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Does Blood Flow Restriction Applied Post High-Load Exercise Augment Skeletal Muscle Growth Following Eight Weeks of Training?

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DOES BLOOD FLOW RESTRICTION APPLIED POST HIGH-LOAD EXERCISE AUGMENT SKELETAL MUSCLE GROWTH FOLLOWING EIGHT WEEKS OF TRAINING?

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ABSTRACT

The application of blood flow restriction during low load exercise has consistently been shown to augment muscle hypertrophy which has been attributed to metabolic accumulation. It remains unknown, however, whether metabolites can augment muscle growth independent of further mechanical tension, specifically when maintained post high-load training. Thirteen untrained individuals performed 24 training sessions. The control arm performed one set of elbow flexion (70% 1RM) exercise to volitional fatigue, while the experimental arm performed the same protocol immediately followed by 3 min of blood flow restriction (70% arterial occlusion). Both conditions completed the same volume (3687 vs. 3638 kg) of exercise. There was an interaction (p=0.031) demonstrating an attenuation of muscle growth at the 60% site in the experimental [pre: 3.1 (0.6), post: 3.1 (0.7) cm] vs. control [pre: 3.1 (0.7), post 3.3 (0.7) cm] condition. Muscle growth at the 50% site did not differ between the experimental [pre: 2.9 (0.6), post 2.9 (0.6) cm] and control [pre: 2.8 (0.7), post: 2.9 (0.6) cm] condition (p=0.31) nor did it differ at the 70% site [experimental pre: 3.3 (0.60), post 3.5 (0.7) cm; control pre: 3.4 (0.7), post 3.6 (0.7) cm]. Although there were no differences at the group level, there were attenuations at the individual level. The number of measured sites displaying growth at or outside the error of the measurement was greater in the control (21) vs. experimental (10) condition. The application of blood flow restriction post high-load exercise did not augment, but appeared to attenuate muscle growth at the group and individual level. With regard to one-repetition maximum strength, increases were observed in both the control [pre: 13.5 (3.8), post: 16.3 (4.5) kg] and experimental [pre: 13.7 (4.1), post: 16.3 (4.6) kg] conditions with no differences between

conditions. No changes were observed for isometric or isokinetic strength for either the control or experimental conditions. These results unveil the possibilities that 1) metabolites do not have anabolic properties per se, and may be detrimental for muscle hypertrophy; 2) immediate post-exercise blood flow is important for muscle hypertrophy; and/or 3) metabolites have anabolic properties but this was masked by the restriction of blood flow.

LIST OF ABBREVIATIONS AND SYMBOLS

1RM	One-repetition maximum
Akt	Protein kinase B
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
BFR	Blood flow restriction
EMG	Electromyography
IGF-1	Insulin like growth factor 1
MAFbx	Muscle atrophy F box
MPB	Muscle protein breakdown
mRNA	Messenger ribonucleic acid
mTOR	Mechanistic target of rapamycin
mTORC1	Mechanistic target of rapamycin complex 1
mTORC2	Mechanistic complex of rapamycin complex 2
MURF1	Muscle ring finger-1
TSC2	Tuberous sclerosis complex 2

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CHAPTER 1: INTRODUCTION

An increase in the size of a muscle fiber is known as muscle hypertrophy and is known to occur after repetitive bouts of resistance training in humans. Muscle hypertrophy involves a variety of complex cellular and molecular mechanisms responsible for the formation of new proteins (90). When the number of proteins synthesized is greater than the number of proteins degraded, a positive net protein balance occurs, causing an increase in muscle size (78).

It was originally thought that heavier loads were necessary to produce muscle hypertrophy (9); a notion further supported by the American College of Sports Medicine (3). However, it has since been shown that muscle hypertrophy can occur through the lifting of lighter loads as long as the exercise is performed to volitional fatigue (66). Furthermore, blood flow restriction training (BFR) has been shown to increase muscle size through the lifting of lighter loads (20-30% 1RM) without the need to exercise to volitional fatigue (54). The increases in muscle size seen from BFR are comparable to that of high load training (43, 55), and provide insight that there may be a number of mechanisms responsible for muscle hypertrophy.

The primary mechanism responsible for muscle hypertrophy during high load training is thought to be mechanical tension. Since BFR training is performed with lighter loads that otherwise do not result in increased muscle size (101), it is unlikely mechanical tension alone is sufficient to stimulate robust muscular hypertrophy. Therefore, increases in muscle size from BFR may be reliant on alternative mechanisms to elicit growth in the absence of high levels of mechanical tension. The application of BFR has not been shown to provide any further muscle adaptation when combined with high load training (44), but this has only been implemented

intermittently due to high levels of participant discomfort (44). This provides some insight that the BFR stimulus may only augment the hypertrophic response when insufficient mechanical tension is present.

It is currently thought that a buildup of metabolites causes an increase in systemic hormones, growth factors, activation of higher threshold motor units (53) and cellular swelling (48). These mechanisms have been hypothesized to occur during commonly implemented BFR protocols and therefore may be acting in conjunction with mechanical tension to stimulate muscle growth. In contrast, applying a restrictive stimulus post exercise would allow for a prolonged period of cell swelling and metabolic accumulation without any additional muscle contraction or mechanical tension. However, the hypertrophic effects of prolonged cell swelling and metabolic accumulation at the conclusion of exercise, but without further mechanical tension, remains to be tested. The aforementioned BFR protocols used in conjunction with low load resistance training have not been designed to differentiate between the potential additive effects of these alternative hypertrophic mechanisms. BFR applied to immobilized limbs has been shown to attenuate disuse atrophy (11) supporting its effectiveness independent of mechanical tension, but this method does not induce muscle hypertrophy. Therefore, applying BFR during low load exercise may be reliant on mechanical tension in addition to some previously mentioned mechanisms (metabolic accumulation, cell swelling, etc.) to further produce increases in muscle size. Inflating a pressure cuff at the conclusion of one set of high load training would differ from traditional unrestricted high load training in that a greater buildup of metabolites would be pooled within the muscle as a result of the initial exercise bout. If metabolic accumulation can stimulate muscle hypertrophy when maintained after exercise, and independent of mechanical tension, inflating a pressure cuff at the conclusion high load training

may further augment the muscle hypertrophic response seen from high load training itself. However, these alternative mechanisms thought to be responsible for muscle hypertrophy during BFR training may only be important in the absence of sufficient mechanical tension during low load training. During high load training the anabolic stimulus provided from mechanical tension alone is likely enough to maximally stimulate muscle hypertrophy, although this has not been previously studied. By implementing a model that restricts blood flow strictly post exercise, it will allow for the effectiveness of alternative muscle hypertrophic mechanisms to be analyzed without further muscle contraction or mechanical tension. If muscle size is further increased with BFR post exercise, it will demonstrate that these alternative mechanisms can augment muscle growth when maintained after exercise. If there is no augmentation in muscle size, it will demonstrate that mechanical tension may be maximally stimulating muscle growth in itself; or, that metabolic accumulation may be reliant on the presence of mechanical tension.

Purpose

The purpose of the study is to see if restricting blood flow for 3 minutes post exercise can augment muscle hypertrophy independent of additional mechanical tension. Although metabolites will not be directly measured via muscle biopsy, acute data from our laboratory would suggest that prolonged fatigue resulting from the BFR stimulus is evident 3 minutes post exercise, which is likely indicative of metabolic accumulation sustained within the muscle.

Research Question

Will restricting blood flow for 3 minutes post-exercise following high load training produce greater increases in muscle size and strength than high load training itself?

Hypothesis

There will be no difference in muscle size and strength between the conditions performing high load training and those performing high load training followed by BFR. This is hypothesized as it seems likely mechanical tension from high load training is already maximally stimulating the muscle hypertrophic response, in which case adding additional mechanisms may provide no further benefit.

Significance of Study

The implementation of BFR in combination with resistance exercise involves both mechanical tension and alternative mechanisms thought to be involved in muscle hypertrophy (cell swelling, metabolic stress, etc.). However, traditional BFR protocols are not designed to determine the effects of these alternative mechanisms as they are always followed by subsequent bouts of mechanical tension. Therefore, by restricting blood flow at the conclusion of exercise, our design allows us to analyze the muscle hypertrophic effects of these alternative mechanisms without further mechanical tension. High load exercise produces metabolites that may then be pooled within the muscle at the conclusion of exercise with the application of a restriction stimulus. If the pooling of metabolites can further augment muscle growth, it will provide insight that alternative mechanisms may signal muscle hypertrophy when maintained after mechanical tension. If the application of BFR post exercise does not augment muscle growth, it will provide insight that alternative mechanisms occurring during BFR cannot further augment the hypertrophic response from high load training; or, that these alternative mechanisms will not induce muscle growth without the presence of additional mechanical tension.

Assumptions

1. Participants performed as many repetitions as they could.

2. Participants were properly hydrated before testing for muscle thickness.

3. Participants truthfully answered all questions on the health history questionnaire.

Delimitations

1. The results of the study are indicative of the effects on untrained people between the ages of 18-35.

2. The application may be limited to the limbs.

Limitations

1. It is possible that a crossover in strength may have occurred where the exercised arm increases the strength of the contralateral arm. However, since both arms were being exercised it is unlikely that this crossover in strength had a large impact on the overall strength of each arm (66).

2. Muscle biopsies were not taken to ensure metabolites were present and elevated during the 3 minute post exercise period; however, acute pilot data from our lab demonstrated a prolonged decrement in torque 3 minutes post exercise which is indicative of an increase in metabolites.

3. We only implemented one set of exercise to avoid having the metabolic stress of blood flow

restriction augment the mechanical tension provided by an additional set of exercise. Despite this, one set of exercise appeared to be sufficient for muscle growth.

4. We retrospectively observed sex differences but were not appropriately powered to analyze them. Future studies could seek to analyze these differences.

5. We cannot infer that trapping metabolites per se is not anabolic given that we were also required to restrict arterial blood flow, potentially limiting the nutrient delivery and associative anabolic signaling(89). Despite this potential limitation, this would seemingly be the only plausible way to pool metabolites post-exercise.

Operational Definitions

1. Blood Flow Restriction (BFR) –Exercise performed with a cuff or wrap placed around the most proximal part of the limb to restrict arterial blood flow and occlude venous return.

2. Arterial Occlusion – The lowest pressure at which no pulse can be detected at the wrist.

3. Muscle Thickness – The distance between the muscle-fat interface and underlying bone will be measured via B-mode ultrasound.

4. 1RM – The maximal load that can be lifted one time with proper form for the dumbbell unilateral elbow flexion exercise.

CHAPTER 2: LITERATURE REVIEW

History of BFR

In 1966 Yoshiaki Sato knelt down at a ceremony and noticed the restricted blood flow in his leg from kneeling produced a numb tingling sensation similar to that felt during resistance exercise (84). He hypothesized a similar restriction of blood flow was playing a role in muscular adaptations seen at the conclusion of weight training. Sato then set out to create a pneumatic device that would allow for the inflation of cuffs to be applied during exercise. Several studies have been published demonstrating the effectiveness of blood flow restriction (BFR) at increasing muscle size during walking (2) and resistance exercise (21). These novel findings have provided the basis for what is today known as BFR training.

Implementation of BFR Stimulus

The pressure applied during BFR should be normalized to the individual and can be estimated based off limb circumference and brachial systolic blood pressure (46). The normalization of BFR can be expressed as a percentage of arterial occlusion and allows for a common stimulus to be applied to all participants. In addition to the applied pressure, the width of the cuff has major implications on the overall restrictive pressure as wider cuffs provide greater restriction stimuli at a given pressure (47). Although a wide range of pressures appear to be effective for the BFR stimulus (49), applying higher pressures may increase the risk of injury, while applying too low of a pressure may be ineffective for stimulating muscle hypertrophy.

Protocols involving BFR training are often classified as either continuous or intermittent. Continuous BFR involves applying the restriction stimulus for the entire duration of exercise, while intermittent allows for unrestricted blood flow during rest periods. There is a distinct difference between these two protocols in that continuous BFR results in a significantly greater metabolic accumulation equivalent to that of high load training; however, intermittent BFR does not (86). This can likely be attributed to the trapping of metabolites within the muscle during continuous BFR, while deflating the cuff intermittently allows metabolites to be flushed out of the muscle. Therefore, continuous BFR results in a greater level of perceived discomfort (18) and makes it a difficult application in conjunction with high load training. To illustrate, Laurentino et al. (44) changed protocols to allow for intermittent BFR as participants were unable to withstand the discomfort of continuous BFR when applied during knee extensor exercises completed with a load corresponding to 80% of their one repetition maximum (1RM).

BFR in the Absence of Exercise

Blood flow restriction applied in the absence of exercise has been shown to attenuate atrophy after periods of disuse (11). The application of BFR in the absence of exercise seems to be effective regardless of the pressure applied as both high (40) and low (39) pressures have successfully attenuated losses in muscular strength. The same protocol used in the previous studies consisting of 5 minute inflations followed by 3 minute deflations, was later shown to have no effect on lactate or EMG activity (48). The authors did note an increase in muscle thickness three minutes after the final deflation which indicated a potential role for cell swelling. Applying BFR without exercise can be a useful tool for people on bed rest or those recovering from major surgery by acting as a stepping stone toward increased physical activity (45).

Aerobic BFR Training

Low intensity aerobic exercise in combination with BFR has been shown to increase muscle size (1, 2, 75, 82) albeit to a lesser extent than when applied in conjunction with low load resistance training. When applied during 2 minute walk intervals lasting 15 minutes in duration, BFR increased quadriceps and lower leg muscle thickness (2, 82). The protocol implemented by Abe et al. was performed at a speed of just 50 m/min and was later shown not to result in increased metabolic accumulation (52). This demonstrates that other mechanisms such as cell swelling and mechanical tension are likely responsible for the hypertrophic effects of slow walking in combination with BFR. Additionally, low intensity cycling performed for 15 minutes at 40% $VO_{2\ max}$ increased muscle size, strength, and $VO_{2\ max}$ in a BFR group but not control group (1). These studies demonstrate increases in muscle size can be seen across various modalities of aerobic exercise when combined with the BFR stimulus.

Low Load BFR Resistance Training

Blood flow restriction is most commonly incorporated in conjunction with low load training and has been shown to elicit muscle growth in athletic (57), elderly (92), clinical (61) and rehabilitative (71) populations. The standard protocol for BFR training involves 4 sets consisting of 30, 15, 15, and 15 repetitions, respectively, completed with a training load of 20% or 30% 1RM (54). Low load training in combination with BFR has been shown to elicit increases in muscle size comparable to that of high load training (101) and low load training to volitional fatigue (16, 17). These findings support the importance of BFR training in at risk populations as low load training to failure is reliant on training protocols consisting of upwards of 165 repetitions, whereas BFR reduces the required workload to around 75 repetitions (49). To

further support the efficacy of BFR training, perceptual responses tend to be similar to that of low load resistance training to failure (49) and lower (36) or equal (31) to that of high load resistance training.

High Load BFR Resistance Training

High load resistance training in combination with intermittent BFR has been shown to provide no further augmentation to muscle growth when compared to high load unrestricted resistance training (44). In the aforementioned study, participants exercised under complete arterial occlusion and thus intermittent BFR was used due to high levels of discomfort reported by the participants. This is of importance because, as previously mentioned, intermittent BFR results in a significantly lower metabolic accumulation than that of continuous BFR (86). Additionally, exercising with higher loads under complete arterial occlusion probably resulted in a lower total volume of exercise, although this was not reported. A separate study concluded that intermittent BFR, implemented with high load training, resulted in significantly greater increases in lower body strength than high load training without BFR (12). While significant, the group undergoing BFR saw an approximate 3kg greater increase in squat strength which was likely within the error of the measurement as the 2% increase in strength did not exceed the 2.6% coefficient of variation reported with 1RM squat testing (85). The lack of a measure for muscle size makes it further difficult to conclude whether BFR had a significant effect on high load resistance training.

High Load Training with Short Rest Intervals

High load resistance training completed with shorter rest intervals provides a modality of training that although different, provides some similarities to that of BFR training. When very short rest periods are implemented, a greater buildup of metabolites occurs as there is less time for its clearance between sets. To demonstrate, high load training performed with only 10 seconds rest between sets increased lactate levels to greater than 21 mmol/L (38); increases have also been observed at the conclusion of BFR exercise (87). Reducing the rest intervals during resistance training has been shown to produce greater increases in muscle size and strength despite similar volumes of completed work (94), indicating metabolic accumulation may have helped to further augment the increases in muscle size. The impact of metabolic accumulation per se cannot automatically be credited for the increases in muscle size as further mechanical tension was present. It is plausible that a decrease in the amount of time allowed for recovery may have helped to recruit an increased number of type II muscle fibers in the absence of sufficient recovery time.

What Causes Muscle Hypertrophy?

When protein synthesis exceeds protein breakdown a positive net protein balance occurs, that when maintained over time, results in muscle hypertrophy. A variety of physiological adaptations and responses to exercise are thought to be responsible for increases in protein synthesis through the mechanistic target of rapamycin (mTOR) signaling pathway. mTOR exists in 2 complexes appropriately named mTORC1 and mTORC2 and are distinguished by their interaction with the drug rapamycin. mTORC1, which is inhibited by rapamycin, is the major signaling pathway for protein synthesis and can be stimulated by growth factors, amino acids and

resistance exercise (59). mTORC2 plays a smaller role in the upward signaling of mTORC1 by allowing for full phosphorylation of protein kinase B (Akt) which is responsible for activating mTORC1 (59). The mTORC1 signaling pathway can be activated through a variety of different mechanisms each taking a unique route based on the initial entry point into the cascade. The mechanisms that are driving muscle hypertrophy are likely all converging on the same mTORC1 pathway and thus may only be necessary for muscle growth in the absence of sufficient mechanical tension; i.e. cell swelling and metabolic accumulation may not be necessary for muscle hypertrophy during high load training due to high levels of mechanical tension already maximizing the hypertrophic response.

The idea that low load training to failure (20-30%1RM), and low load BFR training (20-30%1RM) have both been shown to stimulate muscle hypertrophy provides evidence there are mechanisms other than mechanical tension alone that can promote increases in muscle size (16, 17). One proposed hypothesis is that an accumulation of metabolites, most notably hydrogen ions and lactate, causes a decreased intramuscular pH which may then stimulate muscle growth through a variety of mechanisms (53). However, an increase in metabolites cannot explain the full hypertrophic response occurring during BFR exercise as low intensity walking in combination with BFR elicited muscle growth (2) using a protocol that was later shown not to result in a significant accumulation of metabolites (52). The most robust increases in muscle size are seen when BFR is combined with low load resistance training. When low load resistance exercise is combined with BFR there is an increase in metabolic accumulation, motor unit recruitment and cell swelling. However, it is not presently known whether the robust increases in muscle size from BFR in combination with low load resistance training are a product of increased metabolic accumulation and/or cell swelling per se, or simply increased muscle

activation through metabolically fatigued muscle fibers.

Mechanical Tension

Mechanical tension refers to the load placed on a muscle during resistance exercise and is likely the primary stimulator of muscle hypertrophy during high load resistance training. In the absence of heavier loads, mechanical tension alone is not likely sufficient enough to maximally stimulate muscle hypertrophy during low load/low intensity BFR exercise. Although currently unknown, it seems likely that some level of mechanical tension must be present along with another hypertrophic mechanism to produce increases in muscle size from BFR training. Mechanical tension works to stimulate muscle hypertrophy through mechanoreceptors within the muscle that sense levels of tension and respond by activating a protein kinase that eventually activates mTORC1 (59). Mechanical tension during eccentric contractions in rats has been shown to increase phosphorylation of the Tuberous Sclerosis 2 (TSC2) complex, which serves to suppress mTORC1, in which case the phosphorylation of TSC2 would lead to a greater activation of mTORC1 (34). Additionally, greater increases in muscle strength and size are often reported in eccentric as opposed to concentric contractions (93) further supporting the importance of mechanical tension for driving muscle growth. However, when comparing eccentric and concentric isotonic exercise in combination with BFR, greater increases in both muscle size and strength were seen following concentric only training (100). The discrepancy in size and strength was likely due to the increased metabolic accumulation occurring with concentric exercise (73) further suggesting its involvement in muscle growth.

Satellite Cells

It has long been hypothesized that muscle damage may be important for increases in muscle size (102). Mauro (62) accurately predicted satellite cells, located between the

sarcolemma and basement membrane, become activated when stressed, and enter into the sarcoplasm potentially merging with existing myofibers. While BFR exercise may not result in measurable muscle damage (50, 97), proliferation of myogenic stem cells has been shown to occur from BFR training (70). The authors concluded this proliferation of myogenic stem cells may have been due to muscle cell swelling, a hypoxic like stimulus, and/or may have been due to the release of hepatocyte growth factor by means of nitric oxide. The increase in myogenic stem cells is of importance as myonuclei are responsible for supplying mRNA transcripts, and when an insufficient number of myonuceli are present, a muscle cell can no longer grow as the myonucleur domain becomes too large to maintain (91). Work from Stuart Phillips' laboratory recently noted a direct correlation between accretion of satellite cells and muscle growth following high load exercise (5), suggesting they may be responsible to some extent for increases in muscle size. Removing satellite cells, however, has no negative effect on short term muscle hypertrophy (64), demonstrating they are likely only required for long term growth given the myonuclear domain can expand before muscle growth is limited by insufficient myonuclei.

Systemic Hormones

Systemic hormones released during resistive exercise have been long thought to be responsible for increases in muscle size (37) and have been proposed as a mechanism during BFR exercise (58). Work from Stu Phillips' laboratory has recently demonstrated increases in post-exercise systemic hormones likely have little to no effect on muscle size or strength following traditional high load protocols (96). In contrast to traditional high load training, BFR has been shown to increase growth hormone by 290 times that of resting levels (88). This still seems unlikely to have a large impact on muscle hypertrophy as injecting pharmacological doses of growth hormone has been shown to have no positive benefit on increasing muscle size in

adults (6). Further support against systemic hormones playing a large role is the observation that muscle hypertrophy has been shown to occur in BFR but not in non-exercised control limbs (35) despite both limbs being exposed to elevated systemic hormones. Additionally, the most likely hormone to cause anabolic adaptations, testosterone, is either minimally elevated for less than 15 minutes (58) or unchanged (21, 81) post BFR exercise.

Growth Factors

Insulin like growth factor 1 (IGF-1) is secreted by the liver in response to resistance training, and while not mandatory for muscle growth, has been positively correlated (27). An IGF-1 receptor on the sarcolemma is activated by stretching of the muscle and can initiate the mTORC1 cascade as well as the accretion of myonuclei through satellite cell proliferation (24). While systemic IGF-1 does not appear to be elevated substantially (58, 87) or even at all following BFR exercise (20, 21, 77), local IGF-1 produced in skeletal muscle exists in a different isoform (i.e. mechano-growth factor) and may assist in muscle hypertrophy when activated through heavy loading or stretching (83), with the latter possibly occurring during BFR training.

Cell Swelling

Cell swelling occurs when fluid shifts from the plasma into the muscle, but unlike venous pooling of blood, is maintained for at least several minutes post exercise. Blood flow restriction training to failure has shown to increase muscle cell swelling as much as (16) or more so (17) than low load training to failure. The discrepant findings may be due to the load used (30 vs 40% 1RM) whereas heavier loads in conjunction with BFR may promote greater swelling. It has been proposed that the swelling of a muscle can activate a volume sensing G-protein receptor in similar fashion to that of a mechanical sensor during high load training (56). This activation of the G-Protein receptor would then begin the cascade of phosphorylating proteins down the

mTORC1 pathway stimulating muscle growth.

Muscle Activation

The occurrence of muscle hypertrophy is likely dependent upon a high activation of type II fibers which seems to be the one variable constant across all protocols shown to elicit robust muscle growth. While slow walking in combination with BFR has been shown to produce increases in muscle size (2), these increases are only marginal in comparison to muscle growth seen when BFR is combined with low load resistance training (54); which consequently may be attributed to a limited reliance on type II fiber activation. During muscle contraction, type I fibers are preferentially recruited unless a great enough stimulus is needed to recruit both type I and type II fibers (30). Therefore, during low load resistance exercise, type II fibers are rarely recruited which results in a fewer quantity of fibers to be stimulated for growth. Applying BFR has been shown to increase muscle activation to a greater extent than repetition matched low load training during exercise (67, 88) and immediately after rest intervals (99); demonstrating that BFR both increases and prolongs muscular fatigue. When low load training is performed to volitional fatigue, electromyography (EMG) activity is similar (17, 49) or slightly greater (16) than that of BFR training. Interestingly, even with lower EMG activity (55 vs. 65% of maximal isometric strength recorded during the final set), Fahs et al. noted greater increases in lateral thigh muscle size from BFR training. Additionally, when compared to high load training, BFR has been shown to produce equal (86) or slightly lower levels of muscle activation (49, 98). Regardless, the similarity in muscle activation is likely due to an increase in type II fiber activation to assist in lifting a heavier load during high load training; whereas, in BFR training, the prolonged fatiguing of type I fibers requires the additional recruitment of type II fibers to account for the loss of force production (8).

Reduction in Protein Breakdown

An alteration in protein balance can occur by an increase in muscle protein synthesis or a decrease in muscle protein breakdown (MPB). Some negative regulators of muscle growth such as myostatin serve to inhibit mTORC1, while others are directly involved in the degradation of proteins through the ubiquitin proteasome pathway. Basal levels of myostatin messenger RNA (mRNA) expression appear to decline to a slightly greater extent at the conclusion of low load BFR training when compared to high load training completed over 16 training sessions (43). Differential results have been found when assessing MPB after the completion of traditional high load resistance exercise. One study reported elevated MPB at 3 and 24 hours post exercise in a fasted state (79), while another study reported no increase in MPB when assessed 24 hours post exercise in a fasted state (19) despite implementing similar volumes of exercise. Although statistically different outcomes were reported, these values were similar in that Phillips et al reported an 18% increase in MPB while Fry et al. reported a 16% increase. Contrary to traditional high load training, fractional MPB taken in the fasted state at the conclusion of BFR exercise revealed no change at 6 or 24 hours post exercise (26). Since MPB remained unaltered when measured 6 hours post exercise, it would appear any increase in MPB occurring from low load BFR exercise would be limited to shorter post exercise durations than that of high load training.

CHAPTER 3: METHODS

Participants

Sixteen untrained participants were recruited to participate in the study. The sample size was chosen based on an estimated effect size of 0.79 which was averaged from three similar studies [0.53 (32), 0.63 (17) and 1.2 (100)]. Using G*Power software (GPower 3.1), an estimated sample size of 12 people was recommended to appropriately observe statistical significance at the 0.05 alpha level with a power level of 0.8. Inclusion criteria were as follows: (1) must be untrained in the upper body for at least one year; (2) cannot be using tobacco; (3) cannot have had more than one risk factor for thromboembolism (69); (4) and must have been between the ages of 18 and 35. All participants provided informed written consent for this study which was approved by the university's institutional review board.

Study Design

On visit one participants filled out initial paperwork to ensure they were eligible for participation. Following, and in a counterbalanced fashion, participants had one arm assigned as the experimental arm while the other arm served as the control arm. Participants then had their height and body mass measured before undergoing 10 minutes of seated rest. Following rest, participants had their arterial occlusion pressure measured on their experimental arm and were then familiarized with the isokinetic and isometric strength tests. On visit two, participants had their anterior upper arm and thigh (internal control) muscle thickness measured before being tested for maximal isokinetic, isometric, and isotonic (one repetition maximum (1RM)) strength of both arms. Visits 3-26 consisted of exercise training three times for week with each visit

separated by at least 48 hours. Visit 27 was held 48-72 hours after the final testing visit and consisted of muscle thickness, and isokinetic, isometric and 1RM strength testing.

Arterial Occlusion

Following 10 min of seated rest, participants were asked to stand and a 5 cm nylon cuff (Hokanson, Bellevue, WA, USA) was placed at the most proximal part of the arm. With an MD6 Doppler probe (Hokanson, Bellevue, WA, USA) at the radial artery, the cuff was inflated by one mmHg increments until a pulse was no longer detected at the wrist. The lowest pressure in which a pulse was no longer present was recorded as the individual's arterial occlusion pressure. The arterial occlusion measure was taken to allow for the restrictive stimulus to be made relative to each individual as suggested previously (47).

Isometric and Isokinetic Strength

Participants were seated on a dynamometer (Biodex Medical Systems, Shirley, New York, USA) with the seat and lever arm adjusted appropriately and the settings recorded and standardized for all future tests. The dynamometer was adjusted for each individual and all settings were recorded to ensure a similar testing protocol throughout. After weighing the individuals arm to correct for gravity, participants performed 3 successive isokinetic contractions at 180°/s and then rested for 90 seconds before repeating the test again at the same speed. After another 90 seconds of rest participants performed the same procedure involving 2 sets of 3 isokinetic contractions at 60°/s. Following another 90 seconds of rest, participants performed isometric testing in the same position. The lever arm was locked into place at 60° and participants performed 2 maximal isometric contractions each lasting 3 seconds in duration and separated by 1 minute of rest. The highest value for each test was recorded as the maximum peak torque.

One-Repetition Maximum (1RM) Strength

After a brief warmup consisting of 7-10 repetitions with approximately 30% of the individuals estimated 1RM, the load was increased to approximately 70% 1RM and one repetition was performed. After increasing the load to an estimated 90% 1RM individuals performed a 1RM attempt. The load was then progressively increased until the individual could no longer perform the exercise through a full range of motion with proper form. All 1RM attempts were separated by approximately 90 seconds rest and were performed with the individuals back and heels against a wall to ensure strict form. All 1RMs were measured to the nearest 0.5 kg and were usually obtained in around 5 attempts. The 1RM tests were performed pre and post exercise, as well as during the 13th (i.e. the midpoint) visit in order to readjust the training load.

Muscle Thickness

An Aloka SSD-500 B-mode ultrasound (Aloka Co. Ltd., Tokyo, Japan) was used to measure the distance between the muscle-fat and muscle-bone interface by an experienced tester. All images were printed and analyzed by the same person who was blinded to the condition. Three images were taken at each of three sites including 50%, 60%, and 70% the distance between the lateral epicondyle and the acromion process. An additional measure of thigh muscle thickness was taken at 50% of the distance between the lateral epicondyle of the femur and the greater trochanter and was used to assess the stability of the measurement over time. Participants were asked to refrain from any planned exercise within 24 h of muscle thickness measures. The minimal difference (i.e. reliability) needed to be considered real for the anterior portion of the upper and lower arm was calculated at 0.2 cm prior to the investigation using the procedure detailed previously by Weir (95).

Arm Circumference

The distance from the acromion process to the lateral epicondyle was measured with a standard tape measure and a mark was made 10 cm proximal to the lateral epicondyle. Circumference measures were taken on both arms every training visit prior to exercise.

Training Protocol

In a counterbalanced fashion individuals were assigned one arm to serve as the experimental arm and one arm to serve as the control arm. On training visits individuals performed one set of standing elbow flexion exercise to volitional fatigue using a load corresponding to 70% of the predetermined 1RM for that arm. All exercise was performed to the beat of a metronome allowing 1 second for the concentric and 1 second for the eccentric portion of the exercise. During each training session, individuals alternated which arm exercised first and 5 minutes of rest preceded exercise of the contralateral arm. Both arms exercised with a 5 cm nylon cuff (Hokanson, Bellevue, WA, USA) placed at the most proximal part of the arm. Upon completion of the final repetition, the control arm had the pressure cuff removed immediately, while the experimental arm had the cuff inflated to 70% of their predetermined arterial occlusion pressure for 3 minutes. During the 3 minute post-exercise period individuals were required to remain standing with their arms kept loosely at their side.

Ratings of Perceived Discomfort

The Borg (CR10+) scale was used to assess ratings of discomfort before, immediately post, and 1,2, and 3 min post-exercise. The scale was explained in depth to all participants on the initial training visit and all participants fully understood the scale. As described previously (51), participants were asked ‘‘What are your worst experiences of discomfort? ‘Maximum discomfort (rating of 10)’ is your main point of reference; it is anchored by your previously experienced

worst discomfort. The worst discomfort that you have ever experienced, the 'Maximum discomfort' may not be the highest possible level of discomfort. There may be a level of discomfort that is still stronger than your 10; if this is the case, you will say 11 or 12. If the discomfort is much stronger, for example, 1.5 times 'Maximum Discomfort' you will say 15; any questions?''

Statistical Analysis

Using the SPSS 20 statistical software package (SPSS Inc., Chicago, IL) a 2 (condition) x 3 (time) repeated measures analysis of variance (ANOVA) was used to determine significant changes in 1RM strength. If an interaction was present a one ANOVA way was used to compare differences across time for each condition and a paired t test was used to compare differences between conditions at each time point. If no interaction was present main effects of time and condition were interpreted. Additionally, a 2 (condition) x 2 (time) repeated measures ANOVA was used to compare differences for muscle thickness, isometric strength, isokinetic strength, volume, repetitions, and circumference. If there was an interaction paired t tests were used to compare differences across time and to compare differences between conditions at each time point. If no interaction was present main effects of time and condition were interpreted. Finally, a Wilcoxin signed-rank test was used to compare ratings of discomfort between conditions at each of the 5 time points. The level of significance will be set at $p \leq 0.05$ for all statistical tests.

CHAPTER 4: RESULTS AND DISCUSSION

All data are reported as means (standard deviations) with the exception of discomfort which is reported as 50th percentile (25th percentile, 75th percentile).

Demographics

A total of 16 individuals were recruited for participation in the study. Three individuals withdrew for personal reasons unrelated to participation in the study, and thus data for 13 individuals (6 males and 7 females) was included in the analysis (Table 1). The average age, height and body mass, respectively, were 22 (3) years, 169.1 (9.4) cm and 76.2 (20.0) kg (Table 2). The average arterial occlusion pressure measured before exercise was 152 (25) mmHg which corresponded to 106 (17) mmHg applied as the 70% arterial occlusion post-exercise.

Table 1. Individual Demographics

ID	Sex	Age (years)	Height (cm)	Body Mass (kg)	Total AOC (mmHg)	70% AOC (mmHg)
2	M	20	161.6	56.6	111	78
3	F	20	165.7	48.1	140	98
4	F	23	161.7	53	143	100
5	F	21	163.8	95.4	154	108
7	M	26	183.7	104.2	164	115
8	F	21	168.4	84.2	205	144
9	F	21	153.8	72.3	180	126
10	F	23	162.6	62	133	93
12	M	21	168.2	89	134	94
13	F	24	177.7	66.2	138	97
14	F	25	166.8	73.4	163	114
15	F	20	183.6	113.7	182	127
16	F	32	180.8	73.7	136	95

AOC = arterial occlusion pressure, M=male, F=female

Muscle Thickness

There was no condition x time interaction (p=0.31), main effect of condition (p=0.71) or main effect of time (p=0.19) at the 50% site of the anterior upper arm (Table 3, Figure 1). At the 60% site, there was a condition x time interaction (p=0.03) with the control condition increasing from pre to post (p=0.042), however, no change was observed in the experimental condition from pre to post (Table 3, Figure 1, p=0.74). Additionally, when examining post muscle thickness at the 60% site, there was a trend toward greater muscle thickness in the control vs. experimental condition (p=0.06). At the 70% site, there was no condition x time interaction (p=0.90) or main effect of condition (p=0.177), however there was a main effect of time (Table 3, Figure 1, p=0.006) with muscle thickness increasing from pre to post. For thigh muscle thickness which served as the internal control [control pre: 3.9 (1.4) cm, control post: 3.9 (1.3) cm; experimental pre: 4.0 (1.2) experimental post: 4.1 (1.4)], there was no condition x time interaction (p=0.25), main effect of condition (p=0.55) or main effect of time (p=0.29) suggesting our measurements were stable across time. Interpreting these changes within the context of our reliability, we are confident that we have indeed measured muscle growth as opposed to edema (14) because we did not see increases in arm circumference, did not observe any pre exercise discomfort during any of the training sessions, and performed the post-training muscle thickness measurement 48-72 hours after training which has previously demonstrated to be ample time for swelling to subside when assessed at the fiber level (70).

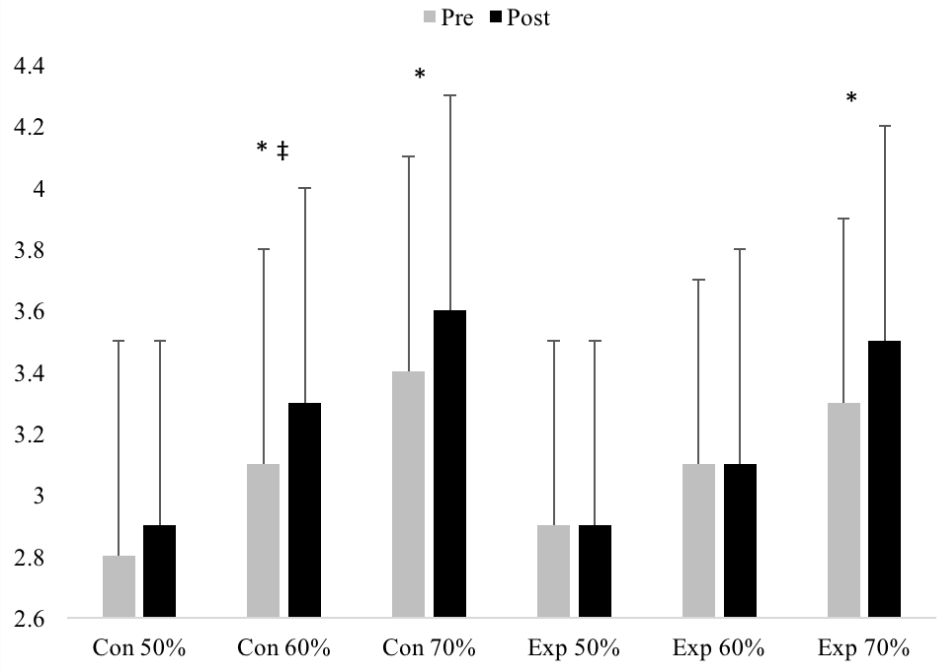
Table 2. Muscle Thickness

	Control			Experimental		
Location	**Pre**	**Post**	Δ	**Pre**	**Post**	Δ
50%	2.8 (0.7)	2.9 (0.6)	0.1	2.9 (0.6)	2.9 (0.6)	0.0
60%	3.1 (0.7)	3.3 (0.7)*	0.2	3.1 (0.6)	3.1 (0.7)	0.0
70%	3.4 (0.7)	3.6 (0.7)*	0.2	3.3 (0.6)	3.5 (0.7)*	0.2
Thigh	3.9 (1.4)	3.9 (1.3)	0.0	4.0 (1.2)	4.1 (1.4)	0.1

All values (cm) are presented as mean (standard deviation) *statistically significant from pre value

When analyzing individual responses at or exceeding 0.2 cm (error of measurement), there were a greater number of participants displaying meaningful increases in the control condition at each the [50% (control = 46% vs. experimental = 23%), 60% (control = 53% vs. experimental = 15%), and 70% sites (control = 61% vs. experimental = 38%) Table 4]. Individual responses displaying the within participant difference in muscle growth between the control and experimental condition at each site are displayed in Figure 2. That is, for each of the 3 sites (i.e. 50, 60, 70%) on all 13 individuals (total of 36 calculations), the pre to post change in muscle thickness of the control condition was subtracted from that of the experimental condition (Δ experimental - Δ control). Therefore, a negative value demonstrates a more advantageous effect of muscle size for the control condition, while a positive value demonstrates a more advantageous effect of for the experimental condition. A value of 0 illustrates a similar response between the experimental and control conditions. Furthermore, the individual pre to post changes for the experimental and control conditions are displayed in Figure 3.

Figure 1. Muscle Thickness



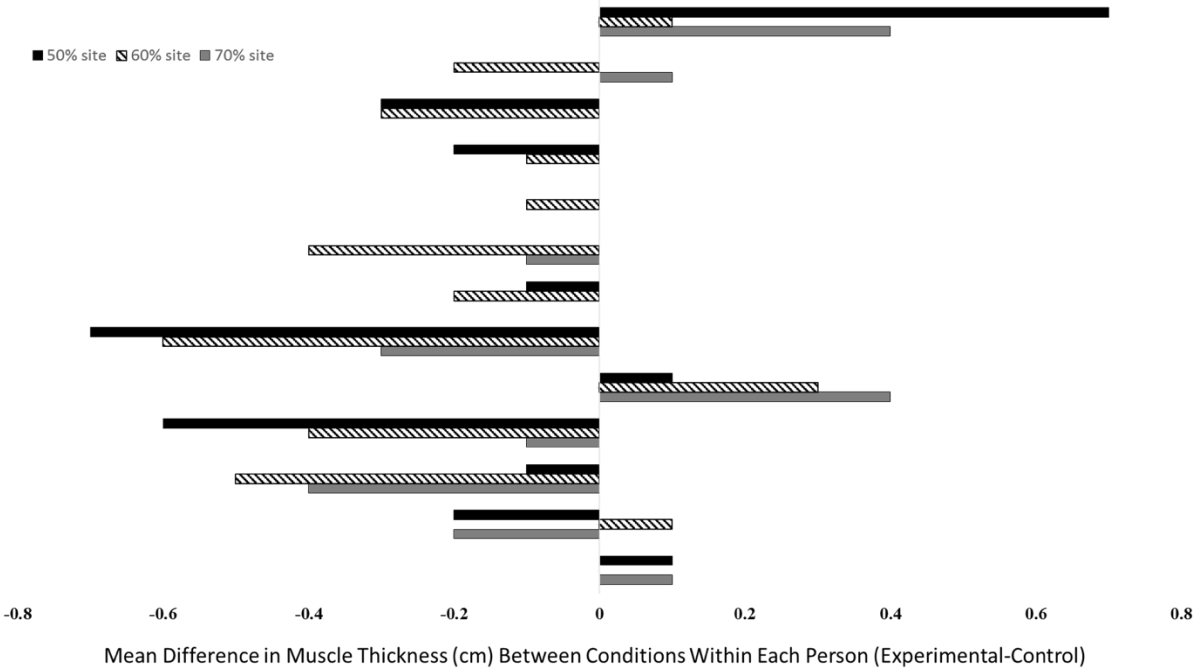
Values are expressed as mean (standard deviation). The 50%, 60% and 70% sites indicate the location of muscle thickness measured as the distance from the lateral epicondyle to the olecranon process. con = control, exp = experimental, *significantly different from pre, ‡ condition x time interaction with the control condition trending toward being greater than the experimental at the post measure (p=0.06).

Table 3. Meaningful Increases in Muscle Thickness

	Control	Experimental
50%	6	3
60%	7	2
70%	8	5

Values are individual participants meeting or exceeding the error (0.2 cm) of the measurement. The 50%, 60% and 70% sites indicate the location of muscle thickness measured as the distance from the lateral epicondyle to the olecranon process.

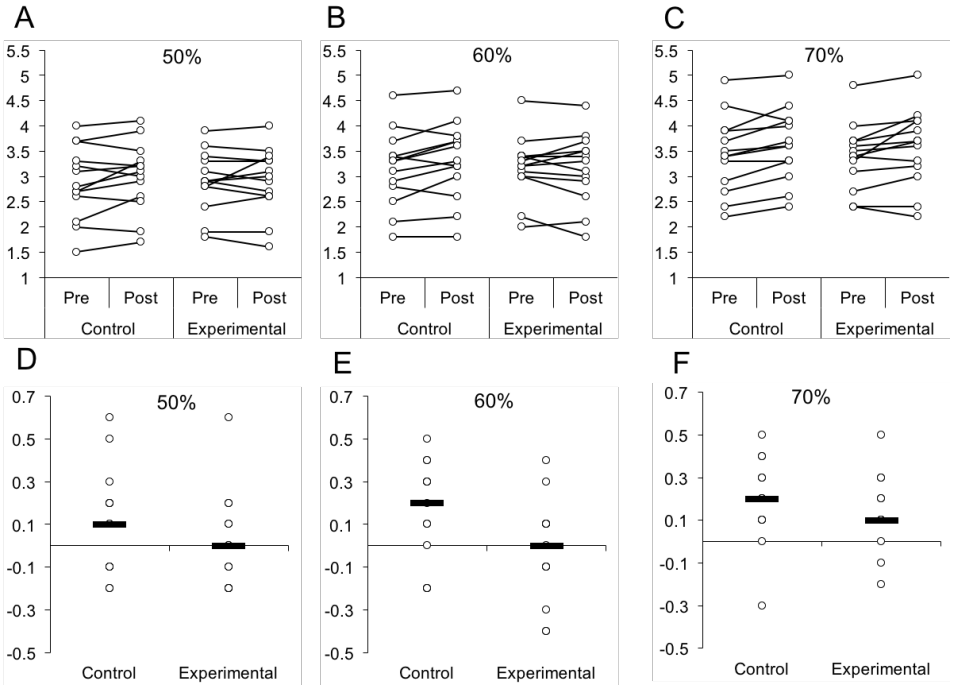
Figure 2. Within Subject Differences in Muscle Thickness Between Conditions



Values are calculated for each individual at each location using the equation (Δ experimental - Δ control). A positive value favors muscle growth in the experimental condition, a negative value favors muscle growth in the control condition, and a value of 0 indicates a similar response between the control and experimental condition.

The 50% site was the only measured site where muscle growth was not observed at the group level in either the control or experimental condition. It has previously been suggested that the application of a pneumatic cuff during resistive exercise may attenuate growth of muscle tissue placed under the cuff (15, 35). While, we applied a cuff that was not inflated, there still was some restriction placed on the anterior upper arm, particularly during the concentric portion of the exercise. Even so, muscle growth has been shown to occur in exercised tissue located under restriction (42), leaving open the possibility that the volume of exercise performed in the present study (1 set) was not sufficient to activate the more proximally located portion of the elbow flexors. We also cannot rule out the possibility that the 50% location was not activated enough during the elbow flexion exercise given the proximal location that was measured.

Figure 3. Individual Changes in Muscle Thickness



A-C illustrate pre to post values (cm) at each the 50, 60 and 70% sites measured for muscle thickness. D-F illustrate the pre to post changes in muscle thickness (cm) with circles representing each individual and the solid black line representing the median pre to post change (some circles may represent more than one individual, if they both had similar median differences); exp=experimental, con=control.

The 60% location clearly displays that the experimental condition appeared to be attenuated by the post-exercise application of BFR (Figure 1, Table 3). While statistically significant at the group level, this was also observed at each of the 3 measured sites when examining individual responses (Figure 2, Figure 3). While we hypothesized there would be no difference in muscle growth between conditions, we thought it would be plausible that the experimental arm would see a greater increase in muscle size given the trapping of metabolites post-exercise. For example, lactate has been shown to induce hypertrophy when administered *in vitro* and in mouse models performing treadmill exercise (72). While unexpected, the attenuated growth in the experimental condition may be partially explained by the location and quantity of

reactive oxygen species produced. For example, reperfusion results in a drastic increase in reactive oxygen species produced within the mitochondria which may serve to inhibit upstream activators of the mechanistic target of rapamycin (mTOR), whereas traditional exercise produces reactive oxygen species at locations along the sarcolemma (e.g. NADPH and Xanthine oxidases) (60) thought to be involved in mechanotransduction (33). Additionally, it has been shown that muscle contraction during BFR alleviates some of the oxidative stress caused by vascular occlusion (22, 23), and therefore, it is possible that the magnitude of oxidative stress caused by high load training combined with post-exercise occlusion exceeded that which has been speculated to be beneficial (7, 60, 65).

In addition to the possibility that metabolites may have been detrimental through oxidative stress, it is possible that metabolic accumulation may have been detrimental through activation of the energy sensing AMP-activated protein kinase (AMPK) complex, which serves to decrease protein synthesis and elevate proteolysis (28). We cannot strictly attribute the detrimental effects observed to the trapping of metabolites, however, as we also restricted arterial blood flow for a 3 minute post-exercise period. It has previously been suggested that an increase in post-occlusive blood flow may be responsible for some of the adaptation provided by blood flow restriction (76), and while this has been refuted elsewhere (25), the elevation in blood flow caused by sodium nitroprusside did not match the immediate post-exercise elevation in blood flow caused by BFR. This restriction of blood flow could have potentially limited post-exercise nutrient delivery and associative anabolic signaling (89). The limited arterial blood flow could also have induced a state of hypoxia which could serve to increase protein degradation through up-regulation of the E3 ligases muscle ring finger-1 (MURF1) and muscle atrophy F box (MAFbx) associated with the ubiquitin proteasome system (10). Despite this finding, the increase

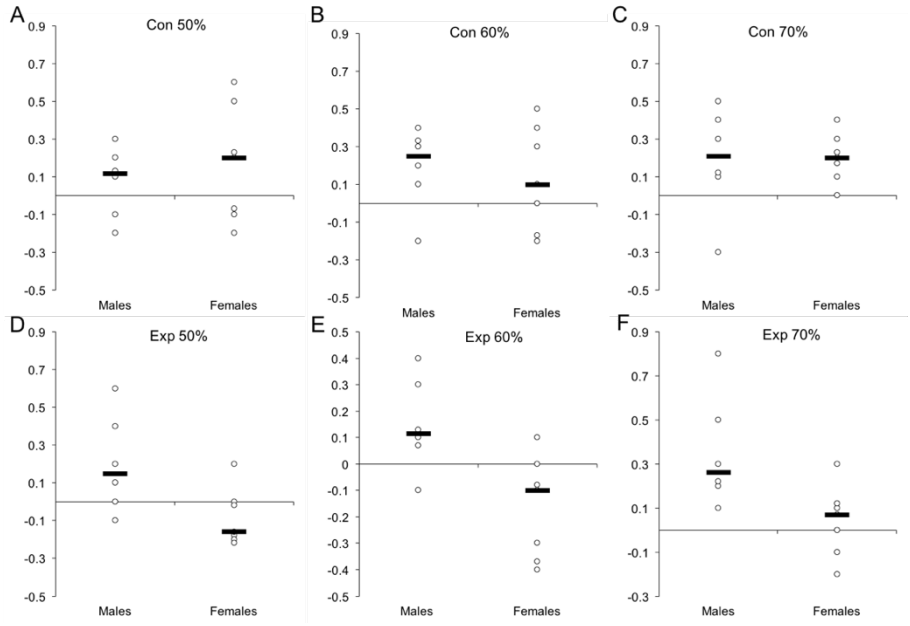
in E3 ligases may be attributed to remodeling associated with increased protein synthesis (4) occurring from the synergistic ablation procedure as opposed to the hypoxic environment. While it has previously been hypothesized that BFR may augment muscle growth by decreasing myostatin mRNA (43), it is possible that our protocol may have actually increased myostatin mRNA from the hypoxic stimulus (29), resulting in a decrease in protein synthesis through inhibition of protein kinase B (Akt), an upstream activator of mTOR. The increase in myostatin mRNA resulting from hypoxia appeared to diminish, however, with the presence of functional overload (10). Additionally, the present study differs from systemic hypoxia in that we analyzed a localized hypoxic-like stimulus specific to the arm, which may have produced different results.

It could be hypothesized that a larger muscle would be capable of producing a greater number of metabolites, and therefore the larger amount of muscle mass present in males may produce different results from the post-exercise application of BFR. For this reason, we retrospectively analyzed differences between males and females in relation to changes in muscle thickness (Figure 4, Table 5), but did not perform additional statistical analyses because we were not powered to do so. However, when examining Figure 4, there were only two experimental conditions that decreased from pre to post in males, whereas 10 conditions decreased in females. Additionally, when analyzing Table 5, it would appear that females were more negatively impacted in the experimental condition when compared to males.

This detrimental effect of post-exercise BFR being specific to females is difficult to explain, but may be related to differences in metabolites and/or fiber type composition between genders. For example, given that females possess a larger percentage of type I fibers in comparison to males, it is possible that females produced a greater number of mitochondrial reactive oxygen species (80). Despite the production of endogenous antioxidants within the

mitochondria(80), it is possible that the ratio of reactive oxygen species exceeded that of antioxidants, thus resulting in detrimental levels of oxidative stress (7, 60, 65), particularly when produced within the mitochondria (60). Another possible explanation is that because males express more type II muscle fibers they produce a greater amount of lactate during exercise. If lactate is indeed anabolic as previously suggested (72), it is possible that the anabolic effects of lactate were sufficient to overcome the catabolic effects caused by restricting blood flow for 3 minutes post-exercise.

Figure 4. Sex Differences in Muscle Thickness



Values are expressed in cm as (post – pre). Circles represent each individual change and the solid black line represents the median pre to post change (some circles may represent more than one individual, if they both had similar median differences); exp = experimental, con=control.

Table 4. Sex Differences in Muscle Thickness

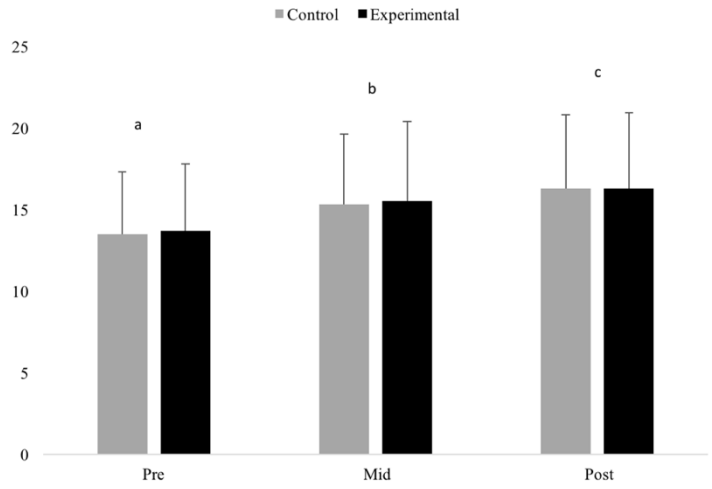
	Pre 50%	**Post 50%**	Δ
Males Control	3.4 (0.4)	3.5 (0.4)	0.1
Male Experimental	3.2 (0.4)	3.3 (0.3)	0.1
Females Control	2.4 (0.5)	2.5 (0.5)	0.1
Females Experimental	2.6 (0.5)	2.5 (0.5)	-0.1
	Pre 60%	**Post 60%**	Δ
Males Control	3.6 (0.5)	3.8 (0.4)	0.2
Male Experimental	3.5 (0.5)	3.7 (0.3)	0.2
Females Control	2.6 (0.5)	2.8 (0.6)	0.2
Females Experimental	2.8 (0.5)	2.7 (0.5)	-0.1
	Pre 70%	**Post 70%**	Δ
Males Control	4.0 (0.5)	4.2 (0.4)	0.2
Male Experimental	3.8 (0.5)	4.1 (0.4)	0.3
Females Control	2.9 (0.5)	3.1 (0.4)	0.2
Females Experimental	3.0 (0.5)	3.0 (0.5)	0

All values (cm) are expressed as mean (standard deviation).

One-Repetition Maximum Strength (1RM)

There was no condition x time interaction ($p=0.94$) or main effect of condition ($p=0.77$), however, there was a main effect of time ($p<0.001$) with 1RM strength increasing from pre to mid ($P<0.001$), mid to post (Table 6, $p=0.002$), and pre to post ($p<0.001$).

Figure 5. One Repetition Maximum (1RM) Strength



Values (kg) are presented as mean (standard deviation). There were no significant differences between conditions. Letters indicate significant differences from one another.

The increase in 1RM strength was similar for both the control and experimental condition, which is not surprising given that both arms performed the identical protocol throughout the study duration. The dissociation between muscle size and strength has been documented previously (66), and is likely related to the principle of specificity. Whereas muscle growth is largely reliant on fatiguing the muscle (66) and increasing muscle activation (68), increases in 1RM strength can be accomplished by adhering to the principle of specificity and performing resistance exercise at or near an individuals 1RM for that particular exercise (74). The individual responses in 1RM strength appear to be fairly consistent across the entire study population (Figure 6). These results demonstrate that pooling metabolites post-exercise did not appear to have any effect at the individual level either.

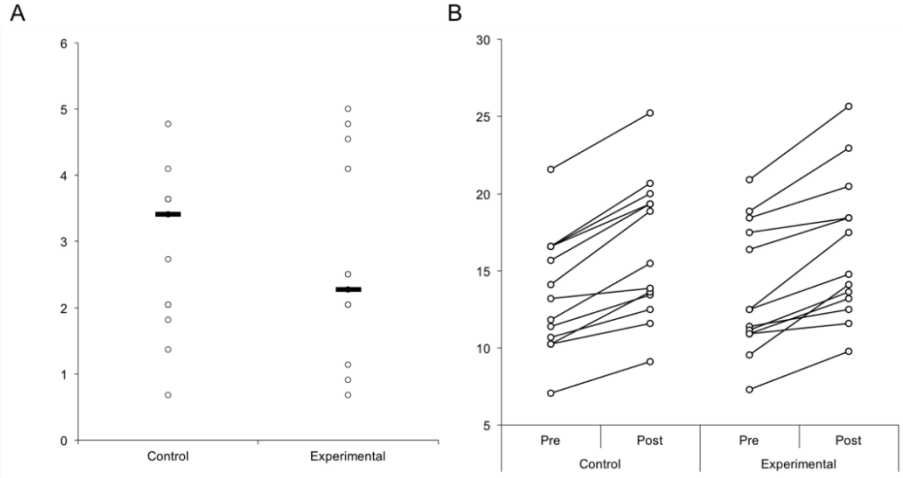
Table 5. One Repetition Maximum (1RM) strength

	Pre [a]	Mid [b]	Post [c]
Control	13.5 (3.8)	15.3 (4.3)	16.3 (4.5)
Experimental	13.7 (4.1)	15.5 (4.9)	16.3 (4.6)

Values are expressed in kg as mean (standard deviation). Letters indicate significant differences. Both conditions increased from pre to mid and mid to post with no differences between conditions.

The differences in 1RM strength were quite large with some individuals increasing 1RM strength by less than 1 kg, while others increased by more than 5 kg (Figure 5B). This variability in individual responses has been observed previously in a large cohort of individuals also performing elbow flexion exercise (32). Furthermore, males in the study saw greater absolute increases in 1RM strength (3.4 vs. 1.6 kg); however, when expressed relative to pre-training values males and females saw similar relative increases (19 vs. 15%) (Table 7), which is supportive of previous research (32).

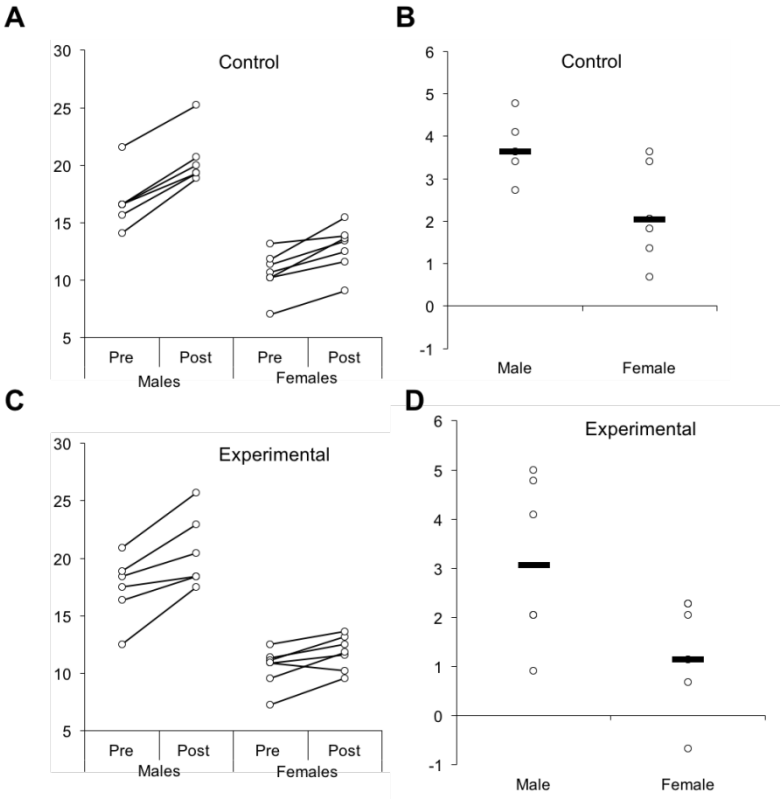
Figure 6. Individual Changes in One-Repetition Maximum (1RM) Strength



Data are expressed in kg. A. Individual changes in 1RM strength. B. Pre to post changes in 1RM strength. Circles represent individual changes and the solid line represents the group median.

The differences in 1RM strength were quite large with some individuals increasing 1RM strength by less than 1 kg, while others increased by more than 5 kg (Figure 5B). This variability in individual responses has been observed previously in a large cohort of individuals also performing elbow flexion exercise (32). Furthermore, males in the study saw greater absolute increases in 1RM strength (3.4 vs. 1.6 kg); however, when expressed relative to pre-training values males and females saw similar relative increases (19 vs. 15%) (Table 7). This is supportive of previous research examining sex differences in elbow flexion 1RM strength (32).

Figure 7. Sex Differences in One-Repetition Maximum (1RM) Strength



All values are expressed in kg. Figures A and C illustrate pre to post changes for each specific individual. Figures B and D illustrate changes in 1RM strength (post – pre) with circles illustrating individual responses and the solid line depicting the median value (some circles may represent more than one individual, if they both had similar median differences).

Given the differences in the control and experimental conditions present in females for

muscle thickness, additional individual plots were created to compare pre to post differences in 1RM strength between conditions and across sex (Figure 7). We did not observe any clear pattern for 1RM strength between conditions for either males or females. Specifically, the detrimental effect observed in the experimental condition of females did not result in reductions in 1RM strength. While Table 7 illustrates a trend toward the experimental condition resulting in greater increases in 1RM strength among both males and females, the individual plots reveal that this may be largely driven by the responses of one male and one female included in the analysis (Figure 7).

Table 6. Sex Differences in One-Repetition Maximum (1RM) Strength

	Pre	Post	Δ
Male Control	16.8 (2.5)	20.5 (2.3)	3.7 (22%)
Male Experimental	17.4 (2.8)	20.5 (3.1)	3.1 (17%)
Female Control	10.6 (1.8)	12.7 (2.0)	2.1 (19%)
Female Experimental	10.5 (1.6)	11.7 (1.4)	1.2 (11%)

Values (kg) are expressed as mean (standard deviation).

Isometric and Isokinetic Strength

For isokinetic strength at 180°/s there was no condition x time interaction (p=0.40) nor was there a main effect of condition (p=0.253) or time (p=0.975) (Table 8, Figure 8). For isokinetic strength at 60°/s there was no condition x time interaction (p=0.81) nor was there a main effect of condition (p=0.138) or time (p=0.562) (Table 8, Figure 8). With regard to isometric strength, there was no condition x time interaction (p=0.285) nor was there a main effect of condition (p=0.507) or time (p=0.963) (Table 8, Figure 8).

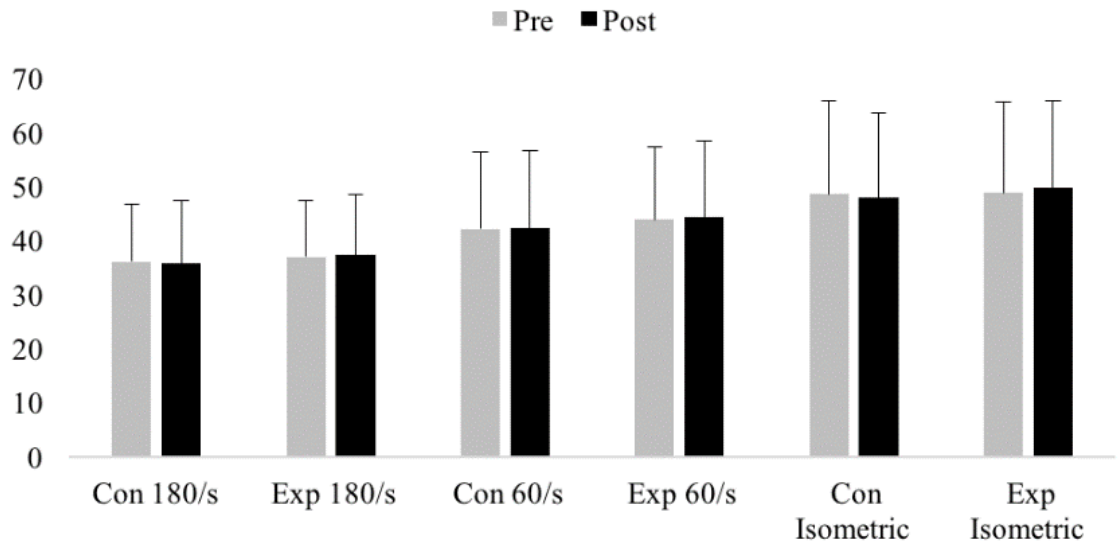
Table 7. Isometric and Isokinetic Strength

	Control		Experimental	
	Pre	Post	Pre	Post
Isokinetic 180°/sec	36.0 (10.6)	35.7 (11.7)	37.0 (10.3)	37.3 (11.2)
Isokinetic 60°/sec	42.1 (14.3)	42.3 (14.4)	43.8 (13.5)	44.3 (14.2)
Isometric at 60°	48.6 (17.2)	47.9 (15.6)	48.8 (16.9)	49.7 (16.1)

All values (Nm) are expressed as mean (standard deviation)

There were no differences observed from pre to post for either the control or experimental condition for isometric or isokinetic strength. The lack of improvement in isometric or isokinetic strength demonstrated in the present study may be largely attributed to the principle of specificity. Given that individuals trained with isotonic exercise it would be expected that isotonic (1RM) strength would increase to a greater extent than that of isometric or isokinetic strength. One set to volitional fatigue has previously been demonstrated to increase isometric strength of the knee extensors to the same extent as three sets (66), however, this may be different in the elbow flexors. To illustrate, a previous study found increases in isometric strength of the elbow flexors with 3 sets (32), suggesting that more repetitions may be necessary to increase isometric/isokinetic strength of the elbow flexors. This hypothesis is speculative, however, as no previous studies to our knowledge have compared isometric/isokinetic strength of the elbow flexors.

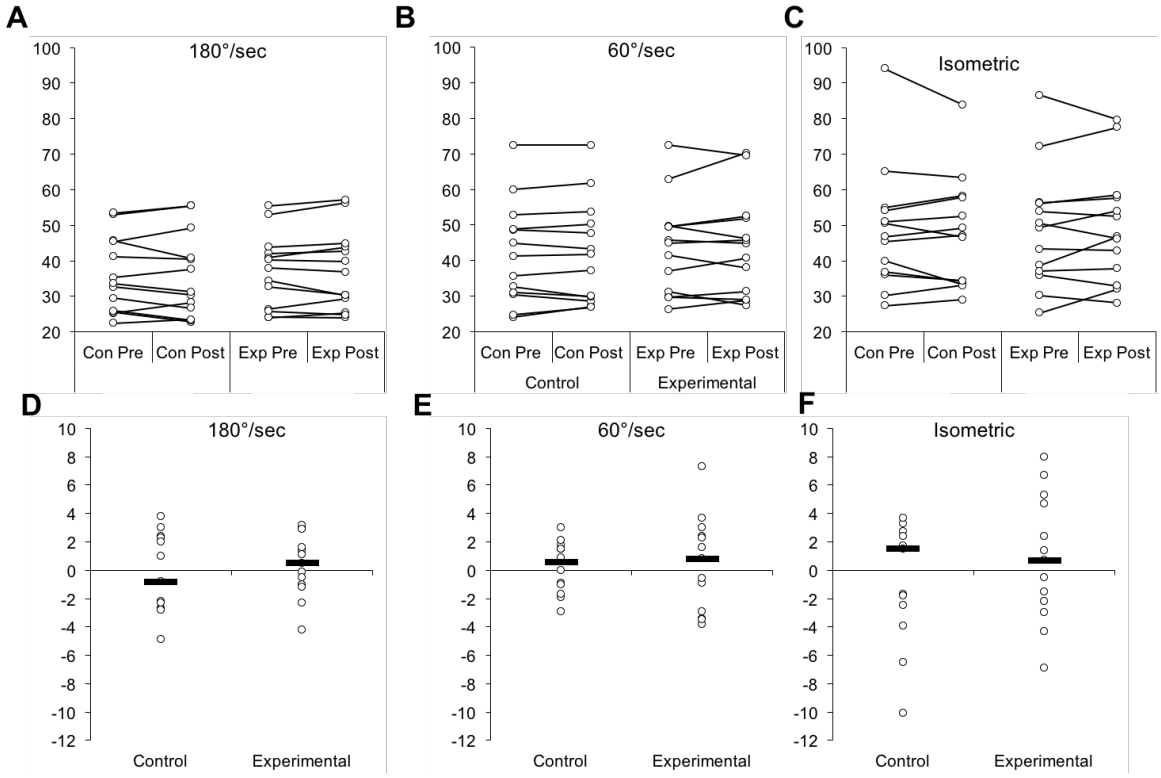
Figure 8. Isometric and Isokinetic Strength



Values (Nm) are expressed as mean (standard deviation). Exp=experimental, con=control.

When examining individual changes in torque (Figure 9) there did not appear to be any trend for the control or experimental condition for any of the tests. As depicted at both the group and individual level, there was an increase in torque production as the speed of the contraction reduced, ultimately resulting in the greatest torque production during the isometric test. The force velocity relationship illustrates that at higher velocities there is less time to apply torque, thus explaining why the lowest torque production was observed during the fastest isokinetic test.

Figure 9. Individual Changes in Isometric and Isokinetic Strength



All values are expressed in nm. A, B and C illustrate individual pre to post changes. Figures D, E and F are presented as (post – pre) with circles representing individual changes and solid lines representing the group median.

Volume

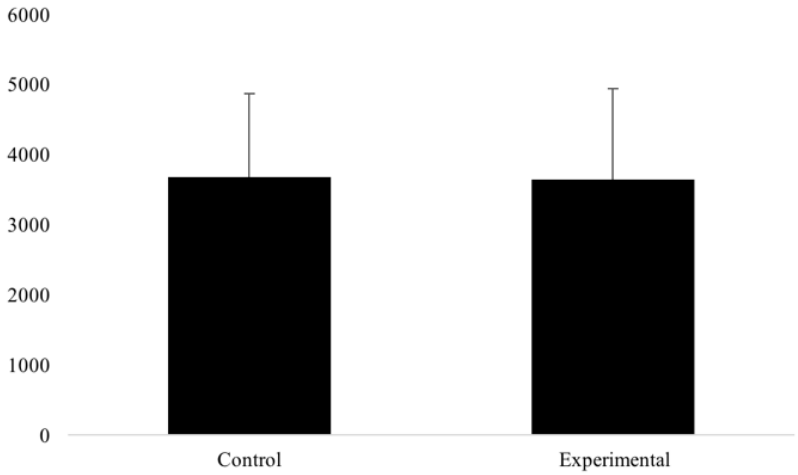
There was no condition x time interaction for volume (p=0.94) nor were there main effects of condition (p=0.74) or time (p=0.88). The total volume completed by the control and experimental arms did not differ during the first 12 or last 12 sessions, nor were there differences in total exercise volume (Table 9, Figure 10).

Table 8. Exercise Volume (repetitions x load)

	First 12 Sessions	Last 12 Sessions	Total
Control	1834.3 (577.9)	1843.9 (642.2)	3678.3 (1183.9)
Experimental	1812.6 (584.9)	1826.0 (740.1)	3638.7 (1297.8)

All values are in kg and are expressed as mean (standard deviation).

Figure 10. Exercise Volume



Values (kg) are expressed as means and standard deviations.

The attenuation of growth that was present in the experimental condition occurred despite performing the same volume of exercise and relative load as the control condition. It has previously been demonstrated that more volume does not always result in greater muscle growth (63) as the benefits gained from a resistance training protocol are undoubtedly finite. With the exclusion of studies implementing BFR, no previous study has demonstrated differential muscle growth involving two protocols implementing the identical protocol (i.e. volume, sets, relative load. Previous studies matching work and volume through the use of different relative loads and repetitions have demonstrated differences in muscle protein synthesis (41) demonstrating that

volume is not necessarily the most important determinant for the hypertrophic potential of an exercise protocol. Nonetheless, in addition to exercise volume, the present study employed the identical exercise protocol involving one set performed to volitional fatigue.

Repetitions

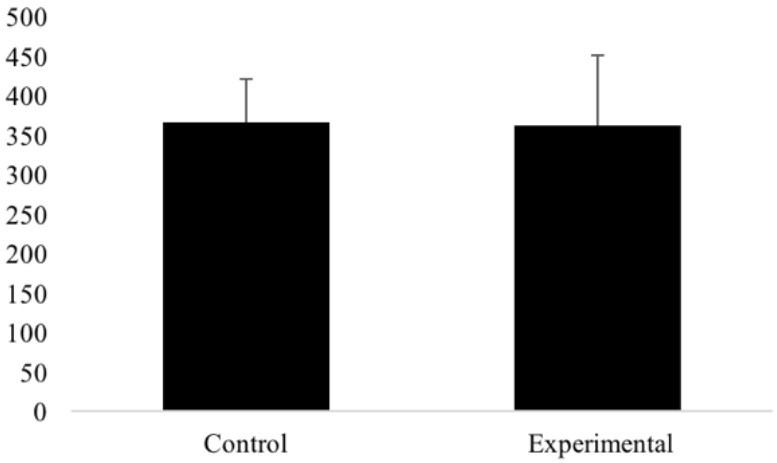
There was no condition x time interaction for repetitions (p=0.74) or main effect of condition (p=0.78), however there was a main effect of time (p=0.01) with repetitions completed decreasing from the first 12 sessions to the final 12 sessions (Table 10, Figure 11).

Table 9. Repetitions

	First 12 Sessions	Last 12 Sessions*	Total
Control	195 (41)	171 (30)	366 (65)
Experimental	194 (52)	167 (45)	362 (89)

Values are in kg and are expressed as mean (standard deviation) *statistically different from the first 12 sessions

Figure 11. Total Repetitions



Values are expressed as means and standard deviations.

Unsurprisingly, both the control and experimental conditions performed nearly the identical number of repetitions over the course of the study (Table 10, Figure 11). When breaking down the number of repetitions comparing the first 12 visits against the last 12 visits, there were more repetitions completed in the last 12 sessions (Table 10). This can be explained by the retesting of 1RMs that were performed prior to training on visit 13. The increase in the load resulted in a decrease in the number of repetitions necessary to reach volitional fatigue. Given that the number of repetitions, relative load, volume, and fatiguing sets of exercise were all similar, the difference in muscle growth observed must be attributed to the application of post-exercise BFR.

Arm Circumference

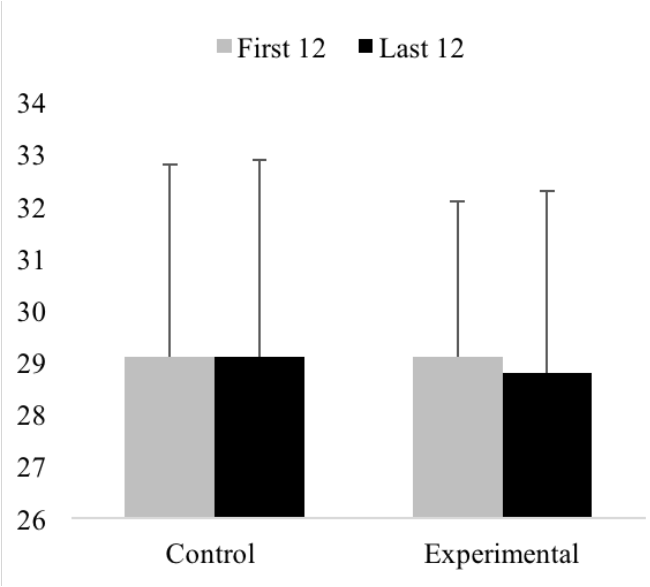
There was no condition x time interaction (p=0.27) nor was there a main effects of condition (p=0.47) or time (p=0.27) for arm circumference (Table 11, Figure 12).

Table 10. Arm Circumference

	First 12 Sessions	Last 12 Sessions	Total Average
Control	29.1 (3.7)	29.1 (3.8)	30.2 (3.7)
Experimental	28.8 (3.0)	291 (3.5)	30.0 (3.3)

Values are in cm and are expressed as mean (standard deviation)

Figure 12. Arm Circumference



Values (cm) are expressed as means and standard deviations

The assessment of arm circumference was primarily used to test whether the muscle growth that was present was not due to edema. Since circumference stayed constant from the first 12 sessions to the last 12 sessions this would suggest that our measure of muscle size was not largely impacted by edema.

Discomfort

The median values over the first 12 and final 12 training sessions were calculated for each individual at all 5 time points (pre, post, 1, 2, 3 min). Each individual's median values for the first 12 and last 12 sessions were then used to calculate the median discomfort at the group level. During the first 12 sessions, there were no differences in the median discomfort at pre ($p=0.99$) or immediately post-exercise ($p=0.28$), however the median discomfort was significantly greater in the experimental arm at 1 ($p=0.002$), 2 ($p=0.002$) and 3 ($p=0.001$) min

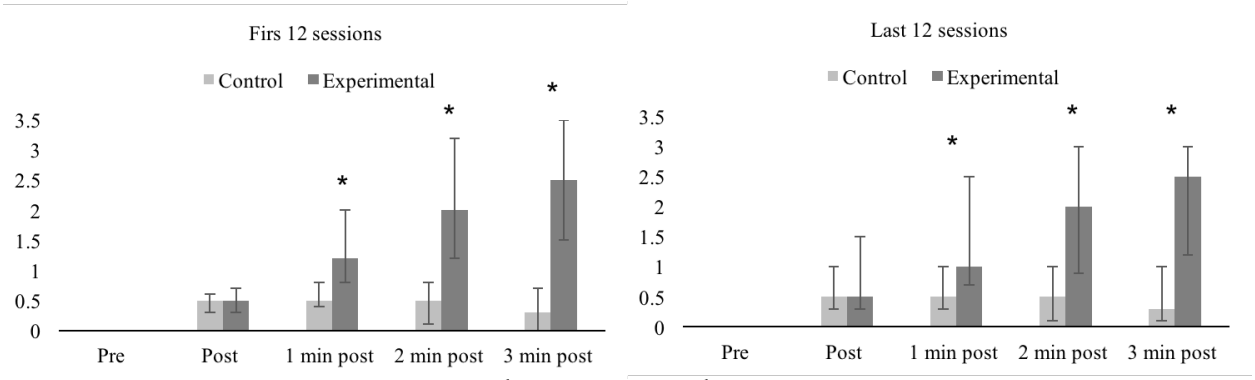
post-exercise (Table 12, Figure 13). The same results were observed for median values during the final 12 sessions of the study with no differences observed at pre (0.31) or immediately post-exercise (p=0.52), however the median discomfort was significantly greater in the experimental arm at 1 (p=0.003), 2 (p=0.002) and 3 (p=0.002) min post-exercise (Table 12, Figure 13).

Table 11. **Borg (CR10+) Ratings of Discomfort**

	First 12 sessions		Last 12 sessions	
	Control	Experimental	Control	Experimental
Pre	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Post	0.5 (0.3, 0.6)	0.5 (0.3, 0.7)	0.5 (0.3, 1.0)	0.5 (0.3, 1.5)
1-min post	0.5 (0.4, 0.8)	1.2 (0.8, 2.0) *	0.5 (0.3, 1.0)	1.0 (0.7, 2.5) *
2-min post	0.5 (0.1, 0.8)	2.0 (1.2, 3.2) *	0.5 (0.1, 1.0)	2.0 (0.9, 3.0) *
3-min post	0.3 (0.0, 0.7)	2.5 (1.5, 3.5) *	0.3 (0.1, 1.0)	2.5 (1.2, 3.0) *

All values are presented as median (25th percentile, 75th percentile) for all individuals across the first 12 and last 12 training sessions. *significantly different than control value at same time point

Figure 13. Borg (CR10+) Ratings of Discomfort



All values are presented as median (25th percentile, 75th percentile) for all individuals across the first 12 and last 12 training sessions. *significantly different than control value at same time point

The levels of discomfort following 3 minutes of post-exercise BFR in the current study (2.5) were similar to what has been observed using a higher restrictive pressure in the upper body (discomfort ranged from 3 to 4) (13). This similarity would be expected given both studies incorporated elbow flexion exercise using similar restrictive pressures (70% vs. 90% arterial occlusion). Additionally, Loenneke et al. observed similar ratings of discomfort when applying BFR in the absence of exercise. This was observed following 3 continuous minutes of BFR (discomfort = 2.5) (51) and following cycles of 5 minute inflations and 3 minute deflations (discomfort = 2.7) (48). Anecdotal reports from participants performing previous studies in our laboratory would suggest that discomfort is greater as the period of BFR is prolonged, and this discomfort is alleviated to some extent by further muscle contraction.

CHAPTER 5: CONCLUSION

The main purpose of the study was to see if performing one set of high load resistance exercise could be augmented by applying blood flow restriction (BFR) for 3 minutes post-exercise. Measures of isotonic (1RM), isometric and isokinetic strength were also analyzed.

Hypotheses

1.There will be no difference in muscle size and strength between the control and experimental conditions.

This hypothesis did not appear to be supported by the data. While there were no significant differences between the experimental and control conditions at either the 50% or 70% sites, there was an interaction at the 60% site demonstrating muscle growth to be attenuated in the experimental condition. Additionally, when analyzing within subject responses between conditions, there appeared to be an attenuation of muscle growth across all sites in the experimental arm in comparison to the control conditions. Furthermore, retrospective sex comparisons demonstrated that, in comparison to the control condition, applying BFR post high load exercise appeared to be more detrimental in females when compared to males.

2. There will be no difference in muscle strength between the control and experimental conditions

This hypothesis was supported by the data given there were no differences in the control or experimental condition at any of the time points examined. Both conditions increased similarly from pre to mid and from mid to post

Significance

The application of BFR allows for individuals to increase muscle size through the use of low load protocols that would otherwise not result in muscle growth. While BFR is thought to work through the pooling of metabolites, previous studies have not been designed to tease out the importance of metabolic accumulation on muscle hypertrophy. All previous BFR studies have been used exclusively with multi-set resistance training protocols, thus allowing for metabolites to augment muscle activation of subsequent sets. By applying BFR at the conclusion of one set of high load training, this study may provide some insight that: 1) metabolites may not have anabolic properties *per se*, and may actually be detrimental for muscle growth when prolonged at the conclusion of high load exercise; 2) the immediate increase in blood flow occurring at the conclusion of exercise may be of great importance for inducing muscle growth; and/or 3) metabolites have anabolic properties but this was masked by the restriction of blood flow post-exercise.

Future Research

Future studies could seek to determine the differences in intramuscular metabolites caused by restricting blood flow for 3 minutes post-exercise. Additionally, future studies could

seek to determine why the application of post-exercise BFR attenuated muscle growth, and further, why this appeared to occur predominantly in females. Finally, studies may seek to determine if the detrimental effects of applying BFR post high-load exercise are still evident when applied post low-load exercise.

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GRANTS

1) Loenneke JP, Dankel SJ (2015). The effects of metabolic stress on muscle size and strength. The Biolayne Foundation. $10,000 (FUNDED). Intellectually contributed to to the conception of the study and assisted with drafting the methods and grant application.
2) Loenneke JP (2015). Can muscle growth occur through resistance training with no external load? American College of Sports Medicine $ (Under Review). Assisted with drafting the methods and grant application.

PEER-REVIEWED PUBLICATIONS

1) Abe T, Counts BR, Barnett BE, **Dankel SJ**, Lee K, and Loenneke JP (2015). Associations between Handgrip Strength and Ultrasound-Measured Muscle Thickness of the Hand and Forearm in Young Men and Women. Ultrasound in medicine & biology.
2) Barnett BE, **Dankel SJ**, Counts BR, Nooe AL, Abe T, and Loenneke JP (2015). Blood flow occlusion pressure at rest and immediately after a bout of low load exercise. Clinical physiology and functional imaging.
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6) **Dankel SJ,** Loenneke JP, and Loprinzi PD (2015). The impact of overweight/obesity duration on the association between physical activity and cardiovascular disease risk: an application of the "fat but fit" paradigm. International Journal of Cardiology.
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10) **Dankel SJ,** Loenneke JP, and Loprinzi PD (2015). Determining the Importance of Meeting Muscle-Strengthening Activity Guidelines: Is the Behavior or the Outcome of the Behavior (Strength) a More Important Determinant of All-cause Mortality?. Mayo Clinic Proceedings.

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SCIENTIFIC ABSTRACTS/ORAL PRESENTATIONS

1) Barnett BE, **Dankel SJ**, Counts BR, Nooe AL, Abe T, Loenneke JP. Predictors of standing upper body arterial occlusion: implications for blood flow restriction research. ACSM National Conference, May 2015, San Diego, California.
2) Counts BR, **Dankel SJ**, Barnett BE, Abe T, Loenneke JP. High relative pressures do not augment changes in early phase muscular adaptations during blood flow restricted exercise. ACSM National Conference, May 2015, San Diego, California
3) **Dankel SJ**, Barnett BE, Counts BR, Nooe AL, Abe T, Loenneke JP. Blood flow occlusion pressure at rest and immediately after a bout of low load exercise. ACSM National Conference, May 2015, San Diego, California.
4) Counts BR, Buckner SL, **Dankel SJ**, Jessee MB, Mattocks KT, Mouser JG, Laurentino GC, and Loenneke JP. The Acute Response to No Load Exercise: Is it Sufficient? ACSM National Conference, May 2016, Boston, Massachusetts.
5) Barnett BE, Buckner SL, **Dankel SJ**, Counts BR, Jessee MB, Mouser JG, Halliday TM and Loenneke JP. Circadian Rhythms in Blood Glucose and Blood Pressure: Are they Reproducible? ACSM National Conference, May 2016, Boston, Massachusetts.
6) Mouser JG, Buckner SL, Counts BR, **Dankel SJ**, Jessee MB, Mattocks KT, Laurentino GC, and Loenneke JP. Venous versus Arterial Blood Flow Restriction: The Impact of Cuff Width. ACSM National Conference, May 2016, Boston, Massachusetts.
7) Ingram JW, Buckner SL, **Dankel SJ**, Counts BR, Mouser JG, Abe T, Laurentino GC, and Loenneke JP. The influence of time on determining blood flow restriction pressure. ACSM National Conference, May 2016, Boston, Massachusetts.
8) Mattocks KT, Buckner SL, **Dankel SJ**, Counts BR, Jessee MB, Mouser JG, Laurentinio GC, Abe T, and Loenneke JP. The Influence of Cuff Material on the Blood Flow Restriction Stimulus in the Upper Body. ACSM National Conference, May 2016, Boston, Massachusetts.
9) Laurentino GC, Mouser JG, Buckner SL, Counts BR, **Dankel SJ**, Jessee MB, Mattocks KT, Loenneke JP, Tricoli V. The influence of cuff width on regional muscle growth:

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11) Loenneke JP, Buckner SL, **Dankel SJ**, Jessee MB, Counts BR, Mouser JG, Mattocks KT, Laurentino GC, and Abe T. The Influence of Cuff Material on the Acute Muscular Response to Blood Flow Restricted Exercise in the Upper Body. ACSM National Conference, May 2016, Boston, Massachusetts.
12) Buckner SL, **Dankel SJ**, Counts BR, Barnett BE, Jessee MB, Mouser JG, Halliday TM, and Loenneke JP. The Influence of Circadian Rhythms on Upper Body Isometric Strength, Muscle Thickness and Body Temperature. ACSM National Conference, May 2016, Boston, Massachusetts.
13) **Dankel SJ**, Counts BR, Barnett BE, Buckner SL, Abe T, Zourdos MC, and Loenneke JP. Muscle adaptation to 21 Straight Days of Elbow Flexor Exercise in Trained Individuals. ACSM National Conference, May 2016, Boston, Massachusetts.

HONORS AND AWARDS

2014 J. Robert Blackburn Graduate Award in Exercise Science

MENTORSHIP

Jeremy Loenneke, PhD
The University of Mississippi (2014 – 2016)