject Date
or Parent if subject is a child

Or

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Patient-Subject, provide a description of the relationship to the Patient-Subject and the Authority to Act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.

IRB No.: 16052

Appendix D

Study Questionnaires

Neuromuscular Research Laboratory

OU Department of Health and Exercise Science

Health Status Questionnaire

Instructions Complete each question accurately. All information provided is confidential.
(NOTE: The following codes are for office use only: RF; MC; SLA; SEP)

Part 1. Information about the individual

1._____
Date

2. _____
Legal name Nickname

3._____
Mailing address

_____
Home phone Business phone

4.Gender (circle one): Female Male (RF)

5. Year of birth: _____ Age _____

6. Number of hours worked per week: Less than 20 20-40 41-60 Over 60

(SLA) More than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

Part 2. Medical history

7. (RF) Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8.Date of: Last medical physical exam: _____Last physical fitness test: _____
Year Year

9. Circle operations you have had:

Back (SLA)	Heart (MC)	Kidney (SLA)	Eyes (SLA)	Joint (SLA)	Neck (SLA)
Ears (SLA)	Hernia (SLA)	Lung (SLA)	Other _____		

10. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Alcoholism (SEP)	Diabetes (SEP)	Kidney problem (MC)
Anemia, sickle cell (SEP)	Emphysema (SEP)	Mental illness (SEP)
Anemia, other (SEP)	Epilepsy (SEP)	Neck strain (SLA)
Asthma (SEP)	Eye problems (SLA)	Obesity (RF)
Back strain (SLA)	Gout (SLA)	Osteoporosis
Bleeding trait (SEP)	Hearing loss (SLA)	Phlebitis (MC)
Bronchitis, chronic (SEP)	Heart problems (SLA)	Rheumatoid arthritis (SLA)
Cancer (SEP)	High blood pressure (RF)	Stroke (MC)
Cirrhosis, liver (MC)	Hypoglycemia (SEP)	Thyroid problem (SEP)
Concussion (MC)	Hyperlipidemia (RF)	Ulcer (SEP)
Congenital defect (SEP)	Infectious mononucleosis (MC)	Other _____

11. Circle all medicine taken in last 6 months:

Blood thinner (MC)	Epilepsy medication (SEP)	Nitroglycerin (MC)
Diabetic pill (SEP)	Heart-rhythm medication (MC)	Estrogen
Digitalis (MC)	High-blood-pressure medication (MC)	Thyroid
Diuretic (MC)	Insulin (MC)	Corticosteroids
Asthma	Other _____	

12. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

1 = Practically never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often

a. Cough up blood (MC)	d. Leg pain (MC)	g. Swollen joints (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
b. Abdominal pain (MC)	e. Arm or shoulder pain (MC)	h. Feel faint (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
c. Low back pain (SLA)	f. Chest pain (RF) (MC)	i. Dizziness (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
j. Breathless with slight exertion (MC)		
1 2 3 4 5		

13. Do any of the following apply:

- A sudden death in your biological father or brother, or mother or sister prior to age 55 or 65, respectively? Yes No
- Current smoker or have you quit smoking within the past 6 months? Yes No
- Do you take hypertensive medication or have a confirmed systolic or diastolic blood pressure ≥140 or 90 mmHg, respectively? Yes No
- Take lipid lowering medication or have high blood cholesterol? Yes No
- You have a confirmed fasting blood glucose of ≥100 mg/dL? Yes No
- Have you recently been diagnosed as clinically obese (BMI > 30)? Yes No
- Are you sedentary? Yes No
- Diagnosed Crohn's or Inflammatory Bowel Disease Yes No
- Past fracture of a hip, pelvis, or femur Yes No
- Major Surgery within the last 6 months Yes No
- Been diagnosed with varicose veins Yes No
- Family history of Deep Vein Thrombosis or Pulmonary Embolism Yes No

Part 3. Health-related behavior

14. (RF) Do you now smoke or chew tobacco? Yes No

15. If you are a smoker, indicate number smoked per day:

Cigarettes:	40 or more	20-39	10-19	1-9
Cigars or pipes only:	5 or more or any inhaled		Less than 5, none inhaled	

16. Weight now: _____lb. One year ago: _____lb.. Age 21: _____lb.

17. Thinking about the things you do at work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

    1. Much more active
    2. Somewhat more active
    3. About the same
    4. Somewhat less active
    5. Much less active
    6. Not applicable

18. Now, thinking about the things you do outside of work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

    1. Much more active

2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

19. Do you regularly engage in strenuous exercise or hard physical labor?

1. Yes (answer question # 19) 2. No (stop)

20. Do you exercise or labor at least three times a week?

1. Yes 2. No

Appendix E

Nutritional Information Sheet

Neuromuscular Research Laboratory
OU Department of Health and Exercise Science
3-Day Dietary Log

Subject ID_____
Date_____

Instructions:

Please record everything that you eat for **the day prior to exercise, the day you exercise, and the day after exercise**. Include the food/drink item with brand names if applicable, the amount ingested (serving size), and method of preparation (baked, fried etc), if applicable. Please be sure to include all beverages including protein/ meal replacements and alcoholic beverages. Please be as specific as possible.

Serving Size Handy Guide: See Attached Appendix

Day 1: _____

Meal/Time	Food/Drink	Amount (1 cup, 8 oz, number of slices, etc.)	How Prepared (fried, baked, etc.)
Breakfast			
Snack			
Lunch			
Snack			
Dinner			

Snack			

Day 2: _____

Meal/Time	**Food/Drink**	**Amount** (1 cup, 8 oz, number of slices, etc.)	**How Prepared** (fried, baked, etc.)
Breakfast			
Snack			
Lunch			
Snack			

Dinner			
Snack			

Day 3: _____

Meal/Time	**Food/Drink**	**Amount** (1 cup, 8 oz, number of slices, etc.)	**How Prepared** (fried, baked, grilled, etc.)
Breakfast			
Snack			
Lunch			

Snack			
Dinner			
Snack			

Appendix F

Raw Data

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	subject_ID	Age	HT_cm	WT_kg	systolic	diastolic	DEXA_BF	LP_1RM	LE_1RM	LC_1RM	Calories_BFR	CHO_BFR	per_cho	PRO_BFR	per_pro
2	RS93	19	189	102.1	128	74	35.1	500	250	250	2909.8	461.4	63.42704	91.56	12.58643
3	JA59	22	176.5	66.7	105	70	20.7	220	162.5	162.5					
4	DC05	28	183	80.7	120	76	21.9	440	250	200	1869.4	236	50.49749	81.7	17.48154
5	NW37	26	184.5	100.9	110	75	26.7	400	275	275	1301.8	107.9	33.15409	113	34.72116
6	GH35	24	189	98.3	110	68	21.7	440	262.5	262.5	2162.5	213.5	39.49133	99.3	18.36763
7	SS32	20	176	55.2	103	75	14.8	240	137.5	137.5	1758.1	236.4	53.78534	57.9	13.17331
8	JC10	21	175	78	116	72	28.5	400	237.5	212.5	2058	264.6	51.42857	88.7	17.24004
9	CC16	22	180	79.2	123	89	26.1	380	200	175	2163.1	266.7	49.31811	87.4	16.16199
10	EK69	19	172.5	66.9	129	84	17.7	340	212.5	212.5	2395.7	328.9	54.91506	72.1	12.03824
11	TW15	20	181	73.9	119	71	19.1	380	225	187.5	2379.8	399.6	67.16531	97.1	16.3207

	P	Q	R	S	T	U	V	W	X	Y	Z	AA
1	FAT_BFR	per_fat	MTQ_PRE_BFR	MTQ_IP_BFR	MTQ_30P_BFR	MQ_60P_BFR	MTH_PRE_BFR	MTH_IP_BFR	MTH_30P_BFR	MTH_60P_BFR	HEM_PRE_BFR	HEM_IP_BFR
2	73.6	22.76445	5.1	6.5	5.7	5.4	5.7	6.7	5.7	5.9	42.5	44
3			5	5.7	5.2	5	5.9	6.1	5.7	5.7	41	48
4	72.4	34.8561	5.5	6.6	6	6	5.1	6.8	5.5	5.3	38	40.5
5	44.2	30.55769	6.6	7.4	6.8	6.6	4.5	5.5	4.9	4.7	42.5	45
6	102	42.45087	5.1	6	5.7	5.3	5.7	6.2	6.4	6.4	43	46
7	69.2	35.42461	4	5.1	4.5	4	4.7	4.7	4.7	4.9	36.5	41
8	74.1	32.40525	6	6.8	6.2	6	4.9	4.9	5.1	4.9	41.5	46
9	68.7	28.58398	4.9	6	5.1	4.9	4.7	5.5	5.3	5.3	37	40
10	96.8	36.36515	5.3	6	5.6	5.1	6.1	6.8	6.3	6	43	45
11	42.5	16.07278	5.5	6.6	6.2	6	5.3	5.5	5.3	5.3	49	51

	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM
1	HEM_60P_BFR	PV_PRE_BFR	PV_IP_BFR	PV_60P_BFR	CSA_PRE_BFR	CSA_15P_BFR	CSA_75P_BFR	CSA_24H_BFR	CSA_48H_BFR	CSA_72H_BFR	CSA_96H_BFR	CIR_PRE_BFR
2	43.5	57.5	-5.93	-4	16166.08	16827.68	16346.56	16191.04	15900.48	15662.24	15925.92	59
3	46	59	-24.72	-18.42	13808.64	14348.16	13973.12	13761.44	13912.8	13647.68	13759.84	51.4
4	37.5	62	-9.96	2.15	17104.8	17997.44	17405.6	17387.2	17440.96	17104	17341.12	55.5
5	40	57.5	-9.66	10.87	19673.6	20801.6	20292.48	19876.48	19779.84	19527.52	19693.12	65.6
6	43	57	-11.44	0	21347.52	21940.32	21485.92	21340.96	21313.76	21122.56	21377.76	60.8
7	37.5	63.5	-17.29	-4.2	10817.44	11687.2	11150.56	11028.32	10768.64	11076.48	10854.24	44.1
8	40	58.5	-16.72	6.4	14903.68	15899.2	15179.52	15104.48	14929.76	15116.8	14889.6	53.9
9	39	63	-11.9	-8.14	13559.52	14131.2	13898.72	13812	13871.36	13636.64	13781.6	52.8
10	43.5	57	-7.8	-2.02	15693.44	16641.92	15867.04	15834.08	15841.12	15789.28	15896.96	52.3
11	49	51	-7.69	0	15824.48	16406.88	15512.16	15804.16	15550.72		15685.44	54.3

	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ
1	CIR_15P_BFR	CIR_75P_BFR	CIR_24H_BFR	CIR_48H_BFR	CIR_72H_BFR	CIR_96H_BFR	Calories_TRE	CHO_TRE	per_cho	PRO_TRE	per_pro	FAT_TRE	per_fat
2	60	59.3	59.2	59.2	59	58.8	2613.6	267.1	40.87848	154	23.56902	102.9	35.43388
3	52	51.4	51.2	51.3	51	51.2	1819.8	253.5	55.72041	63.5	13.95758	65.8	32.54204
4	56.6	55.7	55.6	55.5	55.3	55.1	1649.6	192.7	46.72648	94.2	22.8419	61.5	33.55359
5	66.8	66.2	65.2	65.3	64.9	64.4	722.4	56.8	31.45072	102	56.47841	11.8	14.701
6	61.6	61	61	60.8	60.7	61	2744.5	246.5	35.9264	134	19.52997	139.2	45.64766
7	45.2	44.8	44.5	44.1	44.3	44.3	1593.9	180.4	45.2726	78.1	19.59972	65.4	36.92829
8	55.3	54.4	53.7	54	53.7	53.5	2105.9	210.8	40.03989	127	24.1227	87.5	37.39494
9	54.4	53.5	53.2	53.2	52.9	53.1	2011	274.1	54.52014	91.4	18.18001	64.4	28.82148
10	53.6	52.6	52.9	52.7	52	52.1	2041.4	271.3	53.1596	80.7	15.81268	80.1	35.314
11	56	55	54.4	54.2		54.1	1353.1	136.7	40.41091	47.9	14.16008	69.2	46.02764

	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ
1	MTQ_PRE_TRE	MTQ_IP_TRE	MTQ_30P_TRE	MQ_60P_TRE	MTH_PRE_TRE	MTH_IP_TRE	MTH_30P_TRE	MTH_60P_TRE	HEM_PRE_TRE	HEM_IP_TRE
2	5.1	5.7	5.6	5.4	5.6	6.5	6.1	6.1	44.5	49
3	5.2	5.4	5.3	5.1	6.9	7.1	7.1	6.5	47.5	48
4	5.7	6.4	6.4	6.2	5.1	5.1	5.1	5.3	41.5	43.5
5	6.4	6.8	6.6	6.4	4.7	4.9	4.7	4.5	41	43
6	5.3	6	5.3	5.5	6.4	6.4	6.6	6	46.5	48
7	3.8	4.5	4.3	4.3	4.9	4.9	4.9	4.7	42	45
8	5.7	5.7	6	6	4.9	4.9	4.7	4.9	38	43.5
9	4.6	6.1	5.4	5.4	4.3	4.9	4.8	4.8	36	40
10	5.2	5.8	6	5.7	5.7	6	5.8	6	42.5	45
11	5.7	6	6	6	5.1	5.1	5.1	4.9	45	50

	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT
1	HEM_60P_TRE	PV_PRE_TRE	PV_IP_TRE	PV_60P_TRE	CSA_PRE_TRE	CSA_15P_TRE	CSA_75P_TRE	CSA_24H_TRE	CSA_48H_TRE	CSA_72H_TRE
2	45.5	55.5	-16.55	-3.96	15940.48	16555.36	16200.48	16027.04	16148.8	16001.28
3	47	52.5	-1.98	2.03	14027.04	14639.84	13778.4	13727.84	14019.36	14036.64
4	40.5	58.5	-7.86	4.22	18317.76	19035.84	18523.2	18437.44	18351.52	18257.44
5	42	59	-8.09	-4.14	19379.84	20585.12	20083.2	19110.56	19777.92	19312
6	46	53.5	-5.84	-2.03	21544.8	22491.68	21802.24	21390.56	21521.28	21307.84
7	43.5	58	-11.49	-5.94	11114.24	11845.44	11276.16	11287.2	11273.6	11264.64
8	41	62	-20.39	-11.8	14907.84	15703.04	15124	15012.48	15155.52	15151.68
9	36	64	-15.63	-8.02	14745.92		15424.32	15396.64	15153.44	14990.56
10	41	57.5	-9.66	6.36	15566.88	16276.32	15208.96	15378.56	15454.4	15744.64
11	47	55	-18.18	-7.74	15465.28	16190.88	15773.6	15364	15418.72	15213.92

	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
1	CSA_96H_TRE	CIR_PRE_TRE	CIR_15P_TRE	CIR_75P_TRE	CIR_24H_TRE	CIR_48H_TRE	CIR_72H_TRE	CIR_96H_TRE	Calories_CON	CHO_CON	per_cho
2	16272.48	59	60.4	60.3	59.5	58.8	59	59.1	3591	397.5	44.27736
3		51.1	52.5	51.7	51.7	51.3	51.2		1609.7	209.4	52.034541
4	18369.28	56.7		58.4	57.2	56.8	57.2	57.1	1649.6	192.7	46.726479
5	19353.44	63.6	64.9	64.6	63.8	63.7	63.3	63.4	984.8	106.9	43.419984
6	21419.68	61.4	64	62.2	61.8	61.7	61.8	61.6	1436.2	167.1	46.539479
7	11099.04	44.6	45.6	45	44.5	44.8	44.5	44.4	981.1	117.7	47.986953
8	14984.48	53.3	55	53.8	53.5	53	53.6	53.6	2131.8	241.2	45.257529
9	14545.92	52.8	54.4	53.5	53.2	53.2	52.9	53.1	1554.6	238.3	61.314808
10	15760.64	51.7	54.5	53	52	51.8	51.4	52	1976.4	260.5	52.722121
11	15288.8	52.1	53.7	52.6	52.6	52.3	52.3	52	899.4	77.8	34.600845

	CF	CG	CH	CI	CJ	CK	CL	CM	CN	CO	CP
1	PRO_CON	per_pro	FAT_CON	per_fat	MTQ_PRE_CON	MTQ_IP_CON	MTQ_30P_CON	MQ_60P_CON	MTH_PRE_CON	MTH_IP_CON	MTH_30P_CON
2	175.4	19.537733	145	36.34085	5.4	5.6	5.4	5.6	5.6	5.6	5.7
3	61.8	15.356899	60.9	34.04982	4.8	5	5	5	7.1	6.7	6.7
4	94.2	22.841901	61.5	33.55359	5.7	5.7	5.7	5.5	5.1	5.1	5.1
5	78	31.68156	27.8	25.40617	6.5	6.4	6.6	6.6	4.7	4.9	4.9
6	84.7	23.590029	49.5	31.01936	5.3	5.1	4.9	5.1	5.5	5.7	5.5
7	32.1	13.087351	44.72	41.02334	3.9	4	4	4	4.6	4.9	4.9
8	94	17.637677	47.6	20.09569	6.2	6.2	6	6	5.1	5.1	4.9
9	84.8	21.819117	36.5	21.13084	5.1	5.1	5.1	4.9	4.1	4.4	4.3
10	61.4	12.426634	79.7	36.29326	5.1	5.1	5.1	5.1	6.1	6.1	6.1
11	57.1	25.394708	37.3	37.32488	5.3	5.5	5.5	5.7	5.1	5.3	5.1

	CQ	CR	CS	CT	CU	CV	CW	CX	CY	CZ
1	MTH_60P_CON	HEM_PRE_CON	HEM_IP_CON	HEM_60P_CON	PV_PRE_CON	PV_IP_CON	PV_60P_CON	CSA_PRE_CON	CSA_15P_CON	CSA_75P_CON
2	5.6	43	43.5	42.5	57	-2.02	2.06	15582.56	15110.08	15710.72
3	6.7	42	43	43	58	-4.01	-4.01	13961.44	14090.4	14147.2
4	5.1	38	38	38.5	62	0	-2.09	16718.56	16840.16	16768.64
5	4.9	41	41	40.5	59	0	2.09	19122.08	19083.84	19341.92
6	5.7	44	43	43	56	4.15	4.15	21453.92	21388.16	21201.44
7	4.9	37.5	38	37	62.5	-2.11	2.13	10939.84	10960.16	10943.68
8	4.9	37.5	42	40.5	62.5	-17.14	-11.85	15019.52	15304.32	15120.8
9	4.4	40.5	40.5	39.5	59.5	0	4.26	14917.12	14952.96	14833.12
10	6	42.5	43	42	57.5	-2.02	2.07	15691.52	15620.64	15849.76
11	4.9	47.5	47	46	52.5	2.03	6.21	15323.84	15476.32	15435.36

	DA	DB	DC	DD	DE	DF	DG	DH	DI	DJ	DK
1	CSA_24H_CON	CSA_48H_CON	CSA_72H_CON	CSA_96H_CON	CIR_PRE_CON	CIR_15P_CON	CIR_75P_CON	CIR_24H_CON	CIR_48H_CON	CIR_72H_CON	CIR_96H_CON
2	16030.24	15697.6	15597.12	15820	59	59.2	59.2	59	58.8	59	59
3	14179.84	14044.96	14087.68	13913.6	51.5	51.6	51.5	51.2	51.4	51.3	51.2
4	16779.52	17194.56	17265.28	17273.76	54.5	54.7	54.7	54.8	54.9	54.8	54.8
5	19090.08	19196.16	19300.16	19099.04	62.5	62.6	62.6	62.7	62.3	62.6	62.5
6	21234.56	21197.76	20800.64	20953.76	60.5	60.6	60.4	60.5	60.6	60.3	60.3
7	11009.76	10885.28	10882.4	10894.88	44.7	44.7	44.7	44.7	44.6	44.6	44.5
8	15092.64	14874.4	15070.24	15228.8	53.5	53.7	53.4	53.8	53.3	53.7	53.8
9	14973.92	14649.28	14773.92	14365.92	53.5	53.7	53.7	53.5	53.5	53.7	53.3
10	15584.16	15491.36	15787.2	15705.92	52	52	52.1	51.7	52	52.2	52
11	15470.08	15217.6	14904	15260.8	52.7	52.9	52.9	52.8	52.5	52.5	52.4

Appendix G

Additional Tables/Figures

Table 3. Muscle thickness values before and after exercise across conditions.

	Condition		
	BFR (n = 10)	TRE (n = 10)	CON (n = 10)
MTQ:			
Pre-Exercise	5.3 ± 0.7	5.3 ± 0.7	5.3 ± 0.7
Im Post-Ex	6.3 ± 0.6*†‡	5.8 ± 0.6*†	5.4 ± 0.7
30 min Post-Ex	5.7 ± 0.7*†	5.7 ± 0.7*†	5.3 ± 0.7
60 min Post-Ex	5.4 ± 0.7	5.6 ± 0.6*	5.4 ± 0.7
MTH:			
Pre-Exercise	5.3 ± 0.6	5.4 ± 0.8	5.3 ± 0.8
Im Post-Ex	5.9 ± 0.8*	5.6 ± 0.8	5.4 ± 0.7
30 min Post-Ex	5.5 ± 0.6	5.5 ± 0.9	5.3 ± 0.7
60 min Post-Ex	5.4 ± 0.5	5.4 ± 0.7	5.3 ± 0.7

Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. MTQ: Muscle Thickness Quadriceps. MTH: Muscle Thickness Hamstrings *Significant increase from Pre ($p < 0.05$). †Significantly greater than CON at respective time point ($p < 0.05$). ‡Significantly greater than TRE at respective time point ($p < 0.05$).

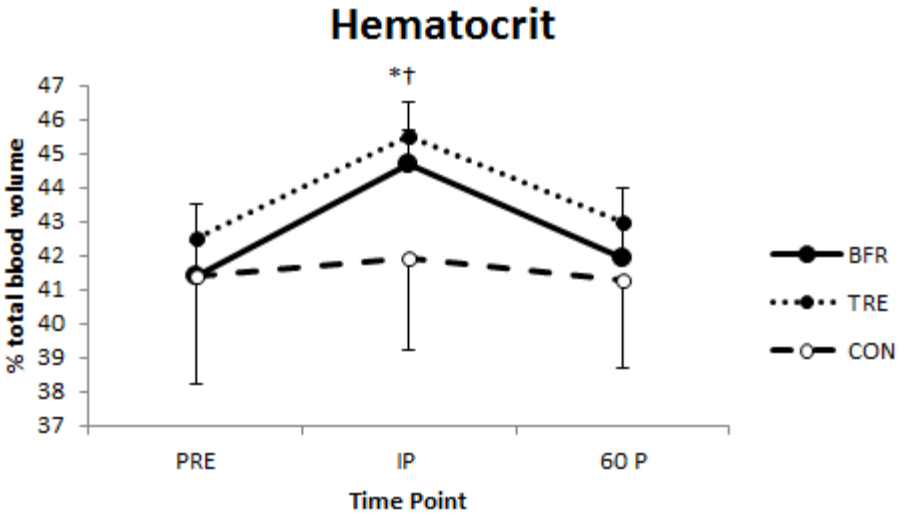


Figure 3. Hematocrit expressed as percent of blood volume. Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. *Significant change from Pre ($p < 0.05$). †Significantly different from CON at respective

Table 5. mCSA and thigh circumference at the 50% femur site before and after exercise across conditions.

	Condition		
	BFR (n = 7)	TRE (n = 7)	CON (n = 7)
mCSA (mm^2):			
Pre-Exercise	16529.5 ± 3404.2	16681.7 ± 3404.5	16361.1 ± 3314.9
15 min Post-Ex	17399.3 ± 3374.4*†	17499.0 ± 3521.0*†	16329.6 ± 3299.2
75 min Post-Ex	16818.2 ± 3419.0*	16888.3 ± 3533.9	16419.6 ± 3266.7
24 hr Post-Ex	16680.4 ± 3362.1	16663.4 ± 3295.6	16403.0 ±3222.8
48 hr Post-Ex	16567.8 ± 3437.3	16811.9 ± 3392.3†	16362.4 ± 3304.5
72 hr Post-Ex	16485.6 ± 3251.5	16719.9 ± 3261.4	16386.1 ± 3208.2
96 hr Post-Ex	16568.4 ± 3411.4	16751.3 ± 3353.2	16425.2 ± 3195.7
Thigh cir (cm):			
Pre-Exercise	55.5 ± 7.0	55.2 ± 6.6	55.1 ± 6.1
15 min Post-Ex	56.7 ± 6.9*†	57.0 ± 6.7*†	55.2 ± 6.1*
75 min Post-Ex	56.0 ± 6.9*	56.1 ± 6.7*	55.2 ± 6.1
24 hr Post-Ex	55.7 ± 6.8	55.5 ± 6.7	55.1 ± 6.1
48 hr Post-Ex	55.7 ± 6.9	55.3 ± 6.5	55.0 ± 6.1
72 hr Post-Ex	55.4 ± 6.8	55.2 ± 6.6	55.2 ± 6.1
96 hr Post-Ex	55.3 ± 6.7	55.3 ± 6.5	55.1 ± 6.1

Values are ± SD. BFR: Low-Intensity Resistance Exercise with Blood Flow Restriction. TRE: Traditional Resistance Exercise. CON: Non-exercise control. mCSA: Muscle Cross-Sectional Area. Thigh Cir: Thigh Circumference. *Significant increase from Pre ($p < 0.05$). †Significantly greater than CON at respective time point ($p < 0.05$).