THE EFFICACY OF BLOOD FLOW RESTRICTION DURING HIGH INTENSITY RESISTANCE EXERCISE

by

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A DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Kinesiology
in the Graduate School of
The University of Alabama

TUSCALOOSA, ALABAMA

2021

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ABSTRACT

Blood flow restriction (BFR) resistance training has demonstrated its effectiveness for inducing hypertrophic adaptations at much lower intensities (20-30% one-repetition maximum (1RM)) compared to traditional high-intensity (>65% 1RM) recommendations. Limited research has examined BFR in conjunction with high-intensity resistance training, with mixed results. The purpose of this dissertation was to expand upon this understudied area with a series of three studies to 1) better understand blood flow responses in the lower limbs with varying occlusion pressures, 2) determine the effect of high-intensity BFR (HI-BFR) resistance exercise on fatigue, ratings of perceived exertion (RPE), and pain, and 3) examine the influence of HI-BFR on metabolic stress, muscle damage, and hypoxia. Study 1 examined the effects of varying BFR occlusion pressures on blood flow volume in the legs. Results indicate a potential 50% limb occlusion pressure (LOP) threshold at which point statistically significant reductions in blood flow volume occur in the posterior tibial artery. An observed plateau in blood flow reductions between 60-80%LOP indicates the potential for reduced occlusion pressure during exercise. Study 2 examined the effects of HI-BFR on inter-set fatigue, RPE, and Pain, in addition to post-exercise neuromuscular fatigue/impairment. Significantly greater number of total repetitions and repetitions during sets 1, 2, and 4 ($p < .05$) were performed in the CTRL condition. Although RPE between conditions was similar across all sets ($p \geq .05$), perceived pain was significantly greater in BFR across all sets ($p < .05$). Changes in neuromuscular performance measures were consistent across exercise conditions. Study 3 investigated the effect of HI-BFR on metabolic stress, muscle swelling, and muscle damage in response to a back-squat protocol. Significantly

lower blood lactate concentrations were measured following the BFR exercise stimulus, compared to CTRL (p = .001). No significant differences in muscle swelling were observed between conditions. Post-exercise interleukin-6 was significantly greater following the BFR exercise (p = .007). The use of BFR during high-intensity resistance exercise seems to be a useful method for advanced induction of fatigue during exercise, although the reduced exercise volume due to fatigue and pain limits the overall acute hypertrophic mechanistic responses.

DEDICATION

This dissertation is dedicated to my family and friends who have supported me throughout this journey. To my parents, Angelika and Jochen Hornikel, and brother, Armin, thank you for your unconditional support in all my endeavors, athletic and academic. Without your support and belief in my ability to succeed I would not have the courage to take on these challenges. To my wife, Jacqueline, thank you for your love, patience, and support during this long process and your ability to brighten my days.

LIST OF ABBREVIATIONS AND SYMBOLS

%Rel	blood flow volume relative to rest
1RM	one-repetition maximum
ACSA	anatomical cross-sectional area
BFR	blood flow restriction
CMJ	countermovement jump
CTRL	control condition
EI	echo intensity
EMG	electromyography
HI-BFR	high-intensity BFR exercise
HI-RT	high-intensity resistance training
IHG	isometric handgrip
IL-6	interleukin-6
LI-BFR	low-intensity BFR exercise
LOP	limb occlusion pressure
MPV	mean propulsive velocity
MVIC	maximal voluntary isometric contraction
PTS II	personalized tourniquet system II
RF	rectus femoris
RMS	root mean square of the electromyographic signal
RPE	ratings of perceived exertion

RT	resistance training
VEGF	vascular endothelial growth factor
VL	vastus lateralis

ACKNOWLEDGMENTS

This project would not have been possible without the help and guidance of my committee, graduate students, and the participants. To my committee chair and advisor, Dr. Lee J. Winchester, thank you for taking me under your wing and introducing me to BFR, biological analyses, and giving me the courage to learn blood draws. You were always optimistic and supportive to me throughout this entire process and motivated me at times when I doubted myself. Dr. Michael R. Esco, thank you for motivating me to pursue this doctoral degree and challenging me to become a better student and researcher throughout this journey. Dr. Jacob A. Mota, I would like to thank you for agreeing to join my committee before your first official day at the University of Alabama. Thank you for your guidance and always being open to chat about the design, analysis, and interpretation throughout this process – you were an amazing help. Drs. Michael V. Fedewa and Stefanie A. Wind, thank you for your time, support, and expertise towards the completion of this project. A special thank you to my fellow graduate student, Keith Saffold, for your countless hours in the lab helping me complete data collections, and always being available to discuss the study or any other topic, keeping things interesting. Thank you also to my undergraduate assistants, Tiffany Adams and Kelvyn Oudjit, for always taking time out of your busy schedules to help with data collections. I know you both will achieve great things, regardless of the paths you decide to pursue in the future. Lastly, of course, a special thank you to all of my participants. I hope we did not scare you away from BFR and are very appreciative of your time and effort.

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INTRODUCTION

LITERATURE REVIEW: A CASE FOR HIGH-INTENSITY RESISTANCE TRAINING WITH BLOOD FLOW RESTRICTION FOR MUSCLE HYPERTROPHY

INTRODUCTION

In the past, resistance training (RT) was primarily used by individuals that participated in strength-based competitions or bodybuilders aiming for muscle hypertrophy. However, RT has gained popularity and is actively recommended by the American College of Sports Medicine (ACSM) and Physical Activity Guidelines Advisory Committee (PAGAC) (1, 2). In addition to strength-based competitors and bodybuilders, RT is now included as part of training for many sports for improving muscular strength, speed, power, and motor performance. RT has become a popular mode of exercise for individuals wanting to increase their muscle strength and size, however, there are numerous additional health benefits. RT induces body composition changes, including reduced fat mass and increased muscle mass, which aid in increasing resting metabolic rate (3). Improved motor performance from RT allows for maintenance of the independence of daily living (4). Lastly, individuals partaking in RT programs exhibit decreased blood pressure, improved blood lipid profiles, improved insulin sensitivity, reduced sarcopenia and risk of osteoporosis (5), which leads to lower mortality rates (6).

While RT has numerous health benefits, this review will focus on muscular hypertrophy adaptations to RT. Muscle hypertrophy, or the enlargement of individual muscle fibers, is an outcome of chronic, heavy-load RT (7, 8). When a muscle hypertrophies, the number of

sarcomeres in parallel increases, and the contractile elements, actin and myosin, increase in size and number. Though muscle proteins are in constant turnover, RT shifts toward a net balance of protein synthesis, rather than protein degradation, allowing for muscle fiber growth (9). This leads to an increase in the individual muscle fiber diameter and muscle cross-sectional area (10).

Contemporary muscular hypertrophy recommendations focus on high-intensity resistance training (HI-RT) (>65% of 1-repetition-max (1RM)) for 8-12 repetitions for 1 to 3 sets (11). However, more recently, low-intensity (20-30% 1RM) blood flow restriction (LI-BFR) training has demonstrated usefulness as an alternative method for producing increases in muscle hypertrophy similar to HI-RT (12).

Blood flow restriction (BFR) is a developing training method that involves the use of a tourniquet or elastic wraps to occlude distal blood flow in a limb. The purpose of BFR training is to reduce the arterial blood supply and eliminate venous return, leading to venous pooling and accumulation of metabolites. BFR training originates from Japan, developed by Dr. Sato, where it is known as "KAATSU training" and has been in public use since 1983 (13). Some of the earliest research using the KAATSU training methods showed increased plasma growth hormone following low-intensity BFR RT and increases in muscle size, strength, and endurance in elite rugby players (14, 15). While the underlying mechanisms of BFR training are still being studied, BFR training has shown great potential for increased strength and muscle hypertrophy using as little as 20% of 1RM (16, 17). Despite the recommendation of 65% of 1RM for promoting hypertrophy (11), LI-BFR has demonstrated the ability to increase muscle mass similar to or exceeding HI-RT (18). Working at this lighter load is especially beneficial for untrained individuals, during rehabilitation, and older persons. Some of the proposed underlying

mechanisms for these BFR training adaptations include metabolite accumulation, increased fiber type activation, hormonal responses, and cell swelling (19, 20).

While the appeal of LI-BFR has grown in both field settings and research, there is a lack of research examining the effect of BFR at higher training intensities. The purpose of this review is to examine the applied benefits and mechanisms of adaptation of both HI-RT and BFR to build a case for the investigation of combining these two training methodologies for high-intensity BFR (HI-BFR) training for muscle hypertrophy.

APPLIED RESISTANCE TRAINING

HI-RT has become the foundational training methodology for inducing muscle hypertrophy. The generally accepted guidelines for muscle hypertrophy training suggest a load of ≥65% 1RM is required for maximal hypertrophic adaptation (5, 21, 22). However, cumulative results from a pooled analysis of 191 subjects and 34 ESs, nested with 17 treatment groups and 8 studies indicated that significant muscle growth can be achieved with as low as 60% 1RM (23). Though the general belief that high resistance loads are required remains.

Type 2, fast-twitch, muscle fibers show a greater hypertrophic response to resistance training compared to type 1, slow twitch, muscle fibers (24, 25). Henneman's size principle is one of the underlying reasons for the recommendation of 60-70% 1RM. The size principle dictates that during resistance exercise, muscle fibers are recruited according to their motor unit size, with the smallest fibers recruited first (26). Accordingly, type 1 fibers (slow twitch) are recruited first as their motor units are the smallest. To recruit the larger, type 2 fiber (fast-twitch) motor units, the exercise intensity and load must be increased accordingly. However, research has also indicated that modifications to RT exercise can achieve hypertrophy under lighter loads

through the use of eccentric loading and increased time under tension by exercising the muscle to volitional fatigue using a lighter load.

A meta-analysis by Schoenfeld et al. indicated that while both low- and high-load resistance training may lead to substantial gains in muscle growth, HI-RT showed greater overall hypertrophy compared to low-load (23). The studies included in the meta-analysis consisted primarily of untrained and recreationally trained participants, thus, results may differ for highly trained individuals. In a study including well-trained men (1.5 – 9 years consistent RT), it was demonstrated that low-load and high-load training programs had similar gains in muscle cross-sectional area measured via ultrasound imaging (27). However, the low load group in the study did perform 3 times the total volume (sets x repetitions) compared to the high load group, indicating that total load is an important factor.

Other factors, in addition to resistance load, impact the hypertrophic response to resistance training; such as intensity, exercise selection, and work to rest intervals (28). Additionally, individual factors such as age may influence an individual's hypertrophic response to resistance training. Younger individuals (22-31 years), when compared to older individuals (62-72 years), have a greater capacity for increasing muscle cross-sectional area with RT (29). While individual differences exist in the extent to which muscles hypertrophy, three primary factors are believed to be responsible for skeletal muscle hypertrophy in response to HI-RT: mechanical tension, muscle damage, and metabolic stress. The next section will discuss the mechanisms of adaptation for each of these factors.

MECHANISMS OF RESISTANCE TRAINING ADAPTATION

Mechanical Tension

Mechanical tension is placed on the muscle by force generation against a resistance load and muscular stretch during RT. The tension placed on the muscle during RT causes perturbations to the tensile integrity of the muscle, leading to the induction of cell signaling cascades (30, 31). Chronic increases in mechanical tension, or overload, enhances muscle hypertrophy, while chronic decreases or absence of mechanical tension leads to muscle atrophy (32). Increased mechanical tension promotes increased rates of intramuscular protein synthesis and therefore, increases in muscle mass (33). The mechanical stimulus must be converted into biochemical signals to regulate protein synthesis, which is known as mechanotransduction. The mechanical stimuli are sensed by costameres and titin in the sarcomere and Z-disk, although more research is needed (34). Burd et al. demonstrated that low-intensity exercise (30% 1RM) produces similar protein synthesis following exercise compared to high intensity (90% 1RM) when both are performed to failure. Additionally, this enhanced protein synthesis is sustained at 24 hours post in the low-intensity protocol but not high-intensity (35). The high-intensity protocol did promote greater protein synthesis immediately following exercise cessation, indicating that the volume of exercise affects the duration of protein synthesis, while overall intensity affects acute responses (35). This finding was confirmed by a later study which showed that prolonged time under tension during RT did not indicate an immediate increase in protein synthesis rates, but rather 24-30 hours post-exercise (36). Importantly, this occurs only if the exercise is performed until volitional fatigue, which requires greater reliance on T2 motor unit recruitment.

Currently, the mammalian target of rapamycin (mTOR) is believed to serve as a central hub for regulating cell signaling pathways that increase RNA translational efficiency, which in part, determines rates of protein synthesis (37). The activation of the mTOR pathway post-resistance exercise upregulates intramuscular protein synthesis and therefore, is a critical pathway for induction of muscular growth due to mechanical stimulation of the tissue (33, 38-40). Mechanical stimuli primarily affect the initiation of genetic translation, or the binding of a ribosome to mRNA (33). Increased rates of ribosomal translation enhance protein synthesis rates for muscular hypertrophy (41). Ribosomal translation is regulated by the mammalian target of rapamycin complex 1 (mTORC1) and overload training enhances mTORC1 activity (41). mTORC1 is the downstream signaling hub for protein synthesis (39, 42). It is believed that initially, the enhanced ribosomal translational efficiency following mechanical tension overload alone can stimulate muscle growth. However, with prolonged exposure to mechanical stimuli, satellite cell proliferation becomes more essential for hypertrophy to continue (41).

Eccentric exercises, during which muscles are forcibly lengthened, produce greater amounts of mechanical tension and force production compared to concentric contractions. Additionally, eccentric contractions increase the phosphorylation of $p70^{S6k}$ by an Akt-independent pathway, while concentric does not (43). $p70^{S6k}$, a nuclear regulator of protein synthesis, is activated by mTORC1 (44). Increased phosphorylation of $p70^{S6k}$ 6 hours post-exercise has been correlated to increases in muscle mass after 6 weeks of RT (45). Additionally, increased NO production by mechanical stretch causes a release of intracellular calcium which can activate the mTOR pathway (46).

Muscle Damage

During HI-RT, and other physical exercise, the muscle tissue can become inflamed and damaged in response to the loads placed upon it. Exercise-induced muscle damage (EIMD) leads to a disruption of sarcomere structural and contractile proteins, the extracellular matrix, basal lamina, and sarcolemma within the muscle fiber (47). Resistance training induced myotrauma peaks 1-3 days following exercise and can remain elevated up to 8 days (48). The extent to which the muscle tissue is damaged is modulated by the exercise type, intensity, and duration (49), with greater force applied concurring with greater muscle damage (50). Additionally, exercise focusing on eccentric loading will have greater muscle damage than isometric or concentric exercise (49, 51). Symptoms of EIMD include delayed onset muscle soreness (DOMS), limited range of motion, decreased force output, and fatigue (52). While EIMD is associated with decreased performance following damage, it leads to an increase in protein turnover that promotes muscle hypertrophy (53). Several mechanisms have been discovered as a response to muscle damage in humans, leading to hypertrophy; including inflammatory cell signaling, satellite cell proliferation, and IGF-1 signaling.

Inflammatory Cell Signaling

Following HI-RT, leukocytes (primarily neutrophils) begin to accumulate in the blood vessels of damaged muscle tissue within 2 hours myotrauma, peaking 6 to 24 hours post and then rapidly declining in numbers (54). Neutrophils aid in degrading cellular debris caused by muscle damage and are capable of contributing to additional tissue damage by direct lysis of muscle cells (55), however, it is suggested that they may aid in signaling other muscle regenerating inflammatory cells (56).

Aside from their ability for phagocytosis, neutrophils can produce reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hypochlorous acid, and hydroxyl radicals (57). While ROS may damage surrounding healthy tissue and further damage skeletal muscle fibers (58), they have also been shown to play a role in the hypertrophy of smooth and cardiac muscle (59). Mechanical load is the primary factor for the production of ROS during RT (60). Proposed mechanisms of ROS-induced hypertrophic effects include possible increased mitogen-activated protein-kinase (MAPK) signaling and IGF-1 signaling (56).

Following neutrophil infiltration, phagocytic macrophages migrate to the area and increase in numbers to assist with clearance of cellular debris prior to remodeling. Approximately 48 hours post-myotrauma, phagocytic macrophages begin to reduce in numbers (61, 62). Concurrently, a shift toward non-phagocytic macrophages occurs and remains elevated for 4 to 7 days post-injury (62). These non-phagocytic macrophages serve to aid in muscle repair and growth and satellite cell differentiation (63, 64). Macrophages produce myokines and growth factors that play a pivotal regulatory role in hypertrophic response. For example, myokines, such as interleukin-6 (IL-6), have been reported to aid in muscle hypertrophy (65) through induction of satellite cell proliferation and by promoting apoptosis of macrophages and neutrophils in the damaged fibers (66).

Satellite Cell Proliferation

Satellite cells are mononucleated myogenic cells, which are capable of proliferating in response to muscle damage, allowing for muscle regeneration and growth through the formation of additional myonuclear domains within the skeletal muscle fiber (67). While a mechanical stimulus alone, without muscle damage, can stimulate satellite cell proliferation (68), it is believed that myotrauma enhances proliferation leading to hypertrophy. When satellite cells

proliferate, they aid in the repair and growth of muscle tissue by fusing to existing myofibers and forming new myonuclei for enhanced myonuclear domain (69). Enhanced myonuclear domain allows for greater synthesis of new contractile proteins during hypertrophy and repair (70, 71). Inflammatory responses to muscle damage are similar to the responses observed with infection (72). Following myofiber damage, neutrophil infiltration into the damaged tissue is observed, which appear to phagocytose cellular debris and promote inflammation (73). Secreted myokines signal macrophage and lymphocyte chemotaxis, which aid in removing cellular debris and enhance cytokine production which further activates macrophages and lymphocytes (74, 75). Satellite cells are believed to be activated by two factors: hepatocyte growth factor (HGF) and nitric oxide (NO) (76). Neuronal nitric oxide synthase (nNOS) is present on quiescent satellite cells and is responsible for increasing NO-induced release of HGF as a result of mechanical stretch of the myofiber (76). HGF is secreted by the damaged tissue, with a linear relationship between the severity of muscle damage and the volume of HGF produced (77). As previously mentioned, HGF binds with the quiescent satellite cell c-met receptor inducing cellular proliferation (78, 79).

IGF-1 signaling

Previous literature indicates that IGF-1 plays an important anabolic role in hypertrophic responses to mechanical loading and overload (80-82). IGF-1 is associated with several hypertrophic mechanisms, satellite cell proliferation, gene transcription, and protein synthesis (83). IGF-1 is part of the IGF-1/PI3K/Akt signaling pathway, which is critical in regulating muscle mass (39). IGF-1 activates AKt-stimulated protein translation as an upstream inducer of the mTOR signaling pathway (39). Importantly, RT has been shown to increase both circulating and muscle IGF-1 concentration post-exercise (80, 84). IGF-1 appears in three isoforms in

humans: IGF-1Ea, IGF-1Eb, and IGF-1Ec. IGF-1Ec is often referred to as mechano growth factor (MGF) as it is detected in muscles following mechanical stimulation (85). Following muscle damage, MGF expression peaks about 1-day post and is followed by an increase in IGF-1Ea and IGF-1Eb isoforms (86, 87). MGF is believed to serve two primary purposes: upregulation of protein synthesis and activation of satellite cells. MGF signals through several different pathways, including phosphatidylinositol 3-kinase-protein kinase B-mammalian target of rapamycin (PI3K-Akt-mTOR), MAPK-ERK 1/2, and various calcium-dependent pathways (88). Following the initial increase in MGF 1-day post, MGF expression is reduced and an increase in IGF-1Ea is observed (86). IGF-1Ea is believed to be essential for long-term protein synthesis, with levels peaking up to 7 days post mechanical muscle damage (86).

Metabolic Stress

Metabolic stress plays an important role as another primary mechanism for muscle hypertrophy. Metabolic stress refers to the accumulation of metabolites (lactate, inorganic phosphate (P_i), and H^+) from reliance on fast glycolysis for ATP production during HI-RT (89-91). During HI-RT, more ATP is hydrolyzed per second compared to LI-RT, thus, depleting phosphocreatine (PCr) and lowering pH as a result of lactate accumulation. In addition to the enhanced energy demand during HI-RT, compression of arterial and venous blood from muscle contractions during RT can decrease oxygen delivery capacity, resulting in a hypoxic environment and increasing metabolic stress (88, 92). The impact of metabolic stress can be seen in increased fast-twitch recruitment, hormonal release, ROS production, and cell swelling, which can stimulate protein synthesis and satellite cell proliferation (88, 93).

When performing HI-RT, shorter rest periods (1 minute) produce greater lactate accumulation and metabolic response compared to longer rest periods (3 minutes) (94, 95).

Additionally, moderate-intensity (60-80% 1RM) resistance training with shorter rest periods has demonstrated greater hypertrophic effects compared to a strength-based program at higher intensities (90% 1RM) with long rest periods (88, 96).

APPLIED BLOOD FLOW RESTRICTION

A large body of literature has suggested that low-intensity resistance training in conjunction with BFR can produce greater muscle hypertrophy results compared to low intensity without BFR (15, 16, 97) and similar hypertrophic increases compared to traditional high-intensity resistance training (97-99). These hypertrophic adaptations have been reported in healthy, young (18, 100) and older (101) adults, and is effective for rehabilitative purposes post-injury (102).

A meta-analysis examining 11 primary studies (31 and 29 effect sizes for LI-BFR and LI-RT, respectively) indicated significant differences between LI-BFR and LI-RT without occlusion for muscular hypertrophy (ES: 0.39 and -0.01, respectively) (100). Yasuda et al. (2010) examined the effect of 30% 1RM bench press training (2x daily/6x week/ 2 weeks /75 repetitions total) combined with BFR on triceps brachii and pectoralis major muscle thickness. Their findings demonstrated significant muscle thickness increases in both muscles following the training program, with no observed significant increases in muscle thickness in the control (non-BFR) group (103). Similarly, Fujita et al. (2008) examined the effect of BFR on quadriceps cross-sectional area during a 6-day (2x daily) training program in 16 young men. Participants performed 4 sets (75 repetitions total) at 20% 1RM in two groups; BFR and non-BFR. The BFR group displayed significant increases in quadricep cross-sectional area (3.5% increase) while the non-BFR control group did not (-1.0% decrease) (104). A meta-analysis by Lixandrão et al. compared the differences in muscle hypertrophy between LI-BFR and HI-RT, findings indicated

that both training protocols produced similar gains in muscle mass (ES difference = 0.10), with HI-RT indicating a slightly greater gain in muscle mass (12).

When prescribing BFR exercise there appear to be several important considerations to be made for consistent results and maximizing user comfort. The most important considerations regarding BFR training is the occlusion pressure placed on the limb. While no standardized procedures exist for determining BFR pressures, previous research used cuff pressures of 140-240mmHg for lower body occlusion and 100-160mmHg for upper body occlusion (105). However, varying cuff widths, cuff materials, and individual characteristics of the user can lead to inconsistencies in the amount of blood flow restriction when using the same absolute occlusion pressure, leading to inconsistencies in hypertrophic results and difficulties interpreting study results (105). Cuff width plays a significant role in predicting relative occlusion pressures (106, 107), with wider cuffs requiring lower pressures to eliminate arterial blood flow (108-110). On the individual level, limb circumference is the largest determinant in pressure requirements (111), as soft tissue depth increases, the drop in pressure beneath a cuff from subcutaneous tissue to bone increases, indicating a need for greater pressures with larger limbs to occlude deeper vessels (112). Individualized relative cuff pressures, rather than absolute pressures, account for individual differences and allow for limited or no differences in blood flow at rest between different cuff widths (110). This relative pressure can be based on an individualized limb occlusion pressure (LOP), which is the minimum pressure required to stop the flow of arterial blood into the limb distal to the cuff (113). Using an individualized relative pressure, rather than arbitrary pressures, ensures the safety of the participants by using the lowest required pressure and ensuring correct occlusion volumes. Research has indicated that the use of high occlusion pressures does not elicit additional benefits compared to lower pressures in low-load resistance

training (114, 115). Additionally, higher occlusion pressures during exercise increase participant discomfort (106).

MECHANISMS OF BLOOD FLOW RESTRICTION ADAPTATION

Mechanical Tension and Muscle Damage

In comparison to HI-RT, muscle damage and mechanical tension play a much smaller role in traditional LI-BFR training. Additionally, the exercise-induced muscle damage (EIMD) associated with HI-RT does not occur to the same extent during LI-BFR (116, 117). The typical low-intensity (20-30% 1RM) load used during BFR training is associated with low mechanical stress and therefore, muscle damage and mechanical tension likely not potent stimulators for hypertrophy in LI-BFR compared to HI-RT. LI-BFR training does not exhibit prolonged decrements in muscle function, muscle soreness ratings, prolonged cell swelling, or elevated blood biomarkers associated with HI-RT muscle damage (118). Although not all BFR research agrees on the extent to which LI-BFR produces muscle damage, the differences in intensity, volume, and mode of exercise likely play a role in the muscle damage associated with the exercise. Most recently, Winchester et al. demonstrated that intermittent, unilateral occlusion during HI back-squats (75% 1RM) did not increase markers of muscle damage (IL-6 and myoglobin) over non-BFR HI back-squats (119). Thus, it is unlikely that BFR induces hypertrophy through the primary mechanisms of hypertrophy associated with HI-RT.

Metabolic Stress

When exercising with BFR at low intensities, the mechanical tension on the muscle is lower compared to HI-RT, however, hypertrophic adaptations can be similar between the two groups. One factor that tends to be different between the two training methodologies is metabolic stress.

During traditional HI-RT, metabolic accumulation may occur due to decreased oxygen delivery capacity from the compression of arterial and venous blood flow during exercise from the contraction of the muscle (88). The metabolic stress is primarily due to the accumulation of lactate, P_i, and H^+ and is an important aspect for promoting muscle hypertrophy (88-90, 120). Previous research has found that BFR training increases the rate of ATP hydrolysis, PCr depletion, lactate response, and decreases pH (121). When compared to LI-RT without BFR, LI-BFR produces greater metabolic stress (89, 122). However, when compared to HI-RT, mixed results exist in the literature. For example, LI-BFR using intermittent occlusion is associated with less metabolic stress compared to HI-RT, while LI-BFR with continuous occlusion during training enhances metabolic stress to the level of HI-RT (122). Indicating that the total time of occlusion and set vs. rest occlusion plays an important role in the metabolic stress accumulation during BFR. Interestingly, blood lactate levels are significantly higher when performing HI-RT with BFR occlusion during the rest interval between sets compared to HI-RT without BFR and HI-RT with intermittent occlusion during the sets (123). Therefore, the addition of BFR during the rest period enhances the accumulation of metabolic stress produced during contraction, by preventing blood flow and oxygen perfusion. Thus, it is very plausible that continuous occlusion during rest and set intervals during HI-RT would further enhance metabolic stress accumulation.

BFR induced metabolic stress is purported to enhance muscle hypertrophy through several mechanisms: increased fast-twitch muscle fiber recruitment, hormonal responses, reactive oxygen species, heat shock proteins, and muscle swelling.

Increased Fast-Twitch Recruitment

The recruitment of muscle fibers follows the "size principle" – small type 1 (T1) muscle fibers are recruited first and type 2 (T2) fast-twitch when the stimulus increases (26). The

recruitment of T2 fibers has a greater ability for protein synthesis as $p70^{S6k}$, a downstream target of mTOR, content is 3 to 4 times higher compared to T1 fibers (124). Previous literature has demonstrated the occurrence of recruitment of higher-threshold motor units (type 2) even during low-intensity RT using BFR (125). Additionally, LI-BFR increases $p70^{S6k}$ phosphorylation following exercise similar to HI-RT, which may be explained by the increase in T2 fiber recruitment (97, 126, 127). It is believed that the metabolic stress associated with BFR training may be the underlying cause of the increased muscle fiber recruitment. Reduced oxygen supply and accumulation of metabolites stimulate group 3 and 4 afferents which inhibit slow-twitch alpha motor neurons, leading to an increase in fast-twitch fiber recruitment to maintain force output (128, 129). Several studies using electromyography (EMG) have found a greater type 2 fiber recruitment during BFR training compared to non-occluded training at equal intensity (97, 125). However, EMG activity during LI-BFR is lower than that experienced during HI-RT (127, 129). Therefore, the mechanical tension produced by HI-RT may play an additional role alongside metabolic stress in recruiting T2 fibers. When comparing HI-RT to HI-BFR, both produce similar muscle activation, with muscle activation decreasing as sets progress (123). In this study by Teixeira et al. (123), blood flow was only occluded intermittently, during the set or rest interval, with rest interval occlusion resulting in a greater decrease in muscle activation as sets progressed and greater metabolic stress response. This greater reduction in muscle activation may be due to the accumulation of protons inhibiting Ca^{2+} release and disrupting excitation-contraction coupling, ultimately resulting in peripheral muscular fatigue (130). Similarly, Dankel et al. measured EMG amplitude during sets of unilateral dumbbell elbow flexion to volitional fatigue. Participants performed two sets of curls to volitional fatigue with 3 minutes rest between sets. The control condition was without BFR, and the BFR condition performed the first set

without occlusion followed by the cuff being inflated immediately after for the rest period and the second set to volitional fatigue. While muscle excitation did not differ between the two conditions during the second set, repetitions to failure was significantly less for the BFR condition during the second set compared to the control condition, 4 vs. 7 repetitions, respectively (131).

Hormonal Responses

The enhanced metabolic stress associated with BFR training may increase secretion of anabolic hormones, leading to greater hypertrophic capacity (132). Numerous studies have indicated that growth hormone (GH) is increased following low-intensity BFR training (14, 133-136). GH concentration following LI-BFR RT has been reported to increase 290 after 5 sets of bilateral leg extension at 20%1RM, when compared to baseline (14). Conversely, during HI-RT, GH may typically increase 1.7-fold following exercise (95). The increase in GH during BFR exercise may be related to the increased accumulation of local metabolites, which stimulates class 3 and 4 afferents through muscle chemoreceptors (129). It is suggested that lactate is directly correlated to GH concentrations following exercise (128, 137). During involuntary isometric knee extension exercise induced by electromyostimulation, GH only increased when the venous flow in the exercising leg was restricted due to metabolite accumulation in the exercising muscle (138). The increase in GH following exercise stimulates the production of hepatic and muscle IGF-1 (139, 140), which is a potent promoter of muscle protein synthesis signaling pathways such as P13K/Akt/mTOR (141).LI-BFR has been observed to have increased IGF-1 response, while LI-RT without BFR has no effect (16, 134).

Reactive Oxygen Species

It is well-established that ROS production by muscles is believed to play a hypertrophic role in response to resistance training and during BFR training (142). The production of ROS may be further increased due to the hypoxic environment and reperfusion created during BFR training (143). However, some research has indicated that LI-BFR produces no significant increases in ROS markers (14, 60). As previously mentioned, high mechanical load is believed to be a primary stimulator of ROS production during RT (60). Therefore, BFR in combination with HI-RT may enhance the ROS response. The increased ROS production in skeletal muscle fibers in response to exercise serve as activators for several redox-sensitive signaling pathways; such as CaMK type IV, ERK ½ and p38 MAPK, which are involved in PGC-1α transcriptional activation (144, 145).

Heat Shock Proteins

Heat shock proteins (HSP), also referred to as stress proteins, function to maintain homeostasis in response to a variety of stressors including increased Ca^{2+} concentrations, ROS, and protein degradation (146). Furthermore, HSP expression has been found to increase following damaging eccentric resistance exercise. Following a bout of traditional eccentric RT, a ten-fold and two-fold increase in HSP70 and HSP27 was observed in the biceps brachii muscle, respectively, which exceeds HSP response to non-damaging endurance exercise (146). IL-6, an anti-inflammatory associated with muscle damage, has also been shown to increase HSP mRNA (147). Therefore, the response of HSP seems to be directly related to the muscle damage brought about by the eccentric exercise. However, this muscle damage does not occur to the same extent in LI-BFR. Fry et al. (148) observed no significant increase in HSP70 or IL-6 following LI-BFR, although this was tested in older individuals (70 ± 2 yrs), which have a blunted HSP and anti-

inflammatory response to exercise. Nielsen et al. (149) found no changes in HSP70 in response to BFR training, but did however, find an increase in HSP27, which has been linked to oxidative stress (150). Interestingly, following LI-BFR, HSP responses are more pronounced in T1 fibers (151), while HI-RT induces greater HSP response in T2 fibers (152). The increase in HSP expression in T1 fibers was correlated with low glycogen content in the muscles (151), which may indicate the HSP response is also related to metabolic stress produced by BFR occlusion (149). Further research is required to determine how HSP production is affected by BFR training and which HSP isoforms have the greatest influence on muscle hypertrophy.

Cell Swelling

While muscle swelling does occur following HI-RT, this is likely due to muscle damage associated with the exercise (153). Interestingly, muscle swelling also occurs during LI-BFR, although exercise-induced muscle damage is not prominent, indicating other underlying mechanisms (116, 117). Previous literature demonstrates an enhanced anabolic response in hepatocytes following acute cell swelling, suggesting that cell swelling of the muscle may be another mechanism contributing to muscle hypertrophic response after LI-BFR training (154). While muscle swelling does occur following HI-RT, this is likely due to muscle damage associated with the exercise (153). Although LI-BFR does not induce muscular damage, Freitas et al. (2017) demonstrated that during a lower body resistance training session, LI-BFR at 20% of 1RM induces acute muscle swelling equivalent to that observed with traditional HI-RT at 80% of 1RM (155). However, these acute increases in muscle swelling can be observed following BFR occlusion without exercise, which attenuates muscle atrophy without an observed increase in metabolites, indicating that cell swelling has a hypertrophic effect on muscle. The blood pooling effect of BFR may shift intra- and extracellular water balance, creating a hydrostatic

pressure gradient which favors a shift of water into the cell, increasing intracellular fluid (38). The increased intracellular fluid upregulates protein synthesis and muscle hypertrophy (156, 157) through stimulation of an intrinsic volume sensor, which activates the mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) pathways (38, 157). Activation of the mTOR pathway is essential for stimulating skeletal muscle growth by promoting myofiber protein synthesis (38, 39).

HIGH-INTENSITY BLOOD FLOW RESTRICTION RESISTANCE TRAINING

The primary focus in BFR has been related to low-intensity resistance training, very limited research exists regarding HI-BFR. Neto et al. examined the effects of HI-BFR in a group of twelve Jiu-Jitsu fighters with RT experience (158). All participants performed back-squats at 80% 1RM until concentric failure. Following the back-squat protocol, three maximal voluntary isometric contractions (MVIC) were performed for five seconds each with the knee flexed at 60° and ankle strapped to the resistance arm. Findings indicate that there were no significant differences between groups following the exercise stimulus in isometric torque production and sEMG amplitude. Although, during the MVICs, a significant reduction in sEMG amplitude was measured in the vastus lateralis of the BFR group. This may be attributed to the onset of neuromuscular fatigue due to the accumulation of metabolites following the BFR squat set. (158)

Biazon et al. (2019) examined the effects of BFR training performed at high and low loads (159). In their study, 30 untrained (no RT and/or endurance training for at least 6 months prior) young men completed 10 weeks of resistance training. Participants were assigned to one of three experimental groups; High-load RT (HL-RT), High-Load BFR (HL-BFR), and Low-Load BFR (LL-BFR). HL-RT and HL-BFR both completed 3 sets of 10 repetitions at 80% 1RM. LL-BFR completed 3 sets of 20 repetitions with 20% 1RM. During both HL-BFR and LL-BFR,

the cuff was inflated intermittently, only inflated during the exercise. All training protocols were performed using a conventional leg extension machine, twice a week for 10 weeks total, with 1RM re-assessed and training load adjusted accordingly at week 5. While all three protocols significantly increased muscle cross-sectional area, there were no significant differences between HL-RT and HL-BFR, indicating that there is no additional benefit from adding intermittent BFR to HL-RT during the sets only. (159)

Laurentino et al. (2008) also examined the effect of HI-BFR, using 16 physically active (non-resistance trained) male college students. Participants were assigned to either HI or MI groups, which worked at a load of 6RM (~80% 1RM) and 12RM (~60% 1RM), respectively (160). Participants performed the unilateral knee extension exercise protocol twice a week for 8 weeks. The right leg of each participant was intermittently occluded (occlusion during exercise), and the left leg served as the control, without occlusion. Quadricep cross-sectional area, measured by MRI, improved among both training groups, with no significant differences between training protocols or occluded and control leg within each protocol. Again, indicating that there is no additive effect from including BFR when performing HL-RT for muscular hypertrophy. (160)

Findings by Teixeira et al. demonstrate that HL-BFR with occlusion performed only during the set does not produce significantly higher lactate response compared to HL-RT (123). However, HL-BFR with occlusion during the rest interval does significantly increase lactate response (123). The two previously mentioned studies by Laurentino et al. and Biazon et al. removed the occlusion to the lower limbs during the rest interval and therefore may not have achieved the additive metabolic stress of the BFR occlusion. Laurentino et al., however, did measure blood deoxyhemoglobin concentration [HHb], a marker of metabolic stress, and found HI-BFR to produce greater muscle deoxygenation compared to HI-RT. It is plausible that this

response would have been enhanced by leaving the limb occluded during the rest period. The underlying reason for the removal of occlusion during the rest period is the discomfort associated with occlusion training, especially during high-intensity exercise (161). The authors hypothesize that leaving the limbs occluded during the sets and rest periods or only during the rest period (to mitigate discomfort) will enhance the metabolic stress response and may produce a greater hypertrophic response to the HI-BFR exercise.

Most recently, Winchester et al. (2020) demonstrated that unilateral occlusion during HI back-squats (75% 1RM) did cause significant reductions in the total number of repetitions performed between BFR and CTRL (47.0 ± 4.25 vs 44.92 ± 3.13, respectively; $p = 0.29$). However, a significant decrease in the number of set repetitions was observed from the second to third set of BFR exercise which was not observed in the CTRL condition, indicating an earlier onset of muscular fatigue in the BFR condition (119). Additionally, no significant increase in markers of muscle damage (IL-6 and myoglobin) was observed in the BFR condition over non-BFR HI back-squats (119). Limitations of this study include only using unilateral intermittent limb occlusion during exercise (pressure released during rest) which has been shown to be less effective at increasing metabolic stress during high-intensity exercise compared to continuous occlusion or occlusion during rest (123).

CONCLUSION AND DISSERTATION AIMS

While the current limited research on HI-BFR training is inconclusive with inconsistent research methodologies, it is reasonable to believe that the combination of the two training modalities may lead to a synergistic increase in hypertrophic response without observed increases in muscle damage. The increased metabolic stress through the occlusion of blood flow would likely further enhance the protein synthesis response to HI-RT, which would otherwise be

physiologically limited. It is clear that further research on this specific area is warranted, therefore, the purpose of this dissertation is to evaluate the effectiveness of HI-BFR. The specific study aims are as follows:

Study 1: to compare the effects of varying BFR pressures based on individualized limb occlusion pressures (LOP) using the Delfi PTS II on blood flow in the leg measured at the posterior tibial artery. We hypothesized that blood flow would decrease in a non-linear fashion with increases in occlusion pressure and that a plateau in blood flow reductions would occur between 50-80%LOP.

Study 2: to compare the effect of BFR during high-intensity resistance training on inter-set fatigue, ratings of perceived exertion (RPE), and Pain, and to assess changes in neuromuscular performance measures following an HI-BFR exercise bout. We hypothesized that HI-BFR would accelerate fatigue during the back-squat protocol. We also hypothesized that the addition of BFR to the back-squat protocol would cause greater impairment in muscle function and force production during performance measures following the exercise.

Study 3: to examine if the underlying mechanisms of BFR-induced hypertrophy (metabolic stress, cell swelling, markers of inflammation) are further enhanced in response to a high load back-squat protocol. We hypothesized that the continuous occlusion during HI-BFR in the would enhance metabolic stress and cause an increase in acute cell swelling, without an increase in markers of muscle damage or inflammation compared to HI-RT following the exercise protocol

REFERENCES

1. Garber, CE, Blissmer, B, Deschenes, MR, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc* 43: 1334-1359, 2011.

2. Powell, KE, King, AC, Buchner, DM, et al. The Scientific Foundation for the Physical Activity Guidelines for Americans, 2nd Edition. *J Phys Act Health*: 1-11, 2018.

3. Campbell, WW, Crim, MC, Young, VR, Evans, WJ. Increased energy requirements and changes in body composition with resistance training in older adults. *Am J Clin Nutr* 60: 167-175, 1994.

4. Rantanen, T, Avlund, K, Suominen, H, et al. Muscle strength as a predictor of onset of ADL dependence in people aged 75 years. *Aging Clin Exp Res* 14: 10-15, 2002.

5. Kraemer, WJ, Ratamess, NA, French, DN. Resistance training for health and performance. *Curr Sports Med Rep* 1: 165-171, 2002.

6. FitzGerald, SJ, Barlow, CE, Kampert, JB, et al. Muscular Fitness and All-Cause Mortality: Prospective Observations. 1: 7, 2004.

7. Cureton, KJ, Collins, MA, Hill, DW, McElhannon, FM, Jr. Muscle hypertrophy in men and women. *Med Sci Sports Exerc* 20: 338-344, 1988.

8. Brown, AB, McCartney, N, Sale, DG. Positive adaptations to weight-lifting training in the elderly. *J Appl Physiol (1985)* 69: 1725-1733, 1990.

9. Sandri, M. Signaling in muscle atrophy and hypertrophy. *Physiology (Bethesda)* 23: 160-170, 2008.

10. Tesch, PA, Larsson, L. Muscle hypertrophy in bodybuilders. *Eur J Appl Physiol Occup Physiol* 49: 301-306, 1982.

11. ACSM. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 41: 687-708, 2009.

12. Lixandrão, ME, Ugrinowitsch, C, Berton, R, et al. Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med* 48: 361-378, 2018.

13. Sato, Y. The history and future of KAATSU Training. *International Journal of KAATSU Training Research* 1: 1-5, 2005.

14. Takarada, Y, Nakamura, Y, Aruga, S, et al. Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J Appl Physiol (1985)* 88: 61-65, 2000.

15. Takarada, Y, Sato, Y, Ishii, N. Effects of resistance exercise combined with vascular occlusion on muscle function in athletes. *Eur J Appl Physiol* 86: 308-314, 2002.

16. Abe, T, Yasuda, T, Midorikawa, T, et al. Skeletal muscle size and circulating IGF-1 are increased after two weeks of twice daily "KAATSU" resistance training. *International Journal of KAATSU Training Research* 1: 6-12, 2005.

17. Yasuda, T, Ogasawara, R, Sakamaki, M, et al. Combined effects of low-intensity blood flow restriction training and high-intensity resistance training on muscle strength and size. *Eur J Appl Physiol* 111: 2525-2533, 2011.

18. Lixandrao, ME, Ugrinowitsch, C, Berton, R, et al. Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med* 48: 361-378, 2018.

19. Loenneke, JP, Wilson, GJ, Wilson, JM. A mechanistic approach to blood flow occlusion. *Int J Sports Med* 31: 1-4, 2010.

20. Pearson, S, Hussain, S. A Review on the Mechanisms of Blood-Flow Restriction Resistance Training-Induced Muscle Hypertrophy. *Sports Med* 45: 187-200, 2015.

21. Kraemer, WJ, Ratamess, NA. Fundamentals of resistance training: progression and exercise prescription. *Med Sci Sports Exerc* 36: 674-688, 2004.

22. McDonagh, MJ, Davies, CT. Adaptive response of mammalian skeletal muscle to exercise with high loads. *Eur J Appl Physiol Occup Physiol* 52: 139-155, 1984.

23. Schoenfeld, BJ, Wilson, JM, Lowery, RP, Krieger, JW. Muscular adaptations in low- versus high-load resistance training: A meta-analysis. *Eur J Sport Sci* 16: 1-10, 2016.

24. McCall, GE, Byrnes, WC, Dickinson, A, Pattany, PM, Fleck, SJ. Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *J Appl Physiol (1985)* 81: 2004-2012, 1996.

25. Kosek, DJ, Kim, JS, Petrella, JK, Cross, JM, Bamman, MM. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *J Appl Physiol (1985)* 101: 531-544, 2006.

26. Henneman, E, Somjen, G, Carpenter, DO. Functional significance of cell size in spinal motoneurons. *J Neurophysiol* 28: 560-580, 1965.

27. Schoenfeld, BJ, Peterson, MD, Ogborn, D, Contreras, B, Sonmez, GT. Effects of Low- vs. High-Load Resistance Training on Muscle Strength and Hypertrophy in Well-Trained Men. *J Strength Cond Res* 29: 2954-2963, 2015.

28. Schoenfeld, BJ. The mechanisms of muscle hypertrophy and their application to resistance training. *J Strength Cond Res* 24: 2857-2872, 2010.

29. Welle, S, Totterman, S, Thornton, C. Effect of age on muscle hypertrophy induced by resistance training. *J Gerontol A Biol Sci Med Sci* 51: M270-275, 1996.

30. Toigo, M, Boutellier, U. New fundamental resistance exercise determinants of molecular and cellular muscle adaptations. *Eur J Appl Physiol* 97: 643-663, 2006.

31. Tidball, JG. Mechanical signal transduction in skeletal muscle growth and adaptation. *Journal of applied physiology (Bethesda, Md : 1985)* 98: 1900-1908, 2005.

32. Goldberg, AL, Etlinger, JD, Goldspink, DF, Jablecki, C. Mechanism of work-induced hypertrophy of skeletal muscle. *Medicine and science in sports* 7: 185-198, 1975.

33. Hornberger, TA. Mechanotransduction and the regulation of mTORC1 signaling in skeletal muscle. *The international journal of biochemistry & cell biology* 43: 1267-1276, 2011.

34. Wackerhage, H, Schoenfeld, BJ, Hamilton, DL, Lehti, M, Hulmi, JJ. Stimuli and sensors that initiate skeletal muscle hypertrophy following resistance exercise. *J Appl Physiol* 126: 30-43, 2019.

35. Burd, NA, West, DWD, Staples, AW, et al. Low-Load High Volume Resistance Exercise Stimulates Muscle Protein Synthesis More Than High-Load Low Volume Resistance Exercise in Young Men. *PLoS One* 5: e12033, 2010.

36. Burd, NA, Andrews, RJ, West, DWD, et al. Muscle time under tension during resistance exercise stimulates differential muscle protein sub-fractional synthetic responses in men. *The Journal of Physiology* 590: 351-362, 2012.

37. McCarthy, JJ, Esser, KA. Anabolic and catabolic pathways regulating skeletal muscle mass. *Curr Opin Clin Nutr Metab Care* 13: 230-235, 2010.

38. Loenneke, JP, Fahs, CA, Rossow, LM, Abe, T, Bemben, MG. The anabolic benefits of venous blood flow restriction training may be induced by muscle cell swelling. *Med Hypotheses* 78: 151-154, 2012.

39. Bodine, SC, Stitt, TN, Gonzalez, M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nature cell biology* 3: 1014-1019, 2001.

40. Glass, DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol* 37: 1974-1984, 2005.

41. Bamman, MM, Roberts, BM, Adams, GR. Molecular Regulation of Exercise-Induced Muscle Fiber Hypertrophy. *Cold Spring Harb Perspect Med* 8: a029751, 2018.

42. Marcotte, GR, West, DW, Baar, K. The molecular basis for load-induced skeletal muscle hypertrophy. *Calcif Tissue Int* 96: 196-210, 2015.

43. Eliasson, J, Elfegoun, T, Nilsson, J, et al. Maximal lengthening contractions increase p70 S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply. *American Journal of Physiology-Endocrinology and Metabolism* 291: E1197-E1205, 2006.

44. Xiao, L, Wang, YC, Li, WS, Du, Y. The role of mTOR and phospho-p70S6K in pathogenesis and progression of gastric carcinomas: an immunohistochemical study on tissue microarray. *Journal of experimental & clinical cancer research : CR* 28: 152-152, 2009.

45. Baar, K, Esser, K. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol* 276: C120-127, 1999.

46. Schiaffino, S, Dyar, KA, Ciciliot, S, Blaauw, B, Sandri, M. Mechanisms regulating skeletal muscle growth and atrophy. *Febs j* 280: 4294-4314, 2013.

47. Newham, DJ, McPhail, G, Mills, KR, Edwards, RH. Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci* 61: 109-122, 1983.

48. Peake, JM, Neubauer, O, Gatta, PAD, Nosaka, K. Muscle damage and inflammation during recovery from exercise. *Journal of Applied Physiology* 122: 559-570, 2017.

49. Malm, C. Exercise-induced muscle damage and inflammation: fact or fiction? *Acta Physiol Scand* 171: 233-239, 2001.

50. Tidball, JG. Mechanisms of muscle injury, repair, and regeneration. *Compr Physiol* 1: 2029-2062, 2011.

51. Proske, U, Morgan, DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of Physiology* 537: 333-345, 2001.

52. Paulsen, G, Mikkelsen, UR, Raastad, T, Peake, JM. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev* 18: 42-97, 2012.

53. Evans, WJ, Cannon, JG. The metabolic effects of exercise-induced muscle damage. *Exerc Sport Sci Rev* 19: 99-125, 1991.

54. Tidball, JG, Villalta, SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 298: R1173-R1187, 2010.

55. Tidball, JG. Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol* 288: R345-353, 2005.

56. Schoenfeld, BJ. Does exercise-induced muscle damage play a role in skeletal muscle hypertrophy? *J Strength Cond Res* 26: 1441-1453, 2012.

57. Koh, TJ, Pizza, FX. Do inflammatory cells influence skeletal muscle hypertrophy? *Front Biosci (Elite Ed)* 1: 60-71, 2009.

58. Nguyen, HX, Tidball, JG. Interactions between neutrophils and macrophages promote macrophage killing of rat muscle cells in vitro. *J Physiol* 547: 125-132, 2003.

59. Suzuki, YJ, Ford, GD. Redox regulation of signal transduction in cardiac and smooth muscle. *J Mol Cell Cardiol* 31: 345-353, 1999.

60. Goldfarb, AH, Garten, RS, Chee, PD, et al. Resistance exercise effects on blood glutathione status and plasma protein carbonyls: influence of partial vascular occlusion. *Eur J Appl Physiol* 104: 813-819, 2008.

61. Tidball, JG, Berchenko, E, Frenette, J. Macrophage invasion does not contribute to muscle membrane injury during inflammation. *J Leukoc Biol* 65: 492-498, 1999.

62. St Pierre, BA, Tidball, JG. Differential response of macrophage subpopulations to soleus muscle reloading after rat hindlimb suspension. *J Appl Physiol (1985)* 77: 290-297, 1994.

63. Merly, F, Lescaudron, L, Rouaud, T, Crossin, F, Gardahaut, MF. Macrophages enhance muscle satellite cell proliferation and delay their differentiation. *Muscle Nerve* 22: 724-732, 1999.

64. Tidball, JG, Wehling-Henricks, M. Macrophages promote muscle membrane repair and muscle fibre growth and regeneration during modified muscle loading in mice in vivo. *The Journal of Physiology* 578: 327-336, 2007.

65. Serrano, AL, Baeza-Raja, B, Perdiguero, E, Jardí, M, Muñoz-Cánoves, P. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell Metab* 7: 33-44, 2008.

66. Cantini, M, Massimino, ML, Rapizzi, E, et al. Human satellite cell proliferation in vitro is regulated by autocrine secretion of IL-6 stimulated by a soluble factor(s) released by activated monocytes. *Biochem Biophys Res Commun* 216: 49-53, 1995.

67. Morgan, JE, Partridge, TA. Muscle satellite cells. *Int J Biochem Cell Biol* 35: 1151-1156, 2003.

68. Wang, XD, Kawano, F, Matsuoka, Y, et al. Mechanical load-dependent regulation of satellite cell and fiber size in rat soleus muscle. *Am J Physiol Cell Physiol* 290: C981-989, 2006.

69. Vierck, J, O'Reilly, B, Hossner, K, et al. Satellite cell regulation following myotrauma caused by resistance exercise. *Cell biology international* 24: 263-272, 2000.

70. Moss, FP, Leblond, CP. Satellite cells as the source of nuclei in muscles of growing rats. *Anat Rec* 170: 421-435, 1971.

71. Allen, DL, Roy, RR, Edgerton, VR. Myonuclear domains in muscle adaptation and disease. *Muscle Nerve* 22: 1350-1360, 1999.

72. Sharp, NC, Koutedakis, Y. Sport and the overtraining syndrome: immunological aspects. *Br Med Bull* 48: 518-533, 1992.

73. Smith, LL, Keating, MN, Holbert, D, et al. The effects of athletic massage on delayed onset muscle soreness, creatine kinase, and neutrophil count: a preliminary report. *J Orthop Sports Phys Ther* 19: 93-99, 1994.

74. Robertson, TA, Maley, MA, Grounds, MD, Papadimitriou, JM. The role of macrophages in skeletal muscle regeneration with particular reference to chemotaxis. *Exp Cell Res* 207: 321-331, 1993.

75. Nathan, CF. Secretory products of macrophages. *J Clin Invest* 79: 319-326, 1987.

76. Tatsumi, R, Hattori, A, Ikeuchi, Y, Anderson, JE, Allen, RE. Release of hepatocyte growth factor from mechanically stretched skeletal muscle satellite cells and role of pH and nitric oxide. *Mol Biol Cell* 13: 2909-2918, 2002.

77. Kimura, F, Miyazaki, M, Suwa, T, et al. Increased levels of human hepatocyte growth factor in serum and peritoneal fluid after partial hepatectomy. *Am J Gastroenterol* 91: 116-121, 1996.

78. Tatsumi, R, Sheehan, SM, Iwasaki, H, Hattori, A, Allen, RE. Mechanical stretch induces activation of skeletal muscle satellite cells in vitro. *Exp Cell Res* 267: 107-114, 2001.

79. Allen, RE, Sheehan, SM, Taylor, RG, Kendall, TL, Rice, GM. Hepatocyte growth factor activates quiescent skeletal muscle satellite cells in vitro. *J Cell Physiol* 165: 307-312, 1995.

80. Brahm, H, Piehl-Aulin, K, Saltin, B, Ljunghall, S. Net fluxes over working thigh of hormones, growth factors and biomarkers of bone metabolism during short lasting dynamic exercise. *Calcif Tissue Int* 60: 175-180, 1997.

81. Barton-Davis, ER, Shoturma, DI, Musaro, A, Rosenthal, N, Sweeney, HL. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proc Natl Acad Sci U S A* 95: 15603-15607, 1998.

82. Musarò, A, McCullagh, K, Paul, A, et al. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 27: 195-200, 2001.

83. Machida, S, Booth, FW. Insulin-like growth factor 1 and muscle growth: implication for satellite cell proliferation. *Proc Nutr Soc* 63: 337-340, 2004.

84. Bamman, MM, Shipp, JR, Jiang, J, et al. Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. *Am J Physiol Endocrinol Metab* 280: E383-390, 2001.

85. Yang, S, Alnaqeeb, M, Simpson, H, Goldspink, G. Cloning and characterization of an IGF-1 isoform expressed in skeletal muscle subjected to stretch. *J Muscle Res Cell Motil* 17: 487-495, 1996.

86. Hill, M, Goldspink, G. Expression and splicing of the insulin-like growth factor gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. *The Journal of physiology* 549: 409-418, 2003.

87. Haddad, F, Adams, GR. Selected Contribution: Acute cellular and molecular responses to resistance exercise. *J Appl Physiol* 93: 394-403, 2002.

88. Schoenfeld, BJ. Potential mechanisms for a role of metabolic stress in hypertrophic adaptations to resistance training. *Sports Med* 43: 179-194, 2013.

89. Suga, T, Okita, K, Morita, N, et al. Intramuscular metabolism during low-intensity resistance exercise with blood flow restriction. *J Appl Physiol (1985)* 106: 1119-1124, 2009.

90. Schott, J, McCully, K, Rutherford, OM. The role of metabolites in strength training. *Eur J Appl Physiol Occup Physiol* 71: 337-341, 1995.

91. Tesch, PA, Colliander, EB, Kaiser, P. Muscle metabolism during intense, heavy-resistance exercise. *Eur J Appl Physiol Occup Physiol* 55: 362-366, 1986.

92. Tamaki, T, Uchiyama, S, Tamura, T, Nakano, S. Changes in muscle oxygenation during weight-lifting exercise. *European journal of applied physiology and occupational physiology* 68: 465-469, 1994.

93. Schoenfeld, BJ. The Mechanisms of Muscle Hypertrophy and Their Application to Resistance Training. *The Journal of Strength & Conditioning Research* 24: 2857-2872, 2010.

94. Kraemer, WJ, Fleck, SJ, Dziados, JE, et al. Changes in hormonal concentrations after different heavy-resistance exercise protocols in women. *J Appl Physiol (1985)* 75: 594-604, 1993.

95. Kraemer, WJ, Marchitelli, L, Gordon, SE, et al. Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol (1985)* 69: 1442-1450, 1990.

96. Schoenfeld, BJ, Contreras, B, Vigotsky, AD, Peterson, M. Differential Effects of Heavy Versus Moderate Loads on Measures of Strength and Hypertrophy in Resistance-Trained Men. *J Sports Sci Med* 15: 715-722, 2016.

97. Takarada, Y, Takazawa, H, Sato, Y, et al. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. *Journal of applied physiology (Bethesda, Md : 1985)* 88: 2097-2106, 2000.

98. Vechin, FC, Libardi, CA, Conceição, MS, et al. Comparisons between low-intensity resistance training with blood flow restriction and high-intensity resistance training on quadriceps muscle mass and strength in elderly. *J Strength Cond Res* 29: 1071-1076, 2015.

99. Lowery, RP, Joy, JM, Loenneke, JP, et al. Practical blood flow restriction training increases muscle hypertrophy during a periodized resistance training programme. *Clin Physiol Funct Imaging* 34: 317-321, 2014.

100. Loenneke, JP, Wilson, JM, Marín, PJ, Zourdos, MC, Bemben, MG. Low intensity blood flow restriction training: a meta-analysis. *Eur J Appl Physiol* 112: 1849-1859, 2012.

101. Centner, C, Wiegel, P, Gollhofer, A, König, D. Effects of Blood Flow Restriction Training on Muscular Strength and Hypertrophy in Older Individuals: A Systematic Review and Meta-Analysis. *Sports Med* 49: 95-108, 2019.

102. Hughes, L, Paton, B, Rosenblatt, B, Gissane, C, Patterson, SD. Blood flow restriction training in clinical musculoskeletal rehabilitation: a systematic review and meta-analysis. *Br J Sports Med* 51: 1003-1011, 2017.

103. Yasuda, T, Fujita, S, Ogasawara, R, Sato, Y, Abe, T. Effects of low-intensity bench press training with restricted arm muscle blood flow on chest muscle hypertrophy: a pilot study. *Clin Physiol Funct Imaging* 30: 338-343, 2010.

104. Fujita, T, Brechue, WF, Kurita, K, Sato, Y, Abe, T. Increased muscle volume and strength following six days of low-intensity resistance training with restricted muscle blood flow. *International Journal of KAATSU Training Research* 4: 1-8, 2008.

105. Fahs, CA, Loenneke, JP, Rossow, LM, Tiebaud, RS, Bemben, MG. Methodological considerations for blood flow restricted resistance exercise. *Journal of Trainology* 1: 14-22, 2012.

106. Jessee, MB, Buckner, SL, Dankel, SJ, et al. The Influence of Cuff Width, Sex, and Race on Arterial Occlusion: Implications for Blood Flow Restriction Research. *Sports Med* 46: 913-921, 2016.

107. Hunt, JE, Stodart, C, Ferguson, RA. The influence of participant characteristics on the relationship between cuff pressure and level of blood flow restriction. *Eur J Appl Physiol* 116: 1421-1432, 2016.

108. Crenshaw, AG, Hargens, AR, Gershuni, DH, Rydevik, B. Wide tourniquet cuffs more effective at lower inflation pressures. *Acta Orthop Scand* 59: 447-451, 1988.

109. Moore, MR, Garfin, SR, Hargens, AR. Wide tourniquets eliminate blood flow at low inflation pressures. *The Journal of Hand Surgery* 12: 1006-1011, 1987.

110. Mouser, JG, Dankel, SJ, Mattocks, KT, et al. Blood flow restriction and cuff width: effect on blood flow in the legs. *Clin Physiol Funct Imaging* 38: 944-948, 2018.

111. Loenneke, JP, Allen, KM, Mouser, JG, et al. Blood flow restriction in the upper and lower limbs is predicted by limb circumference and systolic blood pressure. *Eur J Appl Physiol* 115: 397-405, 2015.

112. Shaw, JA, Murray, DG. The relationship between tourniquet pressure and underlying soft-tissue pressure in the thigh. *J Bone Joint Surg Am* 64: 1148-1152, 1982.

113. McEwen, JA, Owens, JG, Jeyasurya, J. Why is it Crucial to Use Personalized Occlusion Pressures in Blood Flow Restriction (BFR) Rehabilitation? *Journal of Medical and Biological Engineering* 39: 173-177, 2019.

114. Loenneke, JP, Kim, D, Fahs, CA, et al. Effects of exercise with and without different degrees of blood flow restriction on torque and muscle activation. *Muscle Nerve* 51: 713-721, 2015.

115. Counts, BR, Dankel, SJ, Barnett, BE, et al. Influence of relative blood flow restriction pressure on muscle activation and muscle adaptation. *Muscle Nerve* 53: 438-445, 2016.

116. Thiebaud, RS, Yasuda, T, Loenneke, JP, Abe, T. Effects of low-intensity concentric and eccentric exercise combined with blood flow restriction on indices of exercise-induced muscle damage. *Interv Med Appl Sci* 5: 53-59, 2013.

117. Thiebaud, RS, Loenneke, JP, Fahs, CA, et al. Muscle damage after low-intensity eccentric contractions with blood flow restriction. *Acta Physiol Hung* 101: 150-157, 2014.

118. Loenneke, JP, Thiebaud, RS, Abe, T. Does blood flow restriction result in skeletal muscle damage? A critical review of available evidence. *Scand J Med Sci Sports* 24: e415-422, 2014.

119. Winchester, LJ, Morris, CE, Badinger, J, Wiczynski, TL, VanWye, WR. Blood Flow Restriction at High Resistance Loads Increases the Rate of Muscular Fatigue, but Does Not Increase Plasma Markers of Myotrauma or Inflammation. *J Strength Cond Res*, 2020.

120. Suga, T, Okita, K, Morita, N, et al. Dose effect on intramuscular metabolic stress during low-intensity resistance exercise with blood flow restriction. *Journal of applied physiology (Bethesda, Md : 1985)* 108: 1563-1567, 2010.

121. Pope, ZK, Willardson, JM, Schoenfeld, BJ. Exercise and blood flow restriction. *J Strength Cond Res* 27: 2914-2926, 2013.

122. Suga, T, Okita, K, Takada, S, et al. Effect of multiple set on intramuscular metabolic stress during low-intensity resistance exercise with blood flow restriction. *Eur J Appl Physiol* 112: 3915-3920, 2012.

123. Teixeira, EL, Barroso, R, Silva-Batista, C, et al. Blood flow restriction increases metabolic stress but decreases muscle activation during high-load resistance exercise. *Muscle Nerve* 57: 107-111, 2018.

124. Doessing, S, Heinemeier, KM, Holm, L, et al. Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. *J Physiol* 588: 341-351, 2010.

125. Moritani, T, Sherman, WM, Shibata, M, Matsumoto, T, Shinohara, M. Oxygen availability and motor unit activity in humans. *Eur J Appl Physiol Occup Physiol* 64: 552-556, 1992.

126. Fujita, S, Abe, T, Drummond, MJ, et al. Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *J Appl Physiol (1985)* 103: 903-910, 2007.

127. Cook, SB, Murphy, BG, Labarbera, KE. Neuromuscular function after a bout of low-load blood flow-restricted exercise. *Med Sci Sports Exerc* 45: 67-74, 2013.

128. Vanhelder, WP, Radomski, MW, Goode, RC. Growth hormone responses during intermittent weight lifting exercise in men. *Eur J Appl Physiol Occup Physiol* 53: 31-34, 1984.

129. Manini, TM, Clark, BC. Blood flow restricted exercise and skeletal muscle health. *Exerc Sport Sci Rev* 37: 78-85, 2009.

130. Favero, TG, Zable, AC, Colter, D, Abramson, JJ. Lactate inhibits Ca(2+) -activated Ca(2+)-channel activity from skeletal muscle sarcoplasmic reticulum. *J Appl Physiol (1985)* 82: 447-452, 1997.

131. Dankel, SJ, Buckner, SL, Jessee, MB, et al. Can blood flow restriction augment muscle activation during high-load training? *Clin Physiol Funct Imaging* 38: 291-295, 2018.

132. Goto, K, Ishii, N, Kizuka, T, Takamatsu, K. The impact of metabolic stress on hormonal responses and muscular adaptations. *Med Sci Sports Exerc* 37: 955-963, 2005.

133. Reeves, GV, Kraemer, RR, Hollander, DB, et al. Comparison of hormone responses following light resistance exercise with partial vascular occlusion and moderately difficult resistance exercise without occlusion. *J Appl Physiol (1985)* 101: 1616-1622, 2006.

134. Takano, H, Morita, T, Iida, H, et al. Hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with the reduction of muscle blood flow. *Eur J Appl Physiol* 95: 65-73, 2005.

135. Pierce, JR, Clark, BC, Ploutz-Snyder, LL, Kanaley, JA. Growth hormone and muscle function responses to skeletal muscle ischemia. *Journal of applied physiology (Bethesda, Md : 1985)* 101: 1588-1595, 2006.

136. Fujita, S, Abe, T, Drummond, MJ, et al. Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *J Appl Physiol* 103: 903-910, 2007.

137. Chwalbinska-Moneta, J, Krysztofiak, H, Ziemba, A, Nazar, K, Kaciuba-Uściłko, H. Threshold increases in plasma growth hormone in relation to plasma catecholamine and blood lactate concentrations during progressive exercise in endurance-trained athletes. *Eur J Appl Physiol Occup Physiol* 73: 117-120, 1996.

138. Inagaki, Y, Madarame, H, Neya, M, Ishii, N. Increase in serum growth hormone induced by electrical stimulation of muscle combined with blood flow restriction. *Eur J Appl Physiol* 111: 2715-2721, 2011.

139. Florini, JR, Ewton, DZ, Coolican, SA. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev* 17: 481-517, 1996.

140. Hawke, TJ, Garry, DJ. Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol (1985)* 91: 534-551, 2001.

141. Yoshida, T, Delafontaine, P. Mechanisms of IGF-1-Mediated Regulation of Skeletal Muscle Hypertrophy and Atrophy. *Cells* 9: 1970, 2020.

142. Uchiyama, S, Tsukamoto, H, Yoshimura, S, Tamaki, T. Relationship between oxidative stress in muscle tissue and weight-lifting-induced muscle damage. *Pflugers Archiv : European journal of physiology* 452: 109-116, 2006.

143. Clanton, TL. Hypoxia-induced reactive oxygen species formation in skeletal muscle. *J Appl Physiol (1985)* 102: 2379-2388, 2007.

144. Powers, SK, Duarte, J, Kavazis, AN, Talbert, EE. Reactive oxygen species are signalling molecules for skeletal muscle adaptation. *Exp Physiol* 95: 1-9, 2010.

145. Barbieri, E, Sestili, P. Reactive oxygen species in skeletal muscle signaling. *Journal of signal transduction* 2012, 2012.

146. Thompson, HS, Scordilis, SP, Clarkson, PM, Lohrer, WA. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiol Scand* 171: 187-193, 2001.

147. Febbraio, MA, Steensberg, A, Fischer, CP, et al. IL-6 activates HSP72 gene expression in human skeletal muscle. *Biochem Biophys Res Commun* 296: 1264-1266, 2002.

148. Fry, CS, Glynn, EL, Drummond, MJ, et al. Blood flow restriction exercise stimulates mTORC1 signaling and muscle protein synthesis in older men. *Journal of applied physiology (Bethesda, Md : 1985)* 108: 1199-1209, 2010.

149. Nielsen, JL, Aagaard, P, Prokhorova, TA, et al. Blood flow restricted training leads to myocellular macrophage infiltration and upregulation of heat shock proteins, but no apparent muscle damage. *J Physiol* 595: 4857-4873, 2017.

150. Jain, K, Suryakumar, G, Ganju, L, Singh, SB. Differential hypoxic tolerance is mediated by activation of heat shock response and nitric oxide pathway. *Cell Stress Chaperones* 19: 801-812, 2014.

151. Cumming, KT, Paulsen, G, Wernbom, M, Ugelstad, I, Raastad, T. Acute response and subcellular movement of HSP27, αB-crystallin and HSP70 in human skeletal muscle after blood-flow-restricted low-load resistance exercise. *Acta Physiol (Oxf)* 211: 634-646, 2014.

152. Folkesson, M, Mackey, AL, Holm, L, et al. Immunohistochemical changes in the expression of HSP27 in exercised human vastus lateralis muscle. *Acta Physiol (Oxf)* 194: 215-222, 2008.

153. Nosaka, K, Clarkson, PM. Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Med Sci Sports Exerc* 28: 953-961, 1996.

154. Häussinger, D. The role of cellular hydration in the regulation of cell function. *Biochem J* 313 (Pt 3): 697-710, 1996.

155. Freitas, EDS, Poole, C, Miller, RM, et al. Time Course Change in Muscle Swelling: High-Intensity vs. Blood Flow Restriction Exercise. *Int J Sports Med* 38: 1009-1016, 2017.

156. Lang, F, Busch, GL, Ritter, M, et al. Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 78: 247-306, 1998.

157. Loenneke, JP, Fahs, CA, Thiebaud, RS, et al. The acute muscle swelling effects of blood flow restriction. *Acta Physiol Hung* 99: 400-410, 2012.

158. Neto, GR, Santos, HH, Sousa, JB, et al. Effects of high-intensity blood flow restriction exercise on muscle fatigue. *J Hum Kinet* 41: 163-172, 2014.

159. Biazon, T, Ugrinowitsch, C, Soligon, SD, et al. The Association Between Muscle Deoxygenation and Muscle Hypertrophy to Blood Flow Restricted Training Performed at High and Low Loads. *Front Physiol* 10: 446, 2019.

160. Laurentino, G, Ugrinowitsch, C, Aihara, AY, et al. Effects of strength training and vascular occlusion. *Int J Sports Med* 29: 664-667, 2008.

161. Fitschen, PJ, Kistler, BM, Jeong, JH, et al. Perceptual effects and efficacy of intermittent or continuous blood flow restriction resistance training. *Clin Physiol Funct Imaging* 34: 356-363, 2014.

CHAPTER 2

COMPARISON OF BLOOD FLOW RESPONSES TO VARYING BLOOD FLOW RESTRICTION PRESSURES

ABSTRACT

Purpose: The purpose of this study was to compare blood flow in the leg under varying blood flow restriction (BFR) pressures based on individualized limb occlusion pressures (LOP). **Methods:** Twenty-nine healthy participants (65.5% female, 23.8 ± 4.7 yrs., 171.5 ± 8.2 cm, 73.9 ± 14.9 kg) volunteered for this study. An 11.5 cm tourniquet cuff was placed around participant's right proximal thigh, followed by an automated LOP measurement (207.1 ± 29.4 mmHg). Next, volumetric blood flow (Vol Flow) was measured via ultrasound at rest and at 10% increments of LOP (10-90% LOP) in a randomized order. One-way repeated-measures ANOVAs were used to examine potential differences in volumetric blood flow (cc/min) and volumetric flow relative to rest (%Rel) and between relative pressures. **Results:** Vol Flow was significantly lower at 50% (13.91±5.31), 60% (11.95±5.53), 70% (9.67±6.55), 80% (5.88±2.32), and 90% (2.35±2.11) relative pressures (all $p < .05$) when compared to rest. Vol Flow at 80% occlusion pressure, a commonly used occlusion pressure in the legs, were significantly lower than all lower occlusion pressures ($p < .05$) except 70% ($p = .052$). %Rel at 40% (17.57±21.74), 50% (20.31±21.84), 60% (32.28±19.87), 70% (46.85±20.56), 80% (63.71±15.72), and 90% (83.84±16.10) relative pressures were significantly lower (all $p < .05$) when compared to rest. **Conclusion:** A minimal threshold pressure of 50% LOP may be required to elicit significant decreases in arterial blood

flow during BFR exercise. 70% LOP may provide utility in BFR exercise for similar blood flow reductions compared to 80% while reducing occlusion pressure.

INTRODUCTION

Blood flow restriction (BFR) involves the use of an inflatable tourniquet or elastic wraps to occlude distal blood flow in a limb. The purpose of BFR training is to reduce the arterial blood supply and eliminate venous return, leading to venous pooling. BFR training shows great potential for muscle hypertrophy and strength increases using as little as 20% of 1 repetition-maximum (1RM) (1, 2). Traditionally, the American College of Sports Medicine recommends a resistance training load of $\geq$ 65% 1RM to generate increases in muscular strength and hypertrophy (3). Low-load resistance training in conjunction with BFR induces muscular hypertrophy similar to or exceeding high-load resistance training without BFR (4).

Inconsistent BFR research methodologies with the same absolute pressures being used for all participants within a study, rather than individualizing pressures, lead to difficulties in interpreting and comparing study results (5). While procedures for determining occlusion pressures have not been standardized, previous research has used cuff pressures of 140-240 mmHg for lower body occlusion and 100-160 mmHg for upper body occlusion (5). Occlusion pressure can vary from person to person based on cuff sizes and individual characteristics. Cuff width plays a significant role in predicting relative occlusion pressures (6, 7), with wider cuffs requiring lower pressures to fully occlude arterial blood flow (8-10). Limb circumference may also significantly impact the required pressure in the arms and legs (11). Larger limb circumferences and soft tissue thickness require greater pressures to occlude arterial blood flow (12). The composition and muscle thickness of the limb may influence the amount of intramuscular pressure under the cuff. Larger, hypertrophied muscles may compress the blood vessels, occluding blood flow, especially when exercising, and may require lower occlusion pressures compared to a limb with lesser muscle mass (13). Therefore, in addition to thigh

circumference, muscle and fat tissue thickness may introduce variability in blood flow response to relative pressures tested between individuals of different compositions.

If individualized relative cuff pressures are used then there are limited or no differences in blood flow at rest between different cuff widths (10). This relative pressure can be based on an individualized limb occlusion pressure (LOP). LOP is the minimum pressure required to stop the flow of arterial blood into the limb distal to the cuff (14). Using an individualized relative pressure rather than arbitrary pressures ensures the safety of the participants by using the lowest required pressure and ensuring correct occlusion percentage. Additionally, higher occlusion pressures during exercise increase participant discomfort (6).

While personalized pressures are recommended for use with BFR exercise, the blood flow response to the relative pressures needs further research. Mouser et al. (2017) examined the blood flow response in the brachial artery to three different cuff widths on the right arm and concluded blood flow did not decrease linearly as pressure was increased; with blood flow remaining relatively constant between 50 to 90% LOP. Additionally, this trend was observed across three different cuff widths (5cm, 10cm, 12cm) (15). However, conflicting findings by Mouser et al. (2018) suggested that the decrease in blood flow with increasing pressure does follow a linear pattern in the legs. During this study, the blood flow was measured more distally at the posterior tibial artery, which may demonstrate different blood flow characteristics than the brachial artery. However, in agreement with their previous study, two different cuff widths were tested and they similarly reduced blood flow when using relative pressures (10).

The Delfi Personalized Tourniquet System II (PTS II) (Delfi PTS II, Delfi Medical, Vancouver, BC, Canada) benefits from an internal pressure sensor that can determine the arterial occlusion pressure and can self-regulate relative pressures. The PTS II has been previously

validated to be non-significantly different from the gold standard doppler ultrasound technique in determining LOP (16). A previous study has demonstrated the ability to achieve full blood flow occlusion in the legs with the PTS II at a mean occlusion pressure of 239.4mmHg among 30 participants, while another BFR device (KAATSU Master) was not able to achieve full occlusion within its 500mmHg maximal pressure (17). This indicates the uniqueness of each BFR device and cuff combination and the necessity to determine the effectiveness of the devices to ensure safe and accurate usage. To date, no research has examined blood flow responses to relative pressures applied by the PTS II system. Therefore, the purpose of the present study was to examine blood flow responses in the leg during BFR in 10% increments of LOP using the PTS II. The authors hypothesized that blood flow will decrease in a non-linear fashion with increases in occlusion pressure. The results from this study will add to the knowledge of the PTS II and allow practitioners to more accurately make pressure recommendations to maximize BFR potential, while minimizing user discomfort.

METHODS

Participants

Twenty-nine (65.5% female) apparently healthy adults were recruited to participate in this study. Participant characteristics can be seen in Table 2.1. Using data from Mouser et al. (10), an *a priori* power analysis was performed (G*Power, version 3.1.9.6, Universität Kiel, Germany) following the recommendations of Beck (2013) (18). Using occlusion pressures ranging from 10-70% LOP, to exclude pressure extremes, an effect size of 0.29 was determined based on the data from Mouser et al. (10). Using repeated-measures, within-factors ANOVA, effect size of 0.29, with an alpha (α) level of .05 and desired power (1-β) of 0.80 indicated a required total sample size of 11 participants to detect an effect.

Participants were recruited via word-of-mouth advertisement and through recruitment from undergraduate kinesiology courses. Sample characteristics are summarized in Table 1. To be included in this study, participants were not required to partake in regular exercise, but were between the ages 18 and 45 and with no self-reported cardiovascular, pulmonary and metabolic disease, report no musculoskeletal injuries, and been a non-smoker. Participants were asked to refrain from ingestion of caffeine for at least 4 hours prior to reporting to the laboratory. Written, informed consent was obtained prior to participation and all study procedures were approved by the University's Institutional Review Board.

Upon arrival to the laboratory, participants completed a physical activity readiness questionnaire+ (PAR-Q+) and 24-hour history questionnaire, to determine eligibility. Next, to ensure that participants were not hypertensive, after 5 minutes of seated rest, resting blood pressure (BP) was measured with the BPM-100 automated BP monitor (BPtru medical devices; Coquitlam, Canada) three times, 1-min apart in the dominant arm and averaged. For mean resting BP, the three readings were required to agree within 5 mmHg for systolic and diastolic readings. If the first three measurements did not agree within 5 mmHg, then additional recordings will be performed until three measures in agreement were recorded. If the average systolic and/or diastolic BP for a participant was ≥140 for systolic and/or ≥90 mmHg for diastolic, the participant may have stage 2 hypertension (19) and was flagged as not meeting the study inclusion criteria – no participant met this criterion. Two participants were classified elevated BP (120-129 mmHg for systolic, and <80 mmHg for diastolic) and one participant as stage 1 hypertensive (130-139 mmHg for systolic or 80-89 mmHg for diastolic) (19).

Experimental Procedures

Following paperwork and resting blood pressure, standing height was measured without shoes to the nearest 0.1 cm with a manual stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). Body mass (BM) was measured to the nearest 0.1 kg using a digital scale (Tanita BWB-800, Tanita Corporation, Tokyo, Japan). Additionally, skinfold thicknesses were measured from each participant with calibrated skinfold calipers using 7-site skinfold analysis (20). Body density from skinfolds was calculated using the Brozek-equation (21). Next, thigh circumference was measured at the right upper thigh (75% the distance between the proximal border of the patella and superior anterior iliac spine) with a flexible, tension-sensitive, non-elastic vinyl tape measure (Gulick, Lafayette instrument Co. Lafayette, IN). The average of three circumference measurements was used for determining the participant's cuff size.

Resting Blood Flow and Limb Occlusion Pressure

Following these measures, the participants were instructed to lie supine with feet off the ground in a power adjustable examination chair (Ritter 317, Midmark Corporation, Dayton, OH) for five minutes. All ultrasound measurements were obtained in a supine position, as body position may influence LOP measurement (22). At the conclusion of the 5-minute stabilization period a resting blood flow analysis measure was taken using ultrasonography (Philips iU22 ultrasound Doppler imager, L9-3 transducer) at the posterior tibial artery. The analysis of the posterior tibial artery began posterior to the medial malleus and the ultrasound transducer was moved proximal until an acceptable image quality was achieved. The analysis site was marked with a small piece of kinesiology tape (Mueller Sports Medicine, Prairie Du Sac, WI) for consistency in measurement location.

Next, participants were outfitted with the Delfi BFR tourniquet. The PTS II was used with the Delfi Easi-Fit Tourniquet Cuffs (11.5cm width). The Easi-Fit cuffs come in three sizes (18", 24", and 34") and were chosen based on limb circumference for best fit, as per manufacturer recommendation. The BFR cuff was applied as proximal as possible on the right thigh, but distal to the fold of the gluteal muscles. The PTS II was set to self-determine the personalized limb occlusion pressures (LOP). The PTS II increases cuff pressure in increments of 10mmHg and analyzes arterial pressure pulsations following each increase in pressure to determine LOP (23). During this time, no ultrasound measures were taken as the device is very sensitive to movement and sound and may influence the value or cause the device to abort the measurement. Measurements obtained using the PTS II LOP are comparable to the criterion ultrasound doppler technique for determining LOP (23, 24). Following resting blood flow and LOP measurements, the participants remained at rest in the examination chair.

Relative Pressure Measures

Following the resting blood flow analysis and LOP, the relative pressures were tested. To ensure consistency between relative pressure measures, the cuff remained in place on the thigh while deflated and participants remained seated so that the cuff position did not change.

Relative pressures were calculated based on the LOP, in increments of 10%. A total of 9 relative pressures were tested; 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%. The order of relative pressure testing was randomized for each participant using a random number generator (Microsoft Excel 2016 for Windows, Microsoft Corporation, Redmond, WA). The randomization of relative pressures may account for possible time-order effects. Additionally, incrementally increasing pressure from 10% to 90% may not allow for full occlusion due to possible cardiovascular responses as pressure increases (15). For each relative pressure, the cuff

was inflated to the corresponding relative pressure for 2 minutes. A doppler blood flow analysis measure was taken following one minute of inflation. Following the two-minute inflation time, the cuff was deflated for a time period of 3 minutes. This process was repeated until all relative pressures were tested.

Statistical Analysis

Data were managed using Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were completed using SPSS for Windows (Version 25.0, IBM, Somers, NY, USA). One-way repeated-measures ANOVAs were used to examine potential differences in volumetric blood flow (cc/min) and blood flow volume relative to rest (%Rel) between relative pressures**.** Mauchly's test was used to test the assumption of sphericity and a Greenhouse-Geisser correction was applied when the assumption of sphericity was not met. Statistical significance was accepted at $p < .05$. Effect sizes were measured by partial eta square (ηp^2). Tukey post hoc comparisons were used to examine differences between individual occlusion pressures. Bonferroni's correction was used for adjustment for multiple comparisons.

RESULTS

Volumetric Blood Flow

Mauchly's test indicated that the assumption of sphericity was violated ($p < .05$), therefore, a Greenhouse-Geisser correction was applied. The results of the repeated measures ANOVA revealed a significant effect of relative cuff pressure on blood flow volume (cc/min) ($F_{2.142, 59.983)} = 39.771$, $p < .001$, $\eta p^2 = .587$). Pairwise comparisons (See Table 2.3 and Figure 2.1) demonstrated significant reductions in blood flow volume compared to Rest for the 50%, 60%, 70%, 80%, and 90% relative pressures (all $p < .05$). No significant differences in blood flow volume were found between the range of pressures from Rest to 40% occlusion measures

(all $p > .05$). Volumetric flow at 90% (2.35 ± 2.11 cc/min) occlusion was significantly less than all other occlusion pressures and Rest ($p < .05$). Volumetric flow at 80% (5.88 ± 2.32 cc/min) occlusion pressure was found to be significantly different from all other pressures ($p < .05$) except 70% (9.67 ± 6.55 cc/min) ($p = .052$). 60% (11.94 ± 5.53 cc/min) occlusion pressure was significantly different from all other pressures ($p < .05$) except 70% (9.67 ± 6.55 cc/min) ($p = .160$).

Relative Flow

Mauchly's test indicated that the assumption of sphericity was violated ($p < .05$), therefore, a Greenhouse-Geisser correction was applied. The results of the repeated measures ANOVA revealed a significant effect of relative cuff pressure on blood flow relative to Rest (%Rel) ($F_{5.336, 149.405)} = 99.850$, $p < .001$, $\eta p^2 = .781$). Pairwise comparisons (See Table 2.4 and Figure 2.2) demonstrated statistically significant reductions in relative blood flow at 40%, 50%, 60%, 70%, 80%, and 90% relative pressures (all $p < .05$) compared to Rest. No significant differences in relative flow were found between the range of pressures from Rest to 30% relative flow (all $p > .05$). 90% (16.16 ± 16.10 %Rel), 80% (36.29 ± 15.72 %Rel), 70% (53.15 ± 20.56 %Rel), 60% (67.72 ±19.87 %Rel), and 50% (79.70 ± 21.84 %Rel) occlusion pressure were significantly different from all other occlusion pressures and Rest ($p < .05$).

DISCUSSION

The purpose of this study was to examine blood flow responses to relative pressures applied by a common clinical BFR device, the PTS II. While the device has been validated for measuring LOP (16), the current study was the first to examine blood flow occlusion characteristics using the PTS II. Overall findings from this study support the hypothesis that there is a non-linear decrease in blood flow in the posterior tibial artery with increases in

occlusion pressure. The non-linear decrease in blood flow demonstrates the resiliency of the vascular and circulatory system to overcome lower occlusion pressures.

Previous literature by Iida et al. (25, 26) and Hunt et al. (7) observed linear decreases in blood flow with increases in occlusion pressure. However, these studies did not use individual LOPs and rather used incremental absolute pressures for all participants to determine changes in blood flow. While absolute pressures are still widely used in rehabilitative and research settings, personalized occlusion pressures are recommended for increased safety and reliability of BFR (14). Individual differences in BFR, such as limb circumference and cuff width, are known to influence LOP pressures and should be taken into account when prescribing. Measured tissue pressures underneath occlusion cuffs decrease when moving from subcutaneous measures to more central measures near the bone (12). This decrease in central tissue pressure is more pronounced in limbs of greater circumference (12), requiring greater pressure for limb occlusion. Wider cuffs are able to occlude blood flow at lower pressures likely due to the wider cuff causing increased shear stress (frictional resistance) along the length of the artery underneath the cuff and may allow achievement of full arterial occlusion without occlusion of the vessel lumen (27). In agreement with the current study, Mouser et al. previously demonstrated that when using personalized pressures, decreases in blood flow are non-linear and not proportional to the applied relative pressure (15, 28). Only a single cuff width (11.5cm) was examined in this study, but previous research findings indicates that while cuff widths affect LOP, varying cuff widths (5cm, 10cm, and 12cm) all occlude blood flow in a similar manner when using relative personalized occlusion pressures for each cuff width (15).

The cuff pressure needs to overcome the systolic pressure in order to occlude blood flow. Based on the findings of this study, 50% LOP using the 11.5cm Delfi cuffs indicates this

threshold for occlusion at which point the pressure compression and shear stress is able to significantly impact blood flow. At this point, a significant reduction in blood flow is observed and blood flow decreases more linearly with increased cuff pressure.

While no significant reduction in arterial blood flow was observed in the current study at ≤ 40%LOP, the second component of BFR exercise relates to the venous return. Previous research has indicated that venous return is occluded at lower relative pressures, 45-50mmHg, compared to arteries (26, 29). These lower pressures may increase tissue pressure and venous occlusion but are not sufficient for occluding arterial blood supply in the legs.

The use of high occlusion pressures (≥ 60% LOP) does not elicit additional benefits compared to lower pressures in low-load resistance training (30, 31). Reis et al. found that during 4 sets of knee extensions working at 20%1RM, 80% LOP caused no significant additional deoxygenation in the vastus lateralis compared to 60% LOP (32). Additionally, the authors suggest that 60% LOP during exercise may represent a physiological threshold for increased tissue deoxygenation and metabolite accumulation (32). While the current study focused on blood flow responses to BFR occlusion at rest, 50% LOP was the lowest relative pressure to differ significantly from Rest (cc/min) and further supports the idea of an occlusion threshold in lower limbs. Kilgas et al. demonstrated that pressures above 60% LOP cause tissue hypoxia and increased metabolite accumulation, while pressures above 80%LOP do not enhance this effect during rhythmic handgrip exercise (30 contractions, 30% MVC) (33). However, whether exercise using larger muscle groups would exacerbate the hypoxic environment is unknown. Examining blood flow during the handgrip exercise found that 0%, 60%, and 80% LOP all experience increases in blood flow during exercise indicating that the cardiovascular system is able to overcome the mechanical cuff compression (10cm nylon pneumatic cuff) during

muscular contraction (33). While both 60% and 80%LOP experienced increased blood flow during exercise, comparatively, 80%LOP maintained greater reductions in blood flow both at rest and during exercise compared to baseline (33). This indicates the necessity for additional research examining blood flow responses to occlusion during exercise and associated acute and longitudinal on fatigue and hypertrophy.

Limitations and Future Research

A limitation of this study was the position and resting condition of the participant during the data collection. All measurements were taking in a reclined-supine position, rather than seated or standing which would more closely mimic the body position during BFR exercise or research protocols. Seated and standing positions require higher LOPs (22) and may respond differently to relative pressures. Further research is required to examine the effect of postural changes on relative blood flow. Additionally, all blood flow measurements were taken at rest. The muscle contraction and body movement during exercise may alter blood flow responses to relative pressures and needs to be further investigated using the PTS II. Lastly, in this study, the LOP pressures were determined through the built-in automated PTP process rather than manually determined. Although the PTS II has been validated for accurately measuring LOP, there may be instances in which the device over-predicted the personalized LOP, and future research may benefit from the inclusion of a LOP verification measurement.

CONCLUSION

The findings of the current study indicate the potential utility of lower occlusion pressure in the lower limbs (i.e. 60% or 70% LOP) compared to the recommended 80% LOP while achieving similar arterial blood flow reductions. Lower occlusion pressures (i.e. ≤ 40% LOP) minimally occlude arterial blood flow and may not be sufficient for creating a hypoxic

environment in the working muscle, whereas 50% LOP may be the minimum threshold pressure required to elicit significant decreases in arterial blood flow during BFR exercise. Using lower occlusion pressure in the lower limbs (i.e. 60% or 70% LOP) may be beneficial by decreasing discomfort for the participants with similar occlusion effects. Future research should focus on blood flow responses during exercise using varying relative pressures to determine the optimal occlusion pressures for enhanced BFR exercise.

REFERENCES

1. Abe, T, Yasuda, T, Midorikawa, T, et al. Skeletal muscle size and circulating IGF-1 are increased after two weeks of twice daily “KAATSU” resistance training. *International Journal of KAATSU Training Research* 1: 6-12, 2005.

2. Yasuda, T, Ogasawara, R, Sakamaki, M, et al. Combined effects of low-intensity blood flow restriction training and high-intensity resistance training on muscle strength and size. *Eur J Appl Physiol* 111: 2525-2533, 2011.

3. ACSM. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 41: 687-708, 2009.

4. Lixandrao, ME, Ugrinowitsch, C, Berton, R, et al. Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med* 48: 361-378, 2018.

5. Fahs, CA, Loenneke, JP, Rossow, LM, Tiebaud, RS, Bemben, MG. Methodological considerations for blood flow restricted resistance exercise. *Journal of Trainology* 1: 14-22, 2012.

6. Jessee, MB, Buckner, SL, Dankel, SJ, et al. The Influence of Cuff Width, Sex, and Race on Arterial Occlusion: Implications for Blood Flow Restriction Research. *Sports Med* 46: 913-921, 2016.

7. Hunt, JE, Stodart, C, Ferguson, RA. The influence of participant characteristics on the relationship between cuff pressure and level of blood flow restriction. *Eur J Appl Physiol* 116: 1421-1432, 2016.

8. Crenshaw, AG, Hargens, AR, Gershuni, DH, Rydevik, B. Wide tourniquet cuffs more effective at lower inflation pressures. *Acta Orthop Scand* 59: 447-451, 1988.

9. Moore, MR, Garfin, SR, Hargens, AR. Wide tourniquets eliminate blood flow at low inflation pressures. *The Journal of Hand Surgery* 12: 1006-1011, 1987.

10. Mouser, JG, Dankel, SJ, Mattocks, KT, et al. Blood flow restriction and cuff width: effect on blood flow in the legs. *Clin Physiol Funct Imaging* 38: 944-948, 2018.

11. Loenneke, JP, Allen, KM, Mouser, JG, et al. Blood flow restriction in the upper and lower limbs is predicted by limb circumference and systolic blood pressure. *Eur J Appl Physiol* 115: 397-405, 2015.

12. Shaw, JA, Murray, DG. The relationship between tourniquet pressure and underlying soft-tissue pressure in the thigh. *J Bone Joint Surg Am* 64: 1148-1152, 1982.

13. Wernbom, M, Augustsson, J, Thomeé, R. Effects of vascular occlusion on muscular endurance in dynamic knee extension exercise at different submaximal loads. *The Journal of Strength & Conditioning Research* 20: 372-377, 2006.

14. McEwen, JA, Owens, JG, Jeyasurya, J. Why is it Crucial to Use Personalized Occlusion Pressures in Blood Flow Restriction (BFR) Rehabilitation? *Journal of Medical and Biological Engineering* 39: 173-177, 2019.

15. Mouser, JG, Dankel, SJ, Jessee, MB, et al. A tale of three cuffs: the hemodynamics of blood flow restriction. *Eur J Appl Physiol* 117: 1493-1499, 2017.

16. Masri, BA, Day, B, Younger, AS, Jeyasurya, J. Technique for Measuring Limb Occlusion Pressure that Facilitates Personalized Tourniquet Systems: A Randomized Trial. *J Med Biol Eng* 36: 644-650, 2016.

17. Weatherholt, AM, Vanwye, WR, Lohmann, J, Owens, JG. The Effect of Cuff Width for Determining Limb Occlusion Pressure: A Comparison of Blood Flow Restriction Devices. *Int J Exerc Sci* 12: 136-143, 2019.

18. Beck, TW. The importance of a priori sample size estimation in strength and conditioning research. *J Strength Cond Res* 27: 2323-2337, 2013.

19. Whelton, PK, Carey, RM, Aronow, WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 71: e13-e115, 2018.

20. American College of Sports, M, Riebe, D, Ehrman, JK, Liguori, G, Magal, M. *ACSM's guidelines for exercise testing and prescription.* 2018.

21. Brožek, J, Grande, F, Anderson, JT, Keys, A. Densitometric analysis of body composition: revision of some quantitative assumptions. *Ann N Y Acad Sci* 110: 113-140, 1963.

22. Hughes, L, Jeffries, O, Waldron, M, et al. Influence and reliability of lower-limb arterial occlusion pressure at different body positions. *PeerJ* 6: e4697, 2018.

23. McEwen, JA, Masri, BA, Day, B, Younger, AS. Development of Personalized Tourniquet Systems Using a New Technique for Measuring Limb Occlusion Pressure. Presented at World Congress on Medical Physics and Biomedical Engineering, June 7-12, 2015, Toronto, Canada, Cham, 2015//, 2015.

24. Masri, BA, Day, B, Younger, ASE, Jeyasurya, J. Technique for Measuring Limb Occlusion Pressure that Facilitates Personalized Tourniquet Systems: A Randomized Trial. *Journal of Medical and Biological Engineering* 36: 644-650, 2016.

25. Iida, H, Kurano, M, Takano, H, et al. Hemodynamic and neurohumoral responses to the restriction of femoral blood flow by KAATSU in healthy subjects. *Eur J Appl Physiol* 100: 275-285, 2007.

26. Iida, H, Takano, H, Meguro, K, et al. Hemodynamic and autonomic nervous responses to the restriction of femoral blood flow by KAATSU. *International Journal of KAATSU Training Research* 1: 57-64, 2005.

27. Graham, B, Breault, MJ, McEwen, JA, McGraw, RW. Occlusion of arterial flow in the extremities at subsystolic pressures through the use of wide tourniquet cuffs. *Clin Orthop Relat Res*: 257-261, 1993.

28. Mouser, JG, Ade, CJ, Black, CD, Bemben, DA, Bemben, MG. Brachial blood flow under relative levels of blood flow restriction is decreased in a nonlinear fashion. *Clin Physiol Funct Imaging* 38: 425-430, 2018.

29. Patterson, SD, Ferguson, RA. Increase in calf post-occlusive blood flow and strength following short-term resistance exercise training with blood flow restriction in young women. *Eur J Appl Physiol* 108: 1025-1033, 2010.

30. Loenneke, JP, Kim, D, Fahs, CA, et al. Effects of exercise with and without different degrees of blood flow restriction on torque and muscle activation. *Muscle Nerve* 51: 713-721, 2015.

31. Counts, BR, Dankel, SJ, Barnett, BE, et al. Influence of relative blood flow restriction pressure on muscle activation and muscle adaptation. *Muscle Nerve* 53: 438-445, 2016.

32. Reis, JF, Fatela, P, Mendonca, GV, et al. Tissue Oxygenation in Response to Different Relative Levels of Blood-Flow Restricted Exercise. *Front Physiol* 10, 2019.

33. Kilgas, MA, McDaniel, J, Stavres, J, et al. Limb blood flow and tissue perfusion during exercise with blood flow restriction. *Eur J Appl Physiol* 119: 377-387, 2019.

Table 2.1. Descriptive Characteristics of Study Participants

	All (n=29)	**Female (n=19)**	**Male (n=10)**
Age (yrs)	23.8 ± 4.7	22.8 ± 3.9	25.5 ± 5.7
Height (cm)	171.5 ± 8.2	167.4 ± 6.9	179.2 ± 8.2
Weight (kg)	73.9 ± 14.9	66.5 ± 11.1	88.1 ± 10.4
BMI ($kg \cdot m^{-2}$)	25.0 ± 3.8	23.6 ± 3.5	27.5 ± 3.3
BF%	23.7 ± 8.4	27.4 ± 6.0	15.5 ± 7.0
LOP (mmHg)	207.1 ± 29.4	198.6 ± 24.3	223.3 ± 32.5

Notes: Data are presented as mean ± standard deviation. BMI - body mass index. cm - centimeters. kg - kilograms. yrs - years. LOP - limb occlusion pressure. mmHg - millimeter of mercury

Table 2.2. Blood flow Responses to Relative Pressure

Condition	VolFlow (cc/min)	RelativeFlow (%)
Rest	18.66 (10.16)	100 (0)
10%	17.64 (11.64)	93.29 (21.17)
20%	15.68 (7.61)	88.02 (24.33)
30%	15.20 (6.22)	87.51 (26.45)
40%	14.34 (5.48)	82.43 (21.74)*
50%	13.91 (5.31)*	79.70 (21.84)*
60%	11.94 (5.53)*	67.72 (19.87)*
70%	9.67 (6.55)*	53.15 (20.56)*
80%	5.88 (2.32)*	36.29 (15.72)*
90%	2.35 (2.11)*	16.16 (16.10)*

Notes: Data are presented as mean ± standard deviation. VolFlow - volumetric flow. RelativeFlow - blood flow relative to resting value, *significantly different from Rest

Table 2.3. Post Hoc Pairwise Comparisons – Volumetric Flow

	Rest	**10%**	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**	**80%**	**90%**
Rest	-	0.93	2.88	3.48	4.25	4.79*	6.83*	8.91*	12.84*	16.29*
10%		-	1.95	2.55	3.32	3.87	5.91*	7.99*	11.91*	15.36*
20%			-	0.60	1.37	1.92	3.96*	6.04*	9.96*	13.41*
30%				-	0.77	1.31	3.35*	5.43*	9.36*	12.81*
40%					-	0.55	2.59*	4.67*	8.59*	12.04*
50%						-	2.04*	4.12*	8.04*	11.49*
60%							-	2.08	6.00*	9.45*
70%								-	3.92	7.37*
80%									-	3.45*
90%										-

Notes: Mean differences in Volumetric Flow (cc/min) shown. Percentages indicate percentage of Limb Occlusion Pressure (LOP). * indicates statistically significant mean differences ($p < .05$)

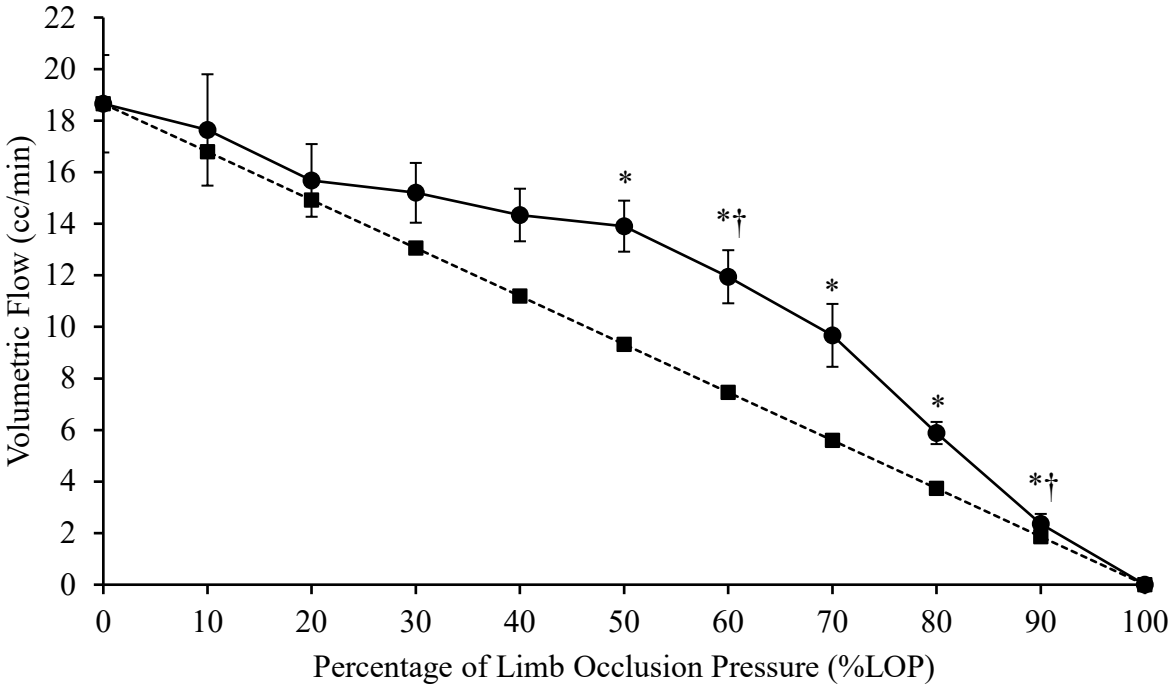

Figure 2.1. Changes in volumetric flow with increases in occlusion pressure. Circles represent measured volumetric flow. Squares and dashed line represent a modeled linear decrease in blood flow volume in relation to relative pressure. Error bars represent standard errors. * significantly different from Rest. † significantly different from previous relative pressure.

Table 2.4. Post Hoc Pairwise Comparisons – Relative Flow

	Rest	**10%**	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**	**80%**	**90%**
Rest	-	6.72	11.98	12.49	17.57*	20.31*	32.28*	46.85*	63.71*	83.84*
10%		-	5.27	5.78	10.86	13.59	25.56*	40.14*	56.99*	77.13*
20%			-	0.51	5.59	8.32	20.30*	34.87*	51.73*	71.86*
30%				-	5.08	7.82	19.79*	34.36*	51.22*	71.53*
40%					-	2.73	14.71*	29.28*	46.14*	66.27*
50%						-	11.97*	26.55*	43.40*	63.54*
60%							-	14.57*	31.43*	51.56*
70%								-	16.86*	36.99*
80%									-	20.13*
90%										-

Notes: Mean differences in relative blood flow. Percentages indicate percentage of Limb Occlusion Pressure (LOP). * indicates statistically significant mean differences at ($p < .05$)

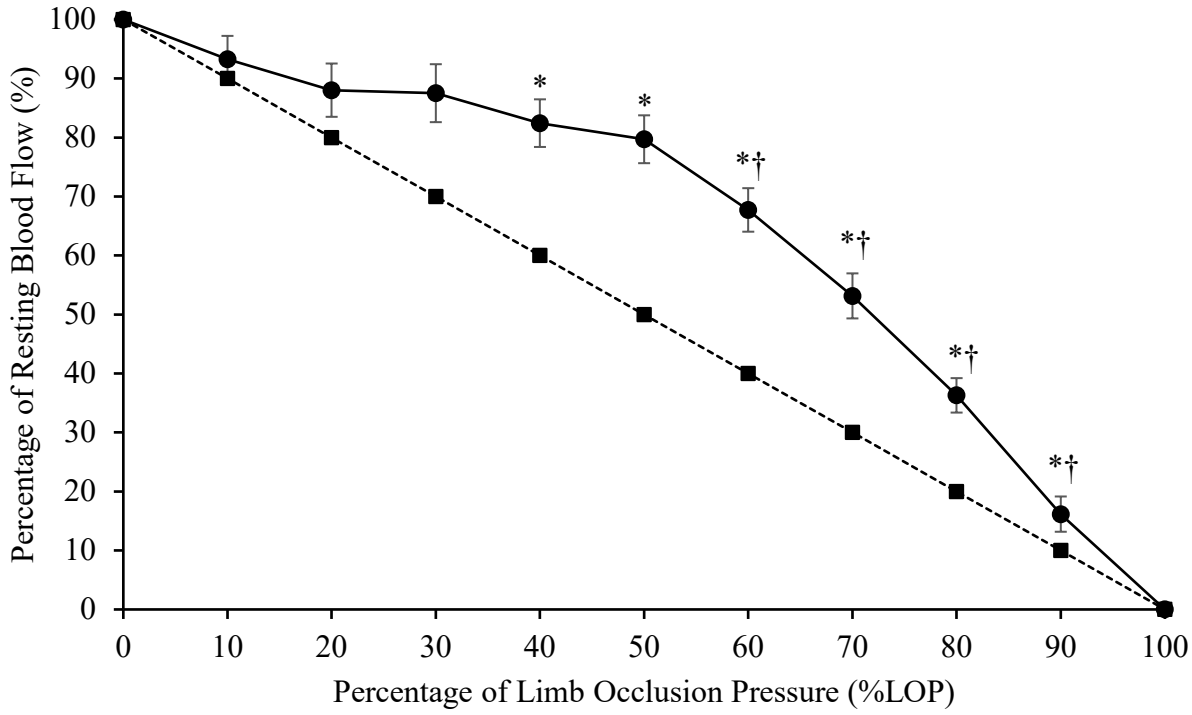

Figure 2.2. Reductions in volumetric flow relative to rest with increases in occlusion pressure. Circles represent measured volumetric flow. Squares and dashed line represent a modeled linear decrease in blood flow volume in relation to relative pressure. Error bars represent standard errors. * significantly different from Rest. † significantly different from previous relative pressure.

CHAPTER 3

ACUTE EFFECTS OF BLOOD FLOW RESTRICTION DURING HIGH-INTENSITY RESISTANCE EXERCISE ON MUSCLE ACTIVATION AND FATIGUE

ABSTRACT

Purpose: The purpose of this study was to compare the effect of blood flow restriction (BFR) during high-intensity resistance exercise (HI-BFR) on inter-set fatigue, ratings of perceived exertion (RPE), and Pain, and to determine if HI-BFR causes greater neuromuscular fatigue/impairment compared to control. **Methods:** Thirteen resistance-trained participants (30.8% female, 24.8 ± 4.7 yrs, 177.8 ± 11.8 cm, 84.3 ± 16.7 kg) performed four sets of barbell back-squats (75% 1RM) to failure under two conditions; HI-BFR (80% occlusion pressure) and control (CTRL) in a counterbalanced order. Set repetitions, RPE, and Pain were recorded for each set. Pre-post neuromuscular performance measures (MVIC, CMJ, IHG, and MPV) were measured. Two-way repeated measures analysis of variance (ANOVAs) were used to examine differences in the number of repetitions, RPE, and Pain perception. Two-way ANOVAs were used to assess changes in neuromuscular performance measures. Paired T-tests and one-way ANOVAs were used, where applicable. An alpha level of $p < .05$ was used to determine significance. **Results:** Greater number of repetitions were performed during the CTRL condition during sets 1, 2, and 4 ($p < .05$) when compared to BFR. Although RPE between conditions was

similar across all sets ($p \geq .05$), perceived pain was significantly greater in BFR across all sets ($p < .05$). Similar post-exercise decreases in MVIC-Flexion, CMJ, MPV, MPV-RMS (all $p < .05$) were observed following both conditions. **Conclusion:** HI-BFR decreases the ability of the exerciser to perform repetitions during the sets and is associated with greater pain. However, no differences in post-exercise fatigue were observed between conditions, although less total work was performed during HI-BFR.

INTRODUCTION

It is well established that resistance training (RT) stimuli cause acute neural, hormonal, and muscular responses which lead to long-term adaptations in muscle hypertrophy and strength (1). The extent to which the RT stimulus is able to cause these adaptations is determined by the resistance load range and total workload performed (2). Previous research has demonstrated that muscle hypertrophy adaptations are related to the mechanical tension, muscle damage, and metabolic stress occurring as a result of the load placed on the muscle fiber during exercise (3).

Muscle hypertrophy, or the enlargement of individual muscle fibers, is an outcome of chronic heavy resistance training (≥ 65% 1RM) (4, 5). This increase in the individual muscle fiber diameter and muscle cross-sectional area (6) is due to a shift toward protein synthesis, rather than protein degradation (7). Additionally, the mechanical tension and muscle damage lead to proliferation of satellite cells which aid in the repair and growth of muscle tissue by donating nuclei and fusing to existing myofibers (8). The donation of the nuclei to the myofiber allows for new contractile proteins to be formed and keep the myonuclear domain steady during hypertrophy and repair (9, 10).

Contemporary muscular hypertrophy recommendations focus on high load resistance training (HI-RT) (>65% of 1-repetition-max (1RM)) for 8-12 repetitions for 1 to 3 sets in order to maximize hypertrophic response. However, more recently, low load (20-30% 1RM) blood flow restriction training has demonstrated usefulness as an alternative method for producing increases in muscle hypertrophy similar to HI-RT (11). BFR exercise involves the use of a tourniquet, pneumatic cuff, or even elastic wraps to occlude distal blood flow in a limb. BFR training induces muscle hypertrophy and strength increases using as little as 20% of 1 repetition-max (1RM), contrary to traditional RT load recommendations (12, 13). Previous literature has demonstrated the occurrence of enhanced recruitment of higher-threshold motor units (T2)

during low-intensity RT using blood flow restriction (BFR) (14-16). The recruitment of T2 fibers has a greater ability for protein synthesis as $p70^{S6k}$ expression, a downstream target of mammalian target of rapamycin (mTOR), is 3 to 4 times higher compared to T1 fibers, indicating a greater potential for hypertrophy (17).The occlusion during BFR exercise increases metabolic stress, which may be an underlying mechanism behind increased muscle fiber recruitment observed during LI-BFR. Reduced oxygen supply and accumulation of metabolites stimulate group 3 and 4 afferents which inhibit slow-twitch alpha motoneurons, this leads to an increase in fast-twitch fiber recruitment to maintain force output (18, 19). However, when comparing electromyography (EMG) for muscle activation, EMG amplitude during LI-BFR is lower than that experienced during HI-RT (19, 20). Therefore, the mechanical tension produced by HI-RT may play an additional role alongside metabolic stress through more potent T2 fiber recruitment.

Although only limited research exists on high-intensity BFR (HI-BFR), Teixeira et al. compared muscle activation during HI-BFR and HI-RT during unilateral knee extension, both produced similar results initially with the addition of BFR not increasing muscle activation. However, a greater decrease in muscle activation in HI-BFR (ES = 1.47) compared to HI-RT (ES = 0.66) was observed across sets (21). The observed decrease in muscle activation may be associated with central fatigue, while nerve conduction is reduced by the elevated metabolite accumulation with BFR, causing the inhibition of alpha motor neurons (22). In the study by Teixeira et al., the decreased muscle activation caused by BFR was also associated with elevated blood lactate levels compared to non-BFR (21).

Neto et al. examined the effects of BFR during a high-intensity back-squat protocol until failure. Following the exercise stimulus, MVIC demonstrated a greater reduction in isometric

torque production in the BFR group (-23.3%) compared to non-BFR (-19.0%), although not statistically significant. Additionally, a greater percentage reduction in median frequency (MF) was observed in the BFR group ($p = 0.03$). While blood lactate was not an outcome variable in the study, the reduction in EMG signal may be due to the underlying elevated metabolite accumulation during the BFR protocol. Additionally, participants only performed a single set of back-squats to failure (80% 1RM), and differences between protocols may have been exacerbated with a greater volume. (23)

The limited research on the effect of BFR during HI-RT provides a good foundation, although more research is needed to understand the interaction BFR and HI-RT on muscle activation and fatigue. The purpose of this study was two-fold, 1) to compare the effect of BFR during high-intensity resistance exercise on inter-set fatigue, ratings of perceived exertion (RPE), and Pain and 2) determine if HI-BFR causes greater neuromuscular fatigue/impairment compared to HI-RT. The authors hypothesized that the addition of BFR to the back-squat protocol would cause greater impairment in muscle function and force production during performance measures following the exercise.

METHODS

Experimental Approach to the Problem

Participants were required to visit the lab a total of three times during this study, separated each by a minimum of 72 hours to one week. Participants reported to the laboratory at the same time (+/- 1 hour) for each visit. The first visit served to complete paperwork, familiarization, measure anthropometrics, assess baseline LOP, and 1RM max on back-squat. During visits 2 and 3, participants completed a barbell back-squat exercise protocol (BFR and CTRL) in a counter-balanced, randomized order. Additionally, participants completed baseline

performance measures before the back-squat protocol, and the same performance measures 10 minutes after the completion of the back-squat exercise protocol to evaluate neuromuscular fatigue.

Participants

Thirteen (30.8% female) apparently healthy adults were recruited to participate in this study. Participant characteristics can be seen in Table 3.1. Using data from Neto et al. (23), an *a priori* power analysis was performed (G*Power, version 3.1.9.6, Universität Kiel, Germany) following the recommendations of Beck (2013) (24). Using the pre-post isometric torque values an effect size of 0.55 was determined based on the data from Neto et al (23). Using repeated-measures, within-factors ANOVA, effect size of 0.55, alpha (α) level of .05, and desired power (1-β) of 0.80 would require a total sample size of 10 participants to detect an effect.

Due to the high-intensity resistance exercise involved with this study, participants were required to be classified as advanced resistance-trained (i.e. minimum of 1-year resistance training experience with at least 3 sessions per week) (25) and must back squat routinely in their resistance training program. Participants were required to be non-smokers between the ages of 18 and 45. Participants were excluded if they did not participate in regular resistance training exercise or if they self-reported cardiovascular, metabolic, or pulmonary conditions or signs and symptoms suggestive of these diseases (26). Blood pressure was assessed following informed consent documentation, participants were excluded if their resting systolic BP (BP) ≥140 mmHg and/or diastolic BP ≥90 mmHg (27, 28), or if it became apparent that they were unable to achieve the full range of motion during back-squats required for this study (29).

Participants were recruited via word of mouth and from undergraduate kinesiology classes. All participants were properly consented and signed an IRB approved informed consent form before beginning the study. All testing protocols and informed consent documents were

reviewed and approved by the University of Alabama Institutional Review Board. All participants were asked to refrain from any moderate-to-vigorous physical activity for 72 hours prior to each meeting during the time course of this study (26).

First Visit

Familiarization

Upon arrival to the laboratory, participants completed the informed consent paperwork, visual analog scale – delayed-onset muscle soreness (VAS-DOMS), and perceptual recovery status (PRS). PRS was used to assess recovery status of the participant in order to ensure similar expected performance during the visits (30). The PRS scale is a 0-10 scale, with 2 sets of verbal anchors defining the numerical indicators of both recovery and expected performance (30). A score of zero indicates that the individual is "very poorly" recovered and may perform poorly whereas a score of 10 would denote that the athlete is fully recovered and will perform optimally (30). VAS-DOMS was measured along a 10-cm line with "no pain" on one end, and "unbearable pain" on the other end (31). Participants were instructed to mark a vertical line along the 10-cm line (31). DOMS was rated as the measured distance between the marked line and the left end (no pain) of the scale (31). Expectations of the study were explained to participants and all questions were answered. After 5 minutes of seated rest, BP was measured with the BPM-100 automated BP monitor (BPtru medical devices) three times, 1-min apart in the dominant arm and averaged (27, 28). If the average systolic and/or diastolic BP for a participant was ≥140 and/or ≥90 mmHg (27, 28) the participant was flagged as possibly not meeting the study inclusion criteria. The participant would return to the lab 24-hr later to repeat baseline/resting BP measurements. If the average BP was still outside of the inclusionary criteria (i.e., ≥140 and/or

≥90 mmHg), the participant would be excluded from the study. No participants met this exclusion criteria.

Anthropometrics

Following paperwork and resting BP, participants had height measured using a stadiometer (SECA 67310, SECA©, Chino, CA). Prior to measuring body weight, the participants were asked for a urine sample to test hydration status. Hydration status was assessed with urine specific gravity (USG) by using a refractometer (Atago SUR-NE, Atago Corp Ltd., Tokyo, Japan). In order to ensure euhydration, a minimum USG of ≤1.020 was required prior to data collection (32, 33). In the case that USG exceeded 1.020, participants were given the opportunity to ingest water *ad libitum* for 30 minutes and have USG reassessed, or reschedule the session for an upcoming day. Body mass was measured on a digital scale (Tanita BWB-800, Tanita©, Arlington Heights, IL) and recorded manually. Next, body composition was assessed via 7 site-skinfolds. Additionally, measurement of thigh circumference was taken with a flexible, tension-sensitive, non-elastic vinyl tape measure (Gulick, Lafayette instrument Co. Lafayette, IN). To ensure consistency in the circumference measures, the measurement was taken at 50% of the distance to the superior patella from the anterior superior iliac spine on the right side of the body. The average of three circumference measurements was used.

Skinfold thicknesses were measured from each participant with calibrated skinfold calipers using 7-site skinfold analysis (26). Body density from skinfolds was calculated using the Brozek-equation (34). Each site measured once and then all measures were repeated. Each site was measured at least two times and the average was calculated. All the measurements were within 2.0 mm of each other for appropriate determination.

Limb Occlusion Pressure Determination

Limb occlusion pressure was measured at the beginning of the familiarization trial, before the determination of the 1RM back squat. LOP was also determined prior to the BFR back squat trial during visits 2 and 3. LOP was measured by the Delfi PTS II and recorded. Each leg was individually measured by the device that will occlude the leg during the back-squat trial. Although devices are calibrated by Delfi, to ensure the same device was used for each leg later in the study, the devices were labeled and noted for future trials. To ensure that cuff placement was similar between visits, the cuff was positioned within 4 inches of the inguinal crease, with the inflation valve located on the medio-lateral portion of the thigh.

Determination of One Repetition Max

Participants had their 1RM tested for barbell back-squat during their first visit following National Strength and Conditioning recommended guidelines (35). Participants were asked to self-report their 1RM based on their resistance training experience. All participants were experienced weightlifters and able to estimate their 1RM if they had directly measured it. In the case that a participant did not know, the estimated 1RM was based on their known 10 repetition maximum and scaled for a 1RM. Prior to beginning the 1RM testing, participants warmed up on a cycle ergometer for 5 minutes, followed by a self-selected dynamic warm-up (i.e. arm circles, lunges, walking hamstring stretch, bodyweight squats). Next, using a goniometer, participants performed a bodyweight squat in the power rack and had their knee flexion measured. Participants were asked to lower their hips to the point that the hip joint between 100° and 90° for each repetition. Participants then performed back-squats with an unloaded barbell (45lbs) for 10 repetitions, followed by 3-5 repetitions at 50% self-reported 1RM, 2-3 repetitions at 70% self-reported 1RM, 1-2 repetitions at 85% self-reported 1RM. 2-3 minutes rest were provided

between warm-up sets. Next, following a 3-minute rest period, the participant attempted the self-reported 1RM. If the participant failed the attempt, then the load was be decreased by 5-10% and the attempt will be repeated following 3-5 minutes rest. If the squat was successful, then the load was increased by 10-20%, and another attempt was made following 3-5 minutes rest. The 1RM was determined by the maximum weight the participant was able to squat while maintaining proper exercise technique.

Visits 2 and 3

Participants reaffirmed consent and completed the PRS and VAS-DOMS measures upon arrival to the laboratory. Following paperwork, resting BP, body weight, and USG were measured following the same protocols as during Visit 1.

Maximal Voluntary Isometric Contraction

A strength assessment of the quadriceps and hamstring musculature was completed via maximal voluntary isometric contraction (MVIC) of leg extension and flexion on the dominant leg. The MVICs were performed using an isokinetic dynamometer (HUMAC NORM, CSMiSolutions, Stoughton, MA). Participants were tested in a seated position at a 90° chair-back angle. A Velcro strap was placed over the most distal portion of the thigh of the dominant leg performing the repetitions, directly superior to the knee joint to allow for greater stability and limit excessive movement of the leg during muscle contractions. The full range of motion (ROM) of the knee joint was first determined from a starting angle of 90° to a full lockout at 180°. Participants completed three repetitions of maximal isometric leg extension at a knee joint angle of 60°, with 0° being full extension (36). Participants were instructed to exert maximum force as fast as possible and maintain that force over the measurement period. Each MVIC was

held for five seconds to attain peak torque (Nm), with one minute of rest given between repetitions. These procedures were conducted both pre- and post-exercise.

Countermovement Jump

Following IHG, participants completed countermovement jump testing. Countermovement jump (CMJ) height was measured using portable force plates (Kistler 9286ba 10kn, Switzerland). Participants were instructed to stand at the center of the force plates and jump as high as possible while maintaining their hands on their hips. Participants performed three jumps with 1-minute rest between each jump. In order to assure the participant's safety during each jump, a researcher stood close to the participant to intervene if the participant was at risk of losing balance and falling. Additionally, the force plates were outlined by a safety platform to decrease the risk of falling off. Flight height (m) was measured by Kistler MARS: Measurement, Analysis, and Reporting Software (Kistler, Kistler Instrument Group, Novi, MI). Flight height was averaged from the three CMJs (37). These procedures were conducted both pre- and post-exercise.

Isometric Handgrip

Participants completed an isometric handgrip (IHG) test. The purpose of the handgrip test was to get an estimate of muscular strength through a non-fatiguing and safe measure. For handgrip testing participants were in a standing position and held the handgrip dynamometer parallel to the side of the body with the elbow flexed at 90 degrees. When ready, the participant squeezed the dynamometer as hard as possible. This procedure was repeated 3 times total for each hand with 1-minute rest between attempts. The average value of the three readings for each

Mean Propulsive Velocity

Following CMJ, participants completed back-squat movement velocity testing. Mean Propulsive Velocity was monitored using a linear position transducer (GymAware PowerTool, Kinematic Performance Technology in Canberra, Australia). Participants performed three back-squats using 60% 1RM, with mean propulsive velocity (m/s) averaged from the three repetitions. The GymAware uses a string component that is tethered between the device itself and the end of the barbell. As the barbell is moved, the length of the string changes, and this information is sent back to the GymAware device to be analyzed. These procedures were performed both pre- and post-exercise.

Surface Electromyography

Electrodes were placed on the dominant-leg rectus femoris (RF) and vastus lateralis (VL) in accordance with SENIAM recommendations. Leg dominance was determined by the side which the participant would use to kick a ball at a target (38). RF electrodes were placed midway between the anterior spine iliac and the top of the patella (39). VL electrodes were placed 2/3 of the way between the anterior spine iliac and the lateral side of the patella (39). The patella served as the reference electrode location. The electrode sites were shaved with a twin-blade single use razor and cleaned/abraded with isopropyl alcohol prep pads prior to placing electrodes. EMG signals were sampled at 2kHz with an electronic signal acquisition system (Biopac MP150 Physiograph, BIOPAC, Goletta, CA, USA), which was connected to a Dell PC. EMG was recorded for MVICs and MPVs pre- and post-exercise. EMG data were managed and analyzed using AcqKnowledge software (Version 4.4, BIOPAC, Goletta, CA, USA). The root mean squared (RMS) function was used for EMG signal analysis. The middle 500ms epoch during the

five second MVIC was analyzed. The middle 250ms during the concentric portion of the MPV was analyzed.

Performance and Perceived Exertion and Limb Pain

The number of back-squat repetitions completed during the back-squat protocol was recorded for each set for both conditions. The total number of repetitions was determined from the sum of completed repetitions of four sets within each condition. Immediately after each set of back-squats, participants were asked about their perceived exertion and limb pain during the set. Ratings of Perceived Exertion (RPE) were assessed using a 0-10 scale (Borg CR-10) scale with 0 anchoring "nothing at all" and 10 being "impossible" (40). Perceived limb pain was assessed using a standard numeric pain scale ranging from 0-10 (Borg CR-10) with 0 anchoring "no pain" and 10 being "extreme pain" (40). Responses for RPE and Pain were recorded following each set on a physical data collection sheet.

Back Squat Protocol

The order of back-squat protocol implemented (CTRL and BFR) was assigned in a counterbalanced order. Only one protocol was performed per visit, with the participant completing the alternate protocol during the subsequent visit to ensure that changes observed were not due to a training effect.

Control Back-Squat Protocol

The back-squat exercise stimulus consisted of 4 sets to volitional fatigue. The relative load was 75% of 1RM with 3 minutes of rest between each set. According to NSCA guidelines at 75%, the participants should be expected to be able to complete roughly 10 repetitions in the first set, and less as the sets progress and fatigue sets in (35). The number of repetitions successfully completed was recorded for each set.

BFR Back-Squat Protocol

Following the warm-up, participants were fitted with the BFR cuffs. Cuffs were placed on both legs for bilateral occlusion during the exercise. The cuffs were positioned within 4 inches of the inguinal crease, with the inflation valve located on the medio-lateral portion of the thigh. The Delfi-PTS was set to self-determine 100% LOP. Average LOP was determined to be 219.6 ± 18.2 mmHg in the right leg and 206.8 ± 25.8 mmHg in the left leg. Following the LOP determination, the cuff was deflated until the exercise began. During the exercise protocol, the BFR cuffs were inflated to 80% occlusion pressure (175.6 ± 14.6 mmHg right leg, 165.4 ± 20.7 mmHg left leg). The cuffs were inflated 30 seconds before beginning the first set. The cuffs remained inflated until the completion of the second set. Upon completion of the second set, the cuffs were deflated and remained deflated during the 3-minute rest period between sets 2 and 3. The cuffs were re-inflated 30 seconds prior to the start of set 3 and remained inflated until 2 minutes after set 4 was completed. The average occlusion time was 341.9 (±24.0) seconds for the first inflation period (sets 1 and 2) and 425.7 (±48.8) seconds for the second inflation period (sets 3 and 4). Total occlusion time during the BFR exercise was 767.6 (±64.9) seconds.

The back-squat exercise stimulus consisted of 4 sets to volitional fatigue. The relative load was 75% of 1RM with 3 minutes of rest between each set. The number of repetitions successfully completed was recorded for each set.

Statistical Analysis

Data were managed using Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were completed using SPSS for Windows (Version 25.0, IBM, Somers, NY, USA). All data is presented as mean ± standard deviation (M ± SD), unless otherwise noted.

Mauchly's test was used to test the assumption of sphericity and a Greenhouse-Geisser correction was applied when the assumption of sphericity was not met. Two-way repeated measures analysis of variance (ANOVA) were used to determine the effects on pre- to post-exercise changes in fatigue measures (MVIC, CMJ, IHG, MPV) and muscle excitation (MVC-EMG and MPV-EMG) (See . Additionally, two-way repeated-measures ANOVAs were used for the analysis of changes in the number of repetitions, RPE, and Pain score across four sets across conditions (See Table 3.3). Post-hoc analysis following significant effects from 2x2 ANOVAs were performed using *t*-tests. A significant result from the 2x4 ANOVA was followed up with one-way ANOVAs. Bonferroni's correction was used for adjustment for multiple comparisons. An alpha level of $p < .05$ was utilized to determine statistical significance**.** Effect sizes were measured by partial eta square (ηp^2).

RESULTS

Comparison Between Testing Conditions

Participants were asked to refrain from any moderate-to-vigorous physical activity for 72 hours prior to each laboratory visit. No significant differences between BFR and CTRL visits in participant pre-performance characteristics, assessed by paired t-tests, VAS-DOMS (0.44 ± 0.54, 0.45 ± 0.58, respectively; p= .933), Perceptual Recovery Status (8.61 ± 1.12, 9.00 ± 0.82 respectively; p= .209), or USG (1.01 ± 0.01, 1.01 ± 0.01, respectively; p= .389) indicating similar participant pre-performance characteristics. Additionally, participants completed a self-paced warm-up (5 minutes cycle ergometer followed by a dynamic warm-up) which was repeated for the following visit. No significant difference exists in warm-up time between BFR and CTRL conditions (559.38 ± 130.65 sec, 555.38 ± 146.66 sec respectively; *p*= .790).

Exercise Protocol

Set Reps

Results of the 2x4 repeated measures ANOVA revealed a si

gnificant time*condition interaction effect: $F(3,36) = 14.010$, $p < .001$, $\eta p^2 = .539$. Significant main effects on number of repetitions for condition ($F(1,12) = 34.040$, $p < .001$, $\eta p^2 = .739$) and time ($F(3,36) = 41.670$, $p < .001$, $\eta p^2 = .776$) were observed. One-way repeated measures ANOVAs were used to examine time effects within conditions. Repeated contrasts within the BFR condition revealed that set repetitions significantly decreased from the first to second set ($p < .001$), and from the third to fourth set ($p < .001$). A non-significant increase in BFR repetitions was observed from the second to third set ($p = .053$). Repeated contrasts revealed that CTRL set repetitions significantly decreased from the first to second set ($p = .001$), and from the second to third set ($p < .001$). No significant difference was observed in CTRL set repetitions from the third to fourth set ($p > .99$). Significantly greater number of repetitions were performed during the CTRL condition during sets 1, 2, and 4 ($p < .05$) compared to BFR. No significant difference in number of repetitions was observed during set 3 ($p > .05$). Significantly more total repetitions (total set 1-4) were performed in the CTRL condition (42.15 ± 13.35 reps) compared to BFR (25.85 ± 8.48 reps) ($p < .001$).

Set RPE

Results of the 2x4 repeated measures ANOVA revealed no significant time*condition interaction on RPE ($F(3,36) = 2.320$, $p = .092$, $\eta p^2 = .162$) or main effect for condition ($F(1,12) = .622$, $p = .445$, $\eta p^2 = .049$). Mauchly's test indicated that the assumption of sphericity was violated ($p = .048$) in the time condition, so a Greenhouse-Geisser correction was applied to the time main effect. A significant main effect for time (sets 1-4) was observed on RPE during the squat stressor: $F(1.747,20.969) = 17.201$, $p < .001$, $\eta p^2 = .589$. One-way repeated measures

ANOVAs were used to examine time effects on RPE within conditions. Repeated contrasts revealed no significant differences in BFR RPE when comparing set pain to the set directly before it ($p > .05$). Repeated contrasts revealed that CTRL RPE significantly increased from the second to third set ($p = .045$), however no other significant differences in RPE were observed when comparing set pain to the set directly before it ($p > .05$). Both BFR and CTRL conditions demonstrated significant increases in RPE from set 1 to set 4 ($p < .05$*).*

Set Pain

Results of the 2x4 repeated measures ANOVA indicated a significant time*condition interaction on pain perception ($F(3,36) = 4.624$, $p = .008$, $\eta p^2 = .278$) and main effects for condition ($F(1,12) = 16.794$, $p = .001$, $\eta p^2 = .583$) and time ($F(3,36) = 24.106$, $p < .001$, $\eta p^2 = .668$). One-way repeated measures ANOVAs were used to examine time effects within conditions. Repeated contrasts revealed that BFR perceived pain perception significantly increased from the first to second set ($p = .001$), and from the third to fourth set ($p = .013$). No significant difference in pain perception was observed from the second to third set ($p > .99$). Repeated contrasts revealed that CTRL pain perception significantly increased from the third to fourth set ($p = .045$), however no other significant differences in pain perception were observed when comparing set pain to the set directly before it ($p > .05$). Significant differences were observed in pain perception between conditions for all sets ($p < .05$), with pain significantly greater in the BFR condition.

Neuromuscular Performance Measures

Maximal Voluntary Isometric Contraction

Knee Extension Peak Torque

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,11) = .000$, $p = .986$, $\eta p^2 = .000$), or main effects for time ($F(1,11) = .676$, $p = .429$, $\eta p^2 = .058$) or condition ($F(1,11) = .000$, $p = .994$, $\eta p^2 = .000$).

Knee Flexion Peak Torque

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,11) = .105$, $p = .751$, $\eta p^2 = .009$), or main effect for condition ($F(1,11) = .198$, $p = .665$, $\eta p^2 = .018$), but there was a significant main effect for time ($F(1,11) = 12.253$, $p = .005$, $\eta p^2 = .527$). Follow-up comparisons indicated that post-exercise peak flexion torque was significantly lower (-7.70 ± 2.20 Nm, $p = .005$) than pre.

Maximal Voluntary Isometric Contraction Electromyography

Rectus Femoris Root Mean Square

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,11) = 1.598$, $p = .232$, $\eta p^2 = .127$), or main effects for time ($F(1,11) = 2.513$, $p = .141$, $\eta p^2 = .186$) or condition ($F(1,11) = .820$, $p = .384$, $\eta p^2 = .069$).

Vastus Lateralis Root Mean Square

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,11) = 2.049$, $p = .180$, $\eta p^2 = .157$), or main effects for time ($F(1,11) = 2.676$, $p = .130$, $\eta p^2 = .196$) or condition ($F(1,11) = 1.791$, $p = .208$, $\eta p^2 = .140$).

Countermovement Jump

Results of the 2x2 repeated measures ANOVA revealed a significant time*condition interaction effect ($F(1,12) = 5.527$, $p = .037$, $\eta p^2 = .315$) and main effect for time ($F(1,12) =$

87.327, $p < .001$, $\eta p^2 = .897$). Follow-up comparisons indicated significant reductions in CMJ height pre to post in the BFR condition (-12.27%, .043 ± .019 m, $p < .001$) and CTRL condition (-16.06%, .057 ± .024 m, $p < .001$). Although a greater decrease in CMJ height was observed in the CTRL condition, no significant difference in post-CMJ height was observed between conditions ($p = .093$). There was no significant main effect for condition ($F(1,12) = .662$, $p = .446$, $\eta p^2 = .049$).

Isometric Handgrip

Results of the 2x2 repeated measures ANOVA revealed no significant main effects for time (pre-post) on isometric handgrip force ($F(1,12) = .027$, $p = .872$, $\eta p^2 = .002$) or condition ($F(1,12) = 2.538$, $p = .137$, $\eta p^2 = .175$). Lastly, the time*condition interaction was found to be non-significant: $F(1,12) = .659$, $p = .433$, $\eta p^2 = .052$.

Mean Propulsive Velocity

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,12) = .410$, $p = .534$, $\eta p^2 = .033$), or main effect for condition ($F(1,12) = .063$, $p = .850$, $\eta p^2 = .005$). A significant main effect for time (pre-post) on MPV performance was observed: $F(1,12) = 29.077$, $p < .001$, $\eta p^2 = .708$. A significant decrease in MPV from PRE to POST-exercise was observed across conditions (-9.66%, .070 ± .013 $m \cdot sec^{-1}$, $p < .001$).

Mean Propulsive Velocity – Electromyography

Rectus Femoris Root Mean Square

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,12) = .758$, $p = .401$, $\eta p^2 = .059$), or main effect for condition ($F(1,12) = 2.891$, $p = .115$, $\eta p^2 = .194$). A significant main effect for time (pre-post) on MPV-RMS was

observed: $F(1,12) = 4.982$, $p = .045$, $\eta p^2 = .293$. A significant decrease in MPV-RMS from PRE to POST-exercise was observed (-7.87%, .020 ± .009 mV , $p = .045$).

Vastus Lateralis Root Mean Square

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,12) = 1.890$, $p = .194$, $\eta p^2 = .136$), or main effect for condition ($F(1,12) = .089$, $p = .771$, $\eta p^2 = .007$). A significant main effect for time (pre-post) on MPV-RMS was observed: $F(1,12) = 9.493$, $p = .010$, $\eta p^2 = .442$. A significant decrease in MPV-RMS from PRE to POST-exercise was observed (-17.65%, .054 ± .018 mV , $p = .010$).

DISCUSSION

To our knowledge, this is the first study to examine the acute effects of bilateral limb occlusion during high-intensity back-squats. Previous BFR research has largely focused on low-intensity RT, single-joint exercises, or unilateral limb occlusion. In the current study, pre- to post-performance metric outcomes were similar between the BFR and CTRL conditions, although significantly less total work was performed during the BFR condition. This indicates that the bilateral BFR during high-intensity back-squats may cause post-exercise fatigue to a similar extent as high-intensity back-squats without BFR while causing earlier onset of fatigue during the exercise protocol. Notable, however, is the elevated RPE and Pain associated with the BFR protocol indicating this training modality may not be suitable for all exercisers.

During the exercise protocols, participants performed significantly greater total repetitions in the CTRL condition compared to the BFR condition (42.2 ± 13.4 reps vs. 25.8 ± 8.5 reps, respectively, $p < .05$). Additionally, repetitions completed during each of the four sets were greater in the CTRL condition. The rapid onset of fatigue following the first set during the BFR condition suggests that the bilateral BFR created a hypoxic environment and lead to

metabolite accumulation in the legs. The decline in repetitions performed during sets is likely linked to the disruption of myofibrillar function. The occlusion of blood flow and reduction in available O_2 has been shown to increase the rate of PCr hydrolysis and P_i accumulation (41, 42). Additionally, the resynthesis of creatine phosphate following the first set in the BFR condition may be disrupted due to the limited O_2 availability and H+ accumulation (43). The increased metabolite accumulation can inhibit Ca^{2+} release and disrupt excitation-contraction coupling, ultimately resulting in peripheral muscular fatigue (44). These mechanisms could potentially also explain the increase in repetitions during the third set following the un-occluded rest period after the second set. Occlusion of blood flow disrupts or completely inhibits the recovery of creatine phosphate in the muscle (43), therefore, during the un-occluded rest period, the metabolites are able to clear from the legs, and oxygenated blood reaches the muscles. This allows for creatine phosphate to regenerate and may be the underlying reason for improved performance during the third set.

To our knowledge, only two other previous studies have examined BFR using high-intensity exercise, and more specifically during back-squats (23, 45). Winchester et al. did not observe reductions in the total number of repetitions performed between BFR and CTRL (47.0 ± 4.25 vs 44.92 ± 3.13, respectively; $p = 0.29$) using 75% of 1RM until fatigue (45). However, a significant decrease in the number of set repetitions was observed from the second to third set of BFR exercise which was not observed in the CTRL condition, indicating an earlier onset of muscular fatigue in the BFR condition (45). Limitations of this study include only using unilateral intermittent limb occlusion during exercise (pressure released during rest) which has been shown to be less effective at increasing metabolic stress during high-intensity exercise compared to continuous occlusion or occlusion during rest (21). Neto et al. used a twofold 206

latex tube to occlude blood flow (~60% femoral artery occlusion) and had participants perform one set of back squats (80% 1RM) to failure (23). However, the authors did not report the total number of repetitions performed under each condition, so no comparisons on set fatigue can be made (23).

Consistent with Winchester et al. (45), pain perception was elevated during the BFR condition across all sets in the current study. Pain perception during the CTRL condition was relatively constant across all sets (5.15 – 6.15), while the BFR condition experienced significantly elevated pain during set 2 (7.92) and set 4 (9.08). While anecdotal, participants had great difficulty managing the pain during the occluded rest periods and this may have affected the ability to perform squat repetitions during the subsequent sets. Rating of discomfort has been found to not be associated with the relative pressure used or reductions in total repetitions (46), however, the high mechanical load (75%1RM) during this study may have exacerbated these effects. The un-occluded rest period following the second set was of great relief to participants and may, in part, explain the increased set performance during the third set. Participants were asked to perform back-squats to failure, therefore RPE was consistently high across sets and between conditions.

In the current study, fatigue was measured through changes in neuromuscular performance assessments and surface electromyography. Reductions in MPV are associated with neuromuscular fatigue (47), and a significant decrease in MPV was observed post-exercise in both conditions. Additionally, significant reductions in CMJ were observed following the exercise protocol in both conditions, indicating neuromuscular fatigue (48). Reductions in MVIC-EXT were non-significant, but consistent across conditions and MVIC-FLEX experienced significant reductions in peak torque which was also not different across conditions.

Despite the exacerbated muscle fatigue during the BFR squat protocol, the condition differences in post-exercise performance measures were minimal. This suggests that the impact of muscular fatigue during the exercise stressor was quickly reduced following the release of occlusion pressure and reperfusion of the legs. These findings further validate the notion that reduced oxygen supply and accumulation of metabolites during BFR exercise results in peripheral muscular fatigue (18, 19, 44) and that reperfusion of the muscles post exercises quickly diminished this fatigue.

In agreement with Neto et al. (23), decreases in MPV-RMS were observed in both RF and VL following the exercise stimulus: BFR (-3.9% and -11.1%, respectively, $p > .05$) and CTRL (-12.0% and -29.2%, respectively, $p < .05$). Larger reductions in MPV-RMS and MVC-RMS were observed following the CTRL condition. These results indicate that the occlusion causes enhanced peripheral fatigue during the squat stressor, however, differences in total mechanical load due to differences in total work (total repetitions across four sets) lead to prolonged fatigue in the CTRL condition. Therefore, while BFR experienced greater fatigue during sets, likely due to local metabolite accumulation, the total workload of the CTRL group potentially caused greater amounts of neuromuscular damage and longer-lasting fatigue.

Limitations

The current study is not without limitations, such as sample characteristics and study design. Inclusion criteria included a minimum resistance training background of one year, and the findings of the current study may not be applicable for untrained individuals. Secondly, total work (number of repetitions) was not matched between conditions and may have influenced the outcome of the post-exercise neuromuscular performance assessments. A matched workload between conditions may allow for a better understanding of fatigue effects of BFR compared to

CTRL and may further support the notion that BFR enhances fatigue. Lastly, the occlusion in the current study was interrupted following set 2 and therefore was not true continuous occlusion during the exercise stimulus. As this was one of the first studies examining high-intensity resistance exercise in conjunction with BFR, the authors were cautious with the BFR application, future research should examine true continuous occlusion with an optimized matched work-load exercise stimulus design.

CONCLUSION

In summary, high-intensity resistance exercise to failure in conjunction with BFR does not increase post-exercise fatigue or lead to additional decrements in performance compared to CTRL. During exercise, however, BFR significantly decreases the ability of the exerciser to perform repetitions during the sets and is associated with greater RPE and Pain throughout the workout. Future research should examine the influence of BFR during high-intensity resistance exercise using a matched total workload (i.e. 4 sets of 5 repetitions) rather than sets to failure. Additionally, future research may benefit from examining if using lower occlusion pressures is able to decrease the pain sensation during high-intensity BFR and allow for greater total work.

REFERENCES

1. Folland, JP, Williams, AG. The adaptations to strength training : morphological and neurological contributions to increased strength. *Sports Med* 37: 145-168, 2007.

2. Schoenfeld, BJ, Peterson, MD, Ogborn, D, Contreras, B, Sonmez, GT. Effects of Low- vs. High-Load Resistance Training on Muscle Strength and Hypertrophy in Well-Trained Men. *J Strength Cond Res* 29: 2954-2963, 2015.

3. Schoenfeld, BJ. The Mechanisms of Muscle Hypertrophy and Their Application to Resistance Training. *The Journal of Strength & Conditioning Research* 24, 2010.

4. Cureton, KJ, Collins, MA, Hill, DW, McElhannon, FM, Jr. Muscle hypertrophy in men and women. *Med Sci Sports Exerc* 20: 338-344, 1988.

5. Brown, AB, McCartney, N, Sale, DG. Positive adaptations to weight-lifting training in the elderly. *J Appl Physiol (1985)* 69: 1725-1733, 1990.

6. Tesch, PA, Larsson, L. Muscle hypertrophy in bodybuilders. *Eur J Appl Physiol Occup Physiol* 49: 301-306, 1982.

7. Sandri, M. Signaling in muscle atrophy and hypertrophy. *Physiology (Bethesda)* 23: 160-170, 2008.

8. Vierck, J, O'Reilly, B, Hossner, K, et al. Satellite cell regulation following myotrauma caused by resistance exercise. *Cell biology international* 24: 263-272, 2000.

9. Moss, FP, Leblond, CP. Satellite cells as the source of nuclei in muscles of growing rats. *Anat Rec* 170: 421-435, 1971.

10. Allen, DL, Roy, RR, Edgerton, VR. Myonuclear domains in muscle adaptation and disease. *Muscle Nerve* 22: 1350-1360, 1999.

11. Lixandrão, ME, Ugrinowitsch, C, Berton, R, et al. Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med* 48: 361-378, 2018.

12. Abe, T, Yasuda, T, Midorikawa, T, et al. Skeletal muscle size and circulating IGF-1 are increased after two weeks of twice daily “KAATSU” resistance training. *International Journal of KAATSU Training Research* 1: 6-12, 2005.

13. Yasuda, T, Ogasawara, R, Sakamaki, M, et al. Combined effects of low-intensity blood flow restriction training and high-intensity resistance training on muscle strength and size. *Eur J Appl Physiol* 111: 2525-2533, 2011.

14. Moritani, T, Sherman, WM, Shibata, M, Matsumoto, T, Shinohara, M. Oxygen availability and motor unit activity in humans. *Eur J Appl Physiol Occup Physiol* 64: 552-556, 1992.

15. Wernbom, M, Jarrebring, R, Andreasson, MA, Augustsson, J. Acute effects of blood flow restriction on muscle activity and endurance during fatiguing dynamic knee extensions at low load. *J Strength Cond Res* 23: 2389-2395, 2009.

16. Yasuda, T, Brechue, WF, Fujita, T, et al. Muscle activation during low-intensity muscle contractions with restricted blood flow. *J Sports Sci* 27: 479-489, 2009.

17. Doessing, S, Heinemeier, KM, Holm, L, et al. Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. *J Physiol* 588: 341-351, 2010.

18. Vanhelder, WP, Radomski, MW, Goode, RC. Growth hormone responses during intermittent weight lifting exercise in men. *Eur J Appl Physiol Occup Physiol* 53: 31-34, 1984.

19. Manini, TM, Clark, BC. Blood flow restricted exercise and skeletal muscle health. *Exerc Sport Sci Rev* 37: 78-85, 2009.

20. Cook, SB, Murphy, BG, Labarbera, KE. Neuromuscular function after a bout of low-load blood flow-restricted exercise. *Med Sci Sports Exerc* 45: 67-74, 2013.

21. Teixeira, EL, Barroso, R, Silva-Batista, C, et al. Blood flow restriction increases metabolic stress but decreases muscle activation during high-load resistance exercise. *Muscle Nerve* 57: 107-111, 2018.

22. Babault, N, Desbrosses, K, Fabre, MS, Michaut, A, Pousson, M. Neuromuscular fatigue development during maximal concentric and isometric knee extensions. *J Appl Physiol (1985)* 100: 780-785, 2006.

23. Neto, GR, Santos, HH, Sousa, JB, et al. Effects of high-intensity blood flow restriction exercise on muscle fatigue. *J Hum Kinet* 41: 163-172, 2014.

24. Beck, TW. The importance of a priori sample size estimation in strength and conditioning research. *J Strength Cond Res* 27: 2323-2337, 2013.

25. Friedman, K. Essentials of Strength Training and Conditioning, 4th Edition. *Med Sci Sports Exerc* 48: 2073-2073, 2016.

26. American College of Sports, M, Riebe, D, Ehrman, JK, Liguori, G, Magal, M. *ACSM's guidelines for exercise testing and prescription.* 2018.

27. Pickering, TG, Hall, JE, Appel, LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation* 111: 697-716, 2005.

28. Whelton, PK, Carey, RM, Aronow, WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. *J Am Coll Cardiol* 71: e127, 2017.

29. Whelton, PK, Carey, RM, Aronow, WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 71: e13-e115, 2018.

30. Laurent, CM, Green, JM, Bishop, PA, et al. A practical approach to monitoring recovery: development of a perceived recovery status scale. *J Strength Cond Res* 25: 620-628, 2011.

31. Waterfield, MJ, Sim, J. Clinical assessment of pain by the visual analogue scale. *British Journal of Therapy and Rehabilitation* 3: 94-97, 1996.

32. Armstrong, LE. Hydration assessment techniques. *Nutr Rev* 63: S40-54, 2005.

33. McDermott, BP, Anderson, SA, Armstrong, LE, et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. *J Athl Train* 52: 877-895, 2017.

34. Brožek, J, Grande, F, Anderson, JT, Keys, A. Densitometric analysis of body composition: revision of some quantitative assumptions. *Ann N Y Acad Sci* 110: 113-140, 1963.

35. Haff, GG, Triplett, NT. *Essentials of strength training and conditioning 4th edition.* Human kinetics, 2015.

36. Murray, MP, Gardner, GM, Mollinger, LA, Sepic, SB. Strength of isometric and isokinetic contractions: knee muscles of men aged 20 to 86. *Phys Ther* 60: 412-419, 1980.

37. Claudino, JG, Cronin, J, Mezêncio, B, et al. The countermovement jump to monitor neuromuscular status: A meta-analysis. *Journal of science and medicine in sport* 20: 397-402, 2017.

38. van Melick, N, Meddeler, BM, Hoogeboom, TJ, Nijhuis-van der Sanden, MWG, van Cingel, REH. How to determine leg dominance: The agreement between self-reported and observed performance in healthy adults. *PLoS One* 12: e0189876, 2017.

39. Hermens, HJ, Commission des Communautés, e, Biomedical, Health Research, P. *SENIAM : European recommendations for surface electromyography : results of the SENIAM project.* [Pays-Bas]: Roessingh Research and Development, 1999.

40. Borg, G. *Borg's perceived exertion and pain scales.* Human kinetics, 1998.

41. Suga, T, Okita, K, Takada, S, et al. Effect of multiple set on intramuscular metabolic stress during low-intensity resistance exercise with blood flow restriction. *Eur J Appl Physiol* 112: 3915-3920, 2012.

42. Sugaya, M, Yasuda, T, Suga, T, Okita, K, Abe, T. Change in intramuscular inorganic phosphate during multiple sets of blood flow-restricted low-intensity exercise. *Clin Physiol Funct Imaging* 31: 411-413, 2011.

43. Sahlin, K, Harris, RC, Hultman, E. Resynthesis of creatine phosphate in human muscle after exercise in relation to intramuscular pH and availability of oxygen. *Scand J Clin Lab Invest* 39: 551-558, 1979.

44. Favero, TG, Zable, AC, Colter, D, Abramson, JJ. Lactate inhibits Ca(2+) -activated Ca(2+)-channel activity from skeletal muscle sarcoplasmic reticulum. *J Appl Physiol (1985)* 82: 447-452, 1997.

45. Winchester, LJ, Morris, CE, Badinger, J, Wiczynski, TL, VanWye, WR. Blood Flow Restriction at High Resistance Loads Increases the Rate of Muscular Fatigue, but Does Not Increase Plasma Markers of Myotrauma or Inflammation. *J Strength Cond Res*, 2020.

46. Loenneke, JP, Kim, D, Mouser, JG, et al. Are there perceptual differences to varying levels of blood flow restriction? *Physiol Behav* 157: 277-280, 2016.

47. Sánchez-Medina, L, González-Badillo, JJ. Velocity loss as an indicator of neuromuscular fatigue during resistance training. *Med Sci Sports Exerc* 43: 1725-1734, 2011.

48. Claudino, JG, Cronin, J, Mezencio, B, et al. The countermovement jump to monitor neuromuscular status: A meta-analysis. *J Sci Med Sport* 20: 397-402, 2017.

Table 3.1. Descriptive Characteristics of Study Participants

	All (n=13)	**Female (n=4)**	**Male (n=9)**
Age (yrs)	24.8 ± 4.7	23.8 ± 4.6	25.2 ± 4.9
Height (cm)	177.8 ± 11.8	164.1 ± 5.5	184.0 ± 7.6
Weight (kg)	84.3 ± 16.7	65.6 ± 3.6	92.5 ± 12.9
BMI ($kg \cdot m^{-2}$)	26.4 ± 3.1	24.5 ± 2.0	27.3 ± 3.2
BF%	16.6 ± 8.2	24.0 ± 6.7	13.2 ± 6.6
Right LOP (mmHg)	219.6 ± 18.2	214.0 ± 10.7	222.1 ± 20.7
Right 80% LOP (mmHg)	175.6 ± 14.6	171.3 ± 8.6	177.6 ± 16.7
Left LOP (mmHg)	206.8 ± 25.8	204.8 ± 28.3	207.8 ± 21.1
Left 80% LOP (mmHg)	165.4 ± 20.7	163.8 ± 22.8	166.1 ± 21.1
1RM (kg)	143.7 ± 50.4	85.8 ± 15.2	169.4 ± 36.1

Notes: Data are presented as mean ± standard deviation. BMI - body mass index. cm - centimeters. BF% - body fat percentage. kg - kilograms. yrs - years. LOP - limb occlusion pressure. mmHg - millimeter of mercury. 1RM - one repetition maximum.

Table 3.2. Pre- and Post-Resistance Exercise Fatigue Measures

Variable	Condition	Pre	Post	Time Effect		Condition Effect		Interaction	
				P	ηp^2	*P*	ηp^2	*P*	ηp^2
MVIC Extension (Nm)	BFR	274.40 ± 81.81	267.25 ± 90.68	.429	.058	.994	.000	.986	.000
	CTRL	274.47 ± 89.95	267.02 ± 88.44						
MVIC Flexion (Nm)	BFR	166.78 ± 58.89	155.24 ± 53.37*	.005	.527	.665	.018	.751	.009
	CTRL	163.65 ± 52.79	154.31 ± 54.39*						
CMJ (m)	BFR	0.35 ± 0.07	0.31 ± 0.06*	< .001	.879	.446	.049	.037	.315
	CTRL	0.35 ± 0.06	0.30 ± 0.05*						
IHG (kg)	BFR	48.51 ± 15.32	48.10 ± 14.85	.872	.002	.137	.175	.433	.052
	CTRL	48.95 ± 13.95	49.56 ± 15.19						
MPV ($m \cdot sec^{-1}$)	BFR	0.71 ± 0.12	0.65 ± 0.14*	< .001	.708	.805	.005	.534	.033
	CTRL	0.72 ± 0.10	0.64 ± 0.12*						
MVIC RMS RF (mV)	BFR	0.15 ± 0.09	0.17 ± 0.09	.141	.186	.384	.069	.232	.127
	CTRL	0.16 ± 0.07	0.19 ± 0.10						
MVIC RMS VL (mV)	BFR	0.17 ± 0.08	0.18 ± 0.09	.130	.196	.208	.140	.180	.157
	CTRL	0.19 ± 0.11	0.21 ± 0.14						
MPV RMS RF (mV)	BFR	0.26 ± 0.10	0.25 ± 0.10	.045	.293	.115	.194	.401	.059
	CTRL	0.25 ± 0.06	0.22 ± 0.06*						
MPV RMS VL (mV)	BFR	0.30 ± 0.13	0.27 ± 0.13	.010	.442	.771	.007	.194	.136
	CTRL	0.31 ± 0.10	0.24 ± 0.07*						

Values are Mean ± SD. BFR - blood flow restriction. CTRL control. MVIC - maximal voluntary isometric contraction. CMJ - countermovement jump. IHG - isometric handgrip. MPV - mean propulsive velocity. RMS - root mean square of the electromyographic signal. RF - rectus femoris. VL - vastus lateralis. * significant difference from Pre within condition at $p < .05$. # sphericity assumption violated (Greenhouse-Geisser correction).

Table 3.3. Performance and Perceptual Outcomes During Exercise

Variable	Condition	Set 1	Set 2	Set 3	Set 4	Time Effect		Condition Effect		Interaction	
						P	ηp^2	*P*	ηp^2	*P*	ηp^2
Reps	BFR	11.69 ± 4.01*	4.08 ± 2.22*	6.92 ± 3.04	3.15 ± 1.99*	< .001	.776	< .001	.739	< .001	.539
	CTRL	13.54 ± 4.22	10.77 ± 3.44	9.15 ± 3.24	8.69 ± 3.45						
Pain	BFR	6.54 ± 2.03*	7.92 ± 1.26*	7.31 ± 2.02*	9.08 ± 0.95*	< .001	.668	.001	.583	.008	.278
	CTRL	5.15 ± 2.08	5.31 ± 2.06	5.92 ± 2.21	6.15 ± 2.44						
RPE	BFR	8.46 ± 1.39	9.31 ± 0.85*	9.15 ± 0.69	9.62 ± 0.51	< .001#	.589	.445	.049	.092	.162
	CTRL	8.54 ± 1.05	8.77 ± 0.83	9.23 ± 0.60	9.62 ± 0.65						

Values are Mean ± SD. Reps - number of repetitions completed. Pain - pain perception (0-10). RPE - rating of perceived exertion. BFR - blood flow restriction. CTRL - control. * significantly different from CTRL condition ($p < .05$). # sphericity assumption violated (Greenhouse-Geisser correction).

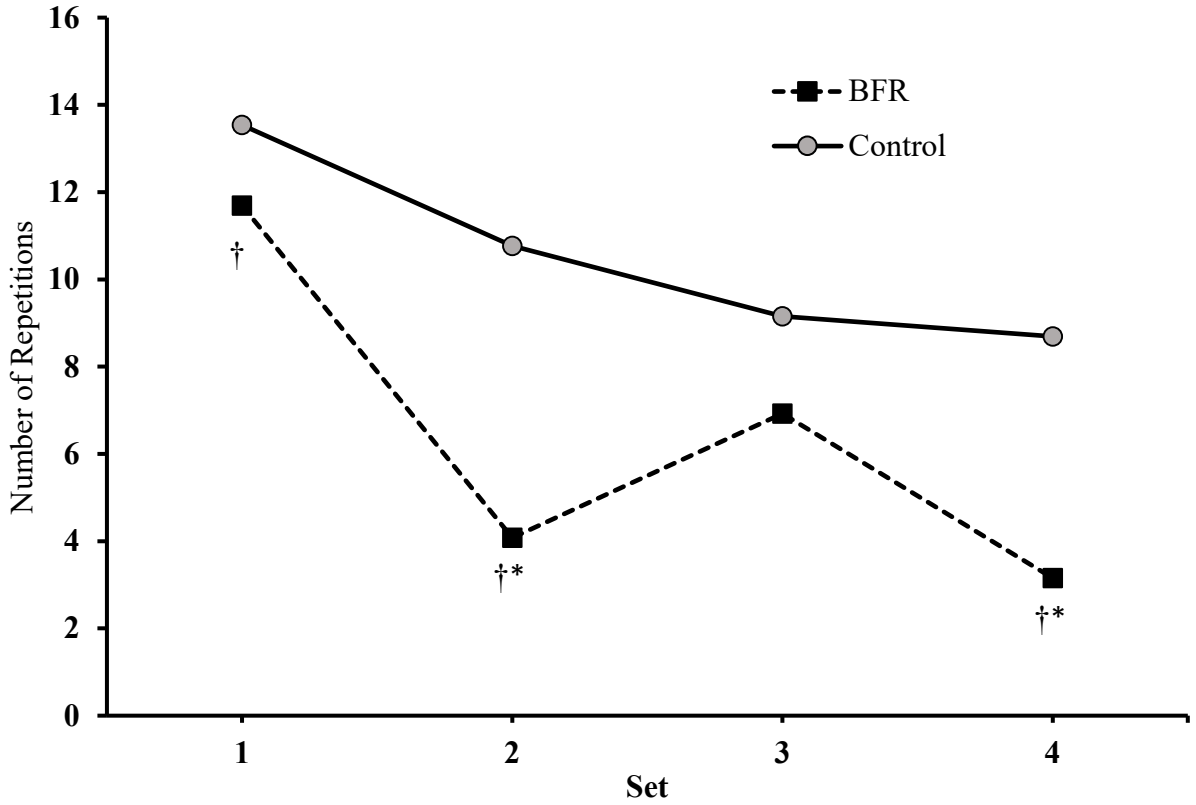

Figure 3.1. Number of repetitions completed during each set. † significant difference ($p < .05$) between conditions. * significant difference ($p < .05$) within condition compared to previous set. Error bars not included for clarity.

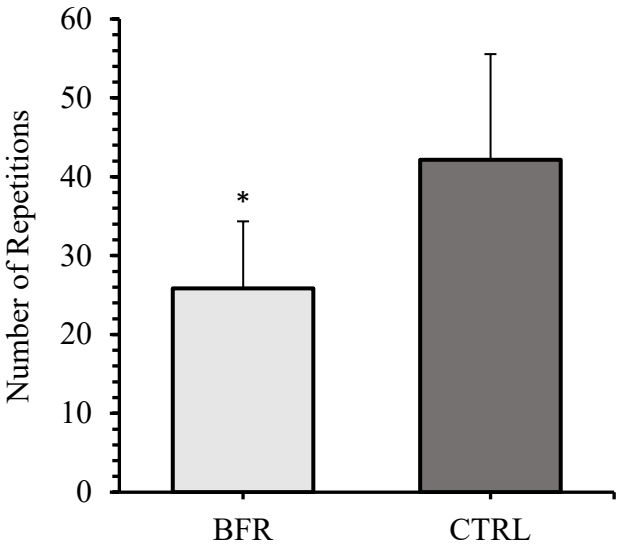

Figure 3.2. Total repetitions across all sets.
* significant difference ($p < .05$) between conditions. Error bars represent standard deviations.

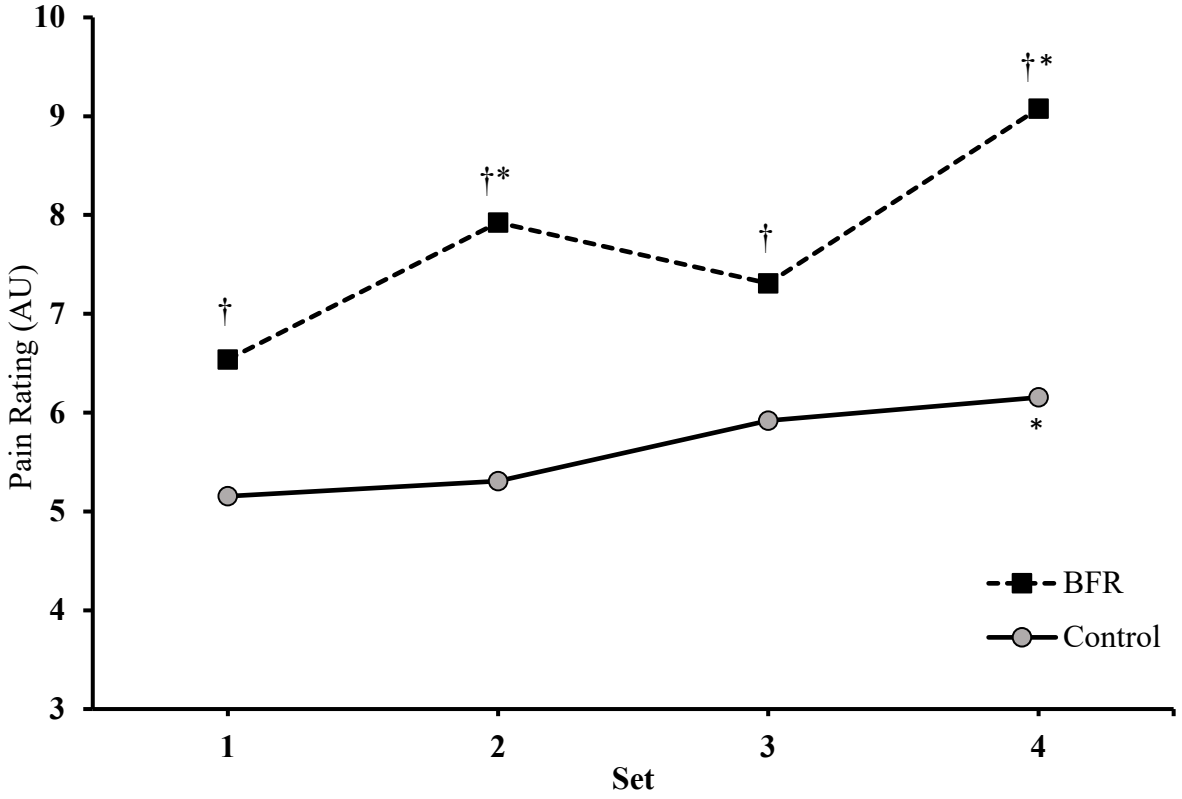

Figure 3.3. Perceived pain during each set. † significant difference ($p < .05$) between conditions; * significant difference ($p < .05$) within condition compared to previous set. Error bars not included for clarity.

CHAPTER 4

INFLUENCE OF BLOOD FLOW RESTRICTION DURING HIGH-INTENSITY RESISTANCE EXERCISE ON MARKERS OF METABOLIC STRESS AND MUSCLE DAMAGE

ABSTRACT

Purpose: The purpose of this study was to examine how blood flow restriction (BFR) during high-intensity resistance exercise (HI-RT) affects physiological responses acutely post-exercise. More specifically, this study investigated the effect of BFR during HI-RT on metabolic stress, cell swelling, and markers of inflammation and muscle damage. **Methods:** Thirteen resistance-trained participants (30.8% female, 24.8 ± 4.7 yrs, 177.8 ± 11.8 cm, 84.3 ± 16.7 kg) performed four sets of barbell back-squats (75% 1RM) to failure under two counterbalanced conditions; blood flow restriction (BFR, 80% occlusion pressure) and control (CTRL). Baseline and 1-hour post-exercise venous blood samples were collected for analysis of interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF). Additionally, baseline finger-prick blood lactate concentrations, anatomical cross-sectional area (ACSA), and Echo Intensity (EI) measures were taken and repeated post-exercise. Two-way repeated measures (2x2) analysis of variance (ANOVA) were used to determine the effects pre- to post-exercise of occlusion condition on markers of muscle damage (IL-6 and EI), hypoxia (VEGF), and muscle swelling (ACSA). Additionally, a two-way ANOVA (2x3) was used for the analysis of changes in blood lactate concentration. Paired T-tests and one-way ANOVAs were used to explore possible differences in pre- to post-exercise measures in each condition (BFR and CTRL), when appropriate. An alpha level of $p < .05$ was used to determine significance.

Results: Significantly elevated blood lactate concentrations were measures immediately and 4-minutes post-exercise in both BFR and CTRL ($p < .001$). Lactate immediately post exercise was significantly greater in CTRL compared to BFR ($p = .001$), but not four minutes post ($p = .063$). BFR post-exercise IL-6 was significantly elevated from baseline ($p = .011$) and significantly greater than CTRL ($p = .007$). No significant changes were observed post-exercise in VEGF or EI in either condition. Thigh circumference and ACSA were significantly greater post-exercise, but consistent across conditions. **Conclusion:** Findings indicate the utility of HI-RT to failure in conjunction with BFR for inducing similar amounts of muscle swelling and increased markers of muscular inflammation (IL-6) without increased blood lactate concentration or indices of hypoxia (VEGF), with significantly lesser total repetitions performed.

INTRODUCTION

Contemporary recommendations for maximizing muscular hypertrophy focus on high load resistance training (HI-RT) [≥65% of 1-repetition-max (1RM)]. Three primary factors are believed to be responsible for the hypertrophic response to HI-RT: mechanical tension, muscle damage, and metabolic stress. Induction of these mechanisms is greatly reliant on the exercise load, which is why a load of >65% 1RM is recommended to cause hypertrophy (1, 2). High resistance loads increase the mechanical tension and subsequent muscle damage associated with RT, which are key anabolic signals for activating the mitogen activated protein kinase (MAPK) protein synthesis pathway, leading to increased protein synthesis and hypertrophy (3). Additionally, the compression of arterial and venous blood flow during high load RT from the contraction of the muscle leads to reduced oxygen delivery capability to the muscle leading to metabolite accumulation and downstream activation of hypertrophying transcriptional inducers (4).

Blood flow restriction (BFR) in combination with low-intensity resistance training (RT) (20-30%1RM) has been shown to produce greater muscle hypertrophy results compared to low intensity RT without BFR (5-7) and similar hypertrophic increases compared to traditional HI-RT (5, 8, 9). However, low intensity BFR (LI-BFR) exercise is associated with neither high mechanical stress or muscle damage due to the typical low exercise load used (10, 11). As previously mentioned, metabolic stress is another mechanism for hypertrophic adaptation to RT and may be the cause of the hypertrophic adaptations seen in response to LI-BFR. Previous research has found that BFR exercise increases the rate of ATP hydrolysis, phosphocreatine (PCr) depletion, decreases pH, and increases lactate response (12). When compared to LI-RT without BFR, LI-BFR produces greater metabolic stress, measured by PCr depletion and decreases in intramuscular pH (13, 14). LI-BFR with continuous occlusion during exercise

enhances metabolic stress to the level of HI-RT (14). In addition to enhanced metabolite accumulation in response to BFR, myofiber swelling may be another mechanism contributing to the muscular adaptations, stimulating anabolism in hepatocytes following acute cell swelling (15). While muscle swelling does occur following HI-RT, this is likely due to muscle damage associated with the heavier loads (16). Exercise-induced muscle damage does not occur following LI-BFR (10, 11), indicating other underlying mechanisms are likely responsible for hypertrophic response. Freitas et al. (2017) demonstrated that LI-BFR (20% 1-RM, 1set of 30 reps, followed by 3sets of 15 reps) induces acute muscle swelling equivalent to that observed with traditional HI-RT (80% 1-RM, 3 sets of 8-10 reps) performing leg press, knee extensions, and leg curl exercises (17). Moreover, these acute increases in muscle swelling are observed following BFR occlusion without exercise, which attenuates muscle atrophy without an increase in metabolites, indicating that myofiber swelling has a hypertrophic effect on muscle. The blood pooling effect of BFR may shift intra- and extracellular water balance creating a hydrostatic pressure gradient which causes a shift of water into the cell, increasing intracellular fluid (18). Increased intracellular fluid upregulates protein synthesis and muscle hypertrophy (19, 20). Muscle cell swelling during BFR is detected by an intrinsic volume sensor, which co-activates the mTOR and MAPK pathways (18, 20).

Both HI-RT and LI-BFR are effective RT modalities for enhancing muscular growth, however the underlying mechanisms for hypertrophy differ due to differences in exercise load, with HI-RT promoting mechanotransduction and muscle damage, while LI-BFR promotes greater metabolic stress and myofiber swelling. Therefore, it may be possible that combining the two modalities, for high intensity BFR (HI-BFR), may maximize the hypertrophic capabilities by enhancing the metabolic stress and cell swelling in the muscle, while simultaneously stimulating

the muscle through mechanotransduction. Teixeira et al. demonstrated that high intensity knee extension with occlusion during the rest period significantly increases blood lactate concentration compared to without occlusion (21). Most recently, Winchester et al. demonstrated that unilateral occlusion during HI back-squats (75% 1RM) did not increase markers of muscle damage (IL-6 and myoglobin) over non-BFR HI back-squats (22). However, muscular fatigue was induced more rapidly with BFR, suggesting that other mechanisms of hypertrophy are plausible.

The purpose of this study was to examine if the underlying mechanisms of BFR-induced hypertrophy can further enhance hypertrophic response to a high load back-squat protocol. More specifically, this study investigated the effect of BFR during HI-RT on metabolic stress, cell swelling, and markers of inflammation and muscle damage. We hypothesized that the continuous occlusion during HI-BFR in the proposed study will enhance metabolic stress and cause an increase in acute cell swelling, without an increase in markers of muscle damage or inflammation compared to HI-RT following the exercise protocol.

METHODS

Experimental Approach to the Problem

Participants were required to visit the lab a total of three times during this study, separated each by a minimum of 72 hours to one week. Participants reported to the laboratory at the same time (+/- 1 hours) for each visit. The first visit served to complete paperwork, familiarization, measure anthropometrics, assess baseline LOP, and 1RM on back-squat. During visits 2 and 3, participants completed the back-squat exercise protocol (BFR and CTRL) in a counter-balanced randomized order. Additionally, before the back-squat protocol, participants had finger prick lactate measured, along with baseline venous blood samples and baseline

ultrasound measures of anatomical cross-sectional area (ACSA) and Echo Intensity (EI). The same measures were performed post-exercise, with finger prick lactate and ultrasound measures performed immediately post-exercise and venous blood samples collected 1-hour post.

Participants

Thirteen (30.8% female) apparently healthy adults were recruited to participate in this study. Participant characteristics can be seen in Table 4.1. Using data from Winchester et al. (22), an *a priori* power analysis was performed (G*Power, version 3.1.9.6, Universität Kiel, Germany) following the recommendations of Beck (2013) (23). Using the pre-post myoglobin and IL-6 values an effect size of 0.46 and 0.85 were determined, respectively, based on the data from Winchester et al. (22). Therefore, it was decided to use a large (0.5) effect size for sample size estimation. Using repeated-measures, within-factors ANOVA, effect size of 0.5, alpha (α) level of .05, and desired power (1-β) of 0.80 would require a total sample size of 12 participants to detect an effect.

Due to the high intensity resistance exercise involved with this study, participants were required to be classified as advanced resistance trained (i.e. minimum of 1 year resistance training experience with at least 3 sessions per week) (24) and must back squat routinely in their resistance training program. Participants were excluded if they did not participate in regular exercise or if they exhibited cardiovascular, metabolic, or pulmonary conditions or signs and symptoms suggestive of these diseases (25). Participants were required to be non-smokers between the ages of 18 and 45 and classified as "low-risk," according to the standards set by the American College of Sports Medicine. Blood pressure was assessed following informed consent documentation, participants were excluded if their resting systolic BP (BP) ≥140 mmHg and/or

diastolic BP ≥90 mmHg (26, 27), or if it became apparent that they will not be able to achieve the full range of motion required for this study.

Participants were recruited via word of mouth and from undergraduate kinesiology classes. All participants were properly consented and signed an IRB approved informed consent form before beginning the study. All testing protocols and informed consent documents were reviewed and approved by the University of Alabama Institutional Review Board. All participants were asked to refrain from any moderate-to-vigorous physical activity for 72 hours prior to each meeting during the time course of this study (25).

First Visit

Familiarization

Upon arrival to the laboratory, participants completed the informed consent paperwork, visual analog scale – delayed-onset muscle soreness (VAS-DOMS), and perceptual recovery status (PRS). PRS was used to assess recovery status of the participant in order to ensure similar expected performance during the visits (28). The PRS scale is a 0-10 scale, with 2 sets of verbal anchors defining the numerical indicators of both recovery and expected performance (28). A score of zero indicates that the individual is "very poorly" recovered and may perform poorly whereas a score of 10 would denote that the athlete is fully recovered and will perform optimally (28). VAS-DOMS was measured along a 10-cm line with "no pain" on one end, and "unbearable pain" on the other end (29). Participants were instructed to mark a vertical line along the 10-cm line (29). DOMS was rated as the measured distance between the marked line and the left end (no pain) of the scale (29). After 5 minutes of seated rest, BP was measured with the BPM-100 automated BP monitor (BPtru medical devices) three times, 1-min apart in the dominant arm and averaged (26, 27). If the average systolic and/or diastolic BP for a participant was ≥140 and/or

≥90 mmHg (26, 27) the participant was flagged as possibly not meeting the study inclusion criteria. The participant would return to the lab 24-hr later to repeat baseline/resting BP measurements. If the average BP was still outside of the inclusionary criteria (i.e., ≥140 and/or ≥90 mmHg), the participant would be excluded from the study. No participants met this exclusion criteria.

Anthropometrics

Following paperwork and resting BP, participants had height measured using a stadiometer (SECA 67310, SECA©, Chino, CA). Prior to measuring body weight, the participants were asked for a urine sample to test hydration status. Hydration status was assessed with urine specific gravity by using a refractometer (Atago SUR-NE, Atago Corp Ltd., Tokyo, Japan). In order to ensure euhydration, a minimum USG of ≤1.020 was required prior to data collection (30, 31). In the case that USG exceeded 1.020, participants were given the opportunity to ingest water *ad libitum* for 30 minutes and have USG reassessed, or reschedule the session for an upcoming day. Weight was measured on a digital scale (Tanita BWB-800, Tanita©, Arlington Heights, IL) and recorded manually. Next, body composition was assessed via 7 site-skinfolds. Additionally, measurement of thigh circumference was taken with a flexible, tension-sensitive, non-elastic vinyl tape measure (Gulick, Lafayette instrument Co. Lafayette, IN). To ensure consistency in the circumference measures, the measurement was taken at 50% of the distance to the superior patella from the anterior superior iliac spine on the right side of the body. The average of three circumference measurements was used.

Skinfold thicknesses were measured from each participant with calibrated skinfold calipers with 7-site skinfold analysis (25). Body density from skinfolds was calculated using the Brozek-equation (32). Each site was measured once and then all measures were repeated. Each

site was measured at least two times and the average was calculated. All the measurements were within 2.0 mm of each other for appropriate determination.

Limb Occlusion Pressure Determination

Limb occlusion pressure was measured at the beginning of the familiarization trial, before the determination of the 1RM back squat. LOP was also determined prior to the BFR back squat trial during visits 2 and 3. LOP was measured by the Delfi PTS II and recorded. Each leg was individually measured by the device that will occlude the leg during the back-squat trial. Although devices are calibrated by Delfi, to ensure the same device was used for each leg later in the study, the devices were labeled and noted for future trials. To ensure that cuff placement was similar between visits, the cuff was positioned within 4 inches of the inguinal crease, with the inflation valve located on the medio-lateral portion of the thigh.

Determination of One Repetition Max

Participants had their 1RM tested for back-squat during their first visit following National Strength and Conditioning recommended guidelines (33). Participants were asked to self-report their 1RM based on their resistance training experience. All participants were experienced weightlifters and were able to estimate their 1RM if they had not directly measured it. In the case that a participant didn't know, the estimated 1RM was based on their known 10 repetition maximum and scaled for a 1RM. Prior to beginning the 1RM testing, participants warmed up on a cycle ergometer for 5 minutes, followed by a self-selected dynamic warm-up (i.e. arm circles, lunges, walking hamstring stretch, bodyweight squats). Next, using a goniometer, participants performed a bodyweight squat in the power rack and had their knee flexion measured. Participants were asked to squat between 100° and 90° for each repetition. Participants then performed back-squats with an unloaded barbell (45lbs) for 10 repetitions, followed by 3-5

repetitions at 50% self-reported 1RM, 2-3 repetitions at 70% self-reported 1RM, 1-2 repetitions at 85% self-reported 1RM. 2-3 minutes rest were provided between warm-up sets. Next, following a 3-minute rest period, the participant attempted the self-reported 1RM. If the participant failed the attempt, then the load was be decreased by 5-10% and the attempt will be repeated following 3-5 minutes rest. If the squat was successful, then the load was increased by 10-20%, and another attempt was made following 3-5 minutes rest. The 1RM was determined by the maximum weight the participant was able to squat while maintaining proper exercise technique.

Visits 2 and 3

Participants reaffirmed consent and completed the PRS and VAS-DOMS measures upon arrival to the laboratory. Following paperwork, resting BP, body weight, and USG were measured following the same protocols as during Visit 1.

Blood Sample Collection and Plasma Protein Analysis

Venous blood samples were collected using a safety winged blood collection needle (0.6mm x 19mm x 305mm) (Henry Schein; Melville, NY) and 10-ml K2 EDTA coated vacutainers (Beckton Dickinson; Franklin Lakes, NJ). Venous blood samples were taken prior to beginning the warm-up for a baseline measure and one-hour post back-squat exercise stimulus. Collected blood samples were centrifuged at room temperature for 15 minutes at 3500rpm (PowerSpin C856 Centrifuge; UNICO; Dayton, NJ) for plasma separation. All plasma samples were stored at -80°C until analysis. Venous blood samples were able to be collected on 12 of the 13 participants in the current study. Enzyme-linked immunosorbent assays (Abcam; Cambridge, MA) were used to assess changes in plasma IL-6 (ab46042) and VEGF (ab222510), according to manufacturer's instructions. Following completion of assay procedures, changes in colorimetric

absorbance were evaluated using a multi-mode plate reader (Synergy LX; BioTek Instruments; Winooski, VT).

In addition to plasma protein analysis, finger-prick blood lactate samples were taken at multiple time points. Lactate measurement were taken prior to beginning the warm-up and twice following the back-squat exercise stimulus: immediately post exercise (before deflating the cuff in BFR condition), and 4 minutes post-exercise (2 minutes post cuff deflation in BFR). Capillary blood samples were drawn from the fingertip using contact-activated lancets (BD Microtainer; Beckton Dickinson; Franklin Lakes, NJ) and were examined using a handheld lactate analyzer (Lactate Plus Meter; Nova Biomedical; Waltham, MA).

Anatomical Cross-Sectional Area and Echo Intensity

Anatomical cross-sectional area (ACSA) and echo intensity (EI) measurements were taken before and immediately post- back squat stimulus in order to assess acute fluid shifts (swelling). Measures of rectus femoris (RF) ACSA and EI were assessed using a B-Mode ultrasound imaging device (iU22; Philips Medical Systems; Bothell, WA) with a broadband linear array transducer (L9-3, 9-3 MHz, 38mm field of view; Philips Medical Systems; Bothell, WA) through panoramic musculoskeletal imaging. Measurements were taken on the anterior aspect of the dominant leg upper-thigh: 50% the distance between the proximal border of the patella and anterior superior iliac spine. Distances between bony landmarks were measured with a vinyl tape measure (Gulick, Lafayette instrument Co. Lafayette, IN) and marked with a pen. The linear transducer probe was coated with transmission gel and placed perpendicular to the tissue interface at the marked sites without depressing the skin. All ultrasound measurements were taken with the participants supine and their arms and legs relaxed and fully extended. Following ultrasound measurements, thigh circumference was taken with a flexible, tension-

sensitive, non-elastic vinyl tape measure (Gulick, Lafayette instrument Co. Lafayette, IN). To ensure consistency in the circumference measures, the measurement took place 50% of the distance to the superior patella from the superior anterior iliac crest on the dominant leg. The average of two circumference measurements was used.

ACSA and EI images were analyzed via ImageJ software (Version 1.53, National Institutes of Health, Bethesda, MD, USA) following the conclusion of data collection. Ultrasound images were scaled from pixels to cm and ACSA was measured using the ImageJ polygon selection tool, selecting the outer border of the RF muscle while excluding surrounding fascia (34-36). Using the selected RF ACSA measure, computer-aided gray-scale analysis (ImageJ) was used to assess echo intensity using the histogram function. The mean echo intensity values were determined as the corresponding index of muscle quality ranging between 0 (black) and 255 (white) arbitrary units. ImageJ straight line tool function was used to measure subcutaneous tissue thickness (cm). Subcutaneous tissue thickness values were used to correct raw EI values using the following equation by Young et al. (37): corrected echo intensity = raw echo intensity + (subcutaneous fat thickness (cm) × 40.5278).

Back Squat Protocol

The order of back-squat protocol implemented (CTRL and BFR) was assigned in a counterbalanced order. Only one protocol was performed per visit, with the participant completing the alternate protocol during the subsequent visit to ensure that changes observed were not due to a training effect. Participants performed a back-squat specific warm-up. They performed 3 to 5 repetitions of BS with the unloaded bar, followed by 30% and then 50% of 1RM.

Control Back-Squat Protocol

The back-squat exercise stimulus consisted of 4 sets to volitional fatigue. The relative load was 75% of 1RM with 3 minutes of rest between each set. According to NSCA guidelines at 75%, the participants were expected to be able to complete roughly 10 repetitions in the first set, and less as the sets progress and fatigue sets in (33). The number of repetitions successfully completed was recorded for each set.

BFR Back-Squat Protocol

Following the warm-up, participants were fitted with the BFR cuffs. Cuffs were placed on both legs for bilateral occlusion during the exercise. The cuffs were positioned within 4 inches of the inguinal crease, with the inflation valve located on the medio-lateral portion of the thigh. The Delfi-PTS was set to self-determine 100% LOP. Average LOP was determined to be 219.6 ± 18.2 mmHg in the right leg and 206.8 ± 25.8 mmHg in the left leg. Following the LOP determination, the cuff was deflated until the exercise begins. During the exercise protocol, the BFR cuffs were inflated to 80% occlusion pressure (175.6 ± 14.6 mmHg right leg, 165.4 ± 20.7 mmHg left leg). The cuffs were inflated 30 seconds before beginning the first set. The cuffs remained inflated until the completion of the second set. Upon completion of the second set, the cuffs were deflated and remained deflated during the 3-minute rest period between sets 2 and 3. The cuffs were re-inflated 30 seconds prior to the start of set 3 and remained inflated until 2 minutes after set 4 was completed. The average occlusion time was 341.9 (±24.0) seconds for the first inflation period (sets 1 and 2) and 425.7 (±48.8) seconds for the second inflation period (sets 3 and 4). Total occlusion time during the BFR exercise was 767.6 (±64.9) seconds. The back-squat exercise stimulus consisted of 4 sets to volitional fatigue. The relative load was 75% of

1RM with 3 minutes of rest between each set. The number of repetitions successfully completed was recorded for each set.

Statistical Analysis

Data were managed using Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were completed using SPSS for Windows (Version 25.0, IBM, Somers, NY, USA). All data is presented as mean ± standard deviation (M ± SD), unless otherwise noted.

Mauchly's test was used to test the assumption of sphericity and a Greenhouse-Geisser correction was applied when the assumption of sphericity was not met. Two-way repeated measures analysis of variance (ANOVA) [2 conditions (HI-RT and HI-BFR) x 2 times (pre vs. post)] were used to determine the effects pre- to post-exercise of occlusion condition on markers of muscle damage (IL-6 and EI), hypoxia (VEGF), and muscle swelling (ACSA), see Table 4.2. Additionally, a two-way analyses of variance (ANOVA) [2 conditions (HI-RT and HI-BFR) x 3 times (baseline vs. immediately post vs. 4-minutes post)] was used for the analysis of changes in blood lactate concentration. Bonferroni's correction was used for adjustment for multiple comparisons. An alpha level of $p < .05$ was utilized to determine statistical significance**.** Post-hoc analysis following significant effects from 2x2 ANOVAs were performed using *t*-tests. A significant result from the 2x3 ANOVA was followed up with one-way ANOVAs. Effect sizes were measured by partial eta square (ηp^2).

RESULTS

Blood Lactate

Results of the 2x3 repeated measures ANOVA revealed a significant time*condition interaction effect: ($F(2,24) = 15.126$, $p = .001$, $\eta p^2 = .558$. Mauchly's test indicated that the

assumption of sphericity was violated in the main effects of time (p = .004) and condition (p = .007), so a Greenhouse-Geisser correction was applied. Significant main effects on blood lactate for condition (F(1,12) = 9.449, p = .010, ηp^2 = .441) and time (F(1.218,14.617) = 108.409, p < .001, ηp^2 = .900 were observed. One-way repeated measures ANOVAs were used to examine time effects within conditions. Repeated contrasts within the BFR condition revealed that blood lactate was significantly increased immediately post (p < .001) and four minutes post exercise (p < .001) compared to baseline. Additionally, a significant increase in blood lactate was observed from immediately post to four minutes post exercise (p = .007). Repeated contrasts revealed that CTRL blood lactate was significantly increased immediately post (p < .001) and four minutes post exercise (p < .001) compared to baseline, however no significant difference was observed from immediately post to four minutes post (p = .835). Paired samples t-tests revealed no significant differences between BFR and CTRL conditions at baseline (1.23 ± .40, 1.06 ± .51 mmol·L^{-1}, respectively, p = .132) or four minutes post exercise (8.95 ± 2.95, 10.72 ± 3.90 mmol·L^{-1}, respectively, p = .063). Lactate immediately post exercise was significantly greater in the CTRL condition (11.09 ± 3.24 mmol·L^{-1}) compared to BFR (7.35 ± 2.53 mmol·L^{-1}) (p = .001). (See Figure 4.1)

Interleukin 6

Due to limited funding at the time of analysis, only the first 10 participants with complete blood samples were used for plasma protein analysis. The remaining participant's plasma samples will be included in future analysis. Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect (F(1,9) = 3.422, p = .097, ηp^2 = .275) or main effect for condition (F(1,9) = 1.285, p = .286, ηp^2 = .125). A significant main effect on IL-6 for time (F(1,9) = 10.803, p = .009, ηp^2 = .546) was observed. A significant increase in IL-6

from PRE to POST-exercise was observed (+11.06%, 1.45 ± .440 pg·ml^{-1}, p = .009). Paired-samples t-tests within conditions indicated a significant increase in IL-6 in the BFR condition (+39.7%, 2.52 ± 0.79 pg·ml^{-1}, p = .011) and non-significant increase in the CTRL condition (+11.11%, 0.37 ± 0.66 pg·ml^{-1}, p = .595). Additionally, post-exercise IL-6 concentrations were significantly greater in the BFR condition (14.08 ± 6.16 pg·ml^{-1}) compared to CTRL (12.06 ± 6.67 pg·ml^{-1}) (p = .007). (See Figure 4.2)

Vascular Endothelial Growth Factor

Due to limited funding at the time of analysis, only the first 10 participants with complete blood samples were used for plasma protein analysis. The remaining participant's plasma samples will be included in future analysis. Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,9) = 2.023$, p = .189, ηp^2 = .184) or main effects for condition ($F(1,9) = .433$, p = .527, ηp^2 = .046) or time ($F(1,9) = 2.188$, p = .173, ηp^2 = .196) on VEGF. (See Figure 4.3)

Anatomical Cross-Sectional Area

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,12) = 1.241$, p = .287, ηp^2 = .094) or main effect for condition ($F(1,12)$ = .548, p = .473, ηp^2 = .044). A significant main effect on ACSA for time ($F(1,12) = 48.160$, $p <$ .001, ηp^2 = .801 was observed. A significant increase in ACSA from PRE to POST-exercise was observed (+9.91%, 1.013 ± .146 cm^2 , $p <$ .001). (See Figure 4.4)

Thigh Circumference

Results of the 2x2 repeated measures ANOVA revealed no significant time*condition interaction effect ($F(1,10) = .331$, p = .578, ηp^2 = .032) or main effect for condition ($F(1,10)$ = .207, p = .659, ηp^2 = .020). A significant main effect on thigh circumference for time ($F(1,10)$ =

66.961, $p < .001$, $\eta p^2 = .870$) was observed. A significant increase in thigh circumference from PRE to POST-exercise was observed (+2.07%, 1.205 ± .147 cm , $p < .001$). (See Figure 4.4)

Echo Intensity

Due to potential methodological inconsistencies (discussed more in the limitations section) in ultrasound measures between testing conditions, no comparisons between conditions can be made. Therefore, only paired samples t-tests were used to analyze pre- to post-changes in EI within conditions (See Figure 4.5). No significant difference was observed pre (99.09 ± 36.18 a.u.) to post (95.69 ± 35.67 a.u.) in the BFR condition ($p = .178$). No significant difference was observed pre (110.66 ± 30.08 a.u.) to post (107.60 ± 26.50 a.u.) in the CTRL condition ($p = .350$).

DISCUSSION

The purpose of this study was to assess the influence of BFR during high-intensity resistance exercise on metabolic stress, inflammation, and muscle damage. It was hypothesized that continuous occlusion during the exercise stimulus in the proposed study would enhance metabolic stress and cause an increase in acute cell swelling, without an increase in markers of muscle damage or inflammation compared to the control exercise stimulus.

Contrary to our hypothesis, blood lactate was significantly greater in the CTRL condition immediately post exercise compared to BFR (11.09 ± 3.24, 7.35 ± 2.53 $mmol \cdot L^{-1}$, respectively), however, although CTRL remained elevated, no significant difference between conditions was observed 4-minutes post exercise (10.72 ± 3.90, 8.95 ± 2.95, $mmol \cdot L^{-1}$, respectively). Of note, however, significantly more total repetitions were performed in the CTRL condition (42.15 ± 13.35 reps) compared to BFR (25.85 ± 8.48 reps) ($p < .001$). While not matching the lactate levels of CTRL, the occlusion may have enhanced the blood lactate response while completing

less total load. Unfortunately, blood lactate was not measured following the second set prior to releasing occlusion pressure. Removing occlusion during the rest period between sets 2 and 3 may have allowed for clearance of accumulated metabolites in the lower limbs. Findings by Teixeira et al. demonstrate that high intensity resistance exercise with limb occlusion performed only during the rest between sets (3 sets of 8 repetitions unilateral knee extension) significantly increases blood lactate concentration compared to non-BFR or occlusion during sets (un-occluded rest) (21). Laurentino et al. measured blood deoxyhemoglobin concentration [HHb], a marker of metabolic stress, and found HI-BFR to produce greater muscle deoxygenation compared to HI-RT (38). It is plausible that post-exercise blood lactate concentrations would have been enhanced by leaving the limb occluded during the rest period in the current study. Average occlusion time during the first inflation period (sets 1 and 2) was 341.9 (±24.0) seconds and 425.7 (±48.8) seconds for the second inflation period (sets 3 and 4). Total occlusion time during the BFR exercise was 767.6 (±64.9) seconds. The underlying reason for the removal of occlusion during the rest period following the second set was the discomfort associated with occlusion training, especially during high-intensity exercise (39).

A larger increase in plasma IL-6 was observed in the BFR condition compared to the CTRL condition (+39.7% vs 11.11%, respectively). Limited research exists regarding IL-6 response to high-intensity BFR resistance exercise. Winchester et al. found no significant differences between BFR (80% LOP) and traditional high-intensity back-squats (75%1RM) in plasma IL-6 protein expression 1-hour post exercise (+31% and +22%, respectively, $p < .05$) (22). In contrast to the (sectioned) continuous limb occlusion during current study, Winchester et al. (22) used unilateral intermittent limb occlusion during exercise (pressure released during rest). The longer, more sustained intramuscular hypoxic environment through (interrupted)

continuous occlusion in the current study may be the underlying difference in IL-6 response. Takarada et al. examined the effect of low-intensity (20% 1RM) resistance exercise in conjunction with bilateral continuous limb occlusion on plasma IL-6 and found a significant increase following 5 sets of knee-extension to fatigue (40). IL-6 is often used as a measure of exercise induced muscle damage (EIMD) (41) however, although no direct measure of EIMD was assessed in the current study, the greater total mechanical load of the CTRL condition in the current study indicates other underlying mechanisms may be responsible for increases in IL-6 post-exercise in the BFR condition.

Vascular endothelial growth factor (VEGF) is a growth factor which promotes angiogenesis and increases in skeletal muscle vascular density following exercise (42). The expression of VEGF is induced by hypoxia (low PO_2) (43) and increases in VEGF expression have been observed acutely following a single bout of exercise (44). Previous research in LI-BFR resistance exercise has shown significantly elevated plasma VEGF concentrations 30, 60, and 120-minutes post exercise, and significantly greater concentrations 60- and 120-minutes post-exercise compared to LI-CTRL (45). Takano et al. observed similar increases in plasma VEGF concentrations 10- and 30-minutes post LI-BFR resistance exercise in untrained adult men (46). Occlusion during low-intensity resistance exercise and the associated reduced tissue oxygenation may promote VEGF secretion, as hypoxia is a stimulus of VEGF production (47). However, no significant increases in VEGF were observed in the current study following HI-BFR. Interestingly, VEGF concentrations decreased following HI-BFR (-3.67%, 4.5 ± 4.5 $pg \cdot ml^{-1}$), while an increase was observed in CTRL (+18.70%, 26.83 ± 18.25 $pg \cdot ml^{-1}$) although neither post-exercise value was significantly different from pre-exercise ($p < .05$). This indicates that the exercise stimulus or occlusion during the BFR exercise potentially was not sufficient for

additional hypoxic benefits for stimulating VEGF. Further research is required determine the effect of BFR on VEGF during high-intensity resistance exercise.

In the current study, post-exercise acute muscle swelling, measured by ACSA and thigh circumference, was not significantly different between conditions. However, as previously mentioned, the exercise stimulus was performed to volitional fatigue and significantly more total repetitions were performed in the CTRL condition. In low-intensity (20% 1RM) resistance exercise performed to volitional fatigue, Yasuda et al. (48) determined acute muscle swelling to be similar between BFR and CTRL conditions, however, similar to the current study, total repetitions in the BFR condition were significantly less compared to the CTRL condition (111 ± 36 reps, 221 ± 67 reps, respectively; $p < .01$) (48). Jenkins et al. demonstrated that low-intensity BFR resistance exercise enhances acute muscle swelling post-exercise compared to high-intensity when both are performed to volitional fatigue, however, in this study the BFR condition performed greater total work across three sets of leg extensions (49). When total work is matched between low-intensity BFR and high-intensity resistance training, acute muscle swelling responses were found to be similar between conditions, and muscle thickness (swelling) was significantly greater in the BFR condition immediately post-exercise, in a study by Freitas et al. (17). Findings of the current study indicate the potential for enhanced acute muscle swelling following HI-BFR, additional research is needed to determine the effects of BFR during high intensity resistance exercise on muscle swelling when total work is matched with the control condition.

Similar reductions in post-exercise EI were observed in both BFR (-3.42%) and CTRL (-2.77%) conditions following the exercise stimulus. Unfortunately, due to methodological inconsistencies between participant visits, no direct comparisons can be made between

conditions and cannot be further extrapolated, therefore, the reductions in EI will be discussed as a general reduction and not condition specific. Increases in EI following resistance exercise are associated with muscle damage. Limited research exists examining the effect of BFR on acute changes in EI, and to our knowledge, this is the first study to examine changes in EI with HI-BFR exercise. A previous study by Alvarez et al. compared acute EI changes following high-load and low-load BFR exercise and observed similar increases in EI in both conditions, attributed to edema-induced muscle swelling (50). However, the first post-exercise measures were taken 24-hours post-exercise and the results may not be applicable to the current study (50).

Limitations

The current study is not without limitations, such as participant characteristics, methodologies/sample analysis, and study design. Inclusion criteria included a minimum resistance training background of one year, and the findings of the current study may not be applicable for untrained individuals. Secondly, total work (number of repetitions) was not matched between conditions and may have influenced the outcome of the post-exercise neuromuscular performance assessments. A matched workload between conditions may allow for a better understanding of effects of BFR compared to CTRL and may further support the utility of BFR during HI-RT. Additionally, occlusion in the current study was interrupted following set 2 and therefore was not true continuous occlusion during the exercise stimulus. As this was one of the first studies examining high-intensity resistance exercise in conjunction with BFR, the authors were cautious with the BFR application. Future research should examine true continuous occlusion with an optimized matched work-load exercise stimulus design. There is potential that ultrasound time gain compensation (TGC) settings were inconsistent between participant visits and EI were not able to be compared between conditions. TGC settings were

consistent pre- to post-exercise and therefore within condition changes were assessed. Lastly, due to funding limitations in the analysis stage of the current study, not all blood samples were analyzed (10 of 12 participants) and statistical analyses may not have been properly powered to assess interaction and main effects for plasma protein analysis. The remaining samples will be analyzed, and findings will be updated once complete.

CONCLUSIONS

Findings of the current study indicate the usefulness of high-intensity resistance exercise to failure in conjunction with BFR for inducing similar amounts of muscle swelling and increased markers of muscular inflammation (IL-6) without increased blood lactate concentration or indices of hypoxia (VEGF). The primary benefit appears to be the lower total workload compared to HI-RT, although, future research should examine the influence of BFR during high-intensity resistance exercise using a matched total workload (i.e. 4 sets of 5 repetitions) rather than sets to failure. Additionally, future research should examine true continuous occluded exercise (i.e. no unoccluded rest period) or include a measure of blood lactate prior to deflating the cuff for the rest period.

REFERENCES

1. Campos, GE, Luecke, TJ, Wendeln, HK, et al. Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. *Eur J Appl Physiol* 88: 50-60, 2002.

2. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 41: 687-708, 2009.

3. Martineau, LC, Gardiner, PF. Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension. *J Appl Physiol* 91: 693-702, 2001.

4. Schoenfeld, BJ. Potential mechanisms for a role of metabolic stress in hypertrophic adaptations to resistance training. *Sports Med* 43: 179-194, 2013.

5. Takarada, Y, Takazawa, H, Sato, Y, et al. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. *Journal of applied physiology (Bethesda, Md : 1985)* 88: 2097-2106, 2000.

6. Takarada, Y, Sato, Y, Ishii, N. Effects of resistance exercise combined with vascular occlusion on muscle function in athletes. *Eur J Appl Physiol* 86: 308-314, 2002.

7. Abe, T, Yasuda, T, Midorikawa, T, et al. Skeletal muscle size and circulating IGF-1 are increased after two weeks of twice daily “KAATSU” resistance training. *International Journal of KAATSU Training Research* 1: 6-12, 2005.

8. Vechin, FC, Libardi, CA, Conceição, MS, et al. Comparisons between low-intensity resistance training with blood flow restriction and high-intensity resistance training on quadriceps muscle mass and strength in elderly. *J Strength Cond Res* 29: 1071-1076, 2015.

9. Lowery, RP, Joy, JM, Loenneke, JP, et al. Practical blood flow restriction training increases muscle hypertrophy during a periodized resistance training programme. *Clin Physiol Funct Imaging* 34: 317-321, 2014.

10. Thiebaud, RS, Yasuda, T, Loenneke, JP, Abe, T. Effects of low-intensity concentric and eccentric exercise combined with blood flow restriction on indices of exercise-induced muscle damage. *Interv Med Appl Sci* 5: 53-59, 2013.

11. Thiebaud, RS, Loenneke, JP, Fahs, CA, et al. Muscle damage after low-intensity eccentric contractions with blood flow restriction. *Acta Physiol Hung* 101: 150-157, 2014.

12. Pope, ZK, Willardson, JM, Schoenfeld, BJ. Exercise and blood flow restriction. *J Strength Cond Res* 27: 2914-2926, 2013.

13. Suga, T, Okita, K, Morita, N, et al. Intramuscular metabolism during low-intensity resistance exercise with blood flow restriction. *Journal of applied physiology (Bethesda, Md : 1985)* 106: 1119-1124, 2009.

14. Suga, T, Okita, K, Takada, S, et al. Effect of multiple set on intramuscular metabolic stress during low-intensity resistance exercise with blood flow restriction. *Eur J Appl Physiol* 112: 3915-3920, 2012.

15. Häussinger, D. The role of cellular hydration in the regulation of cell function. *Biochem J* 313 (Pt 3): 697-710, 1996.

16. Nosaka, K, Clarkson, PM. Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Med Sci Sports Exerc* 28: 953-961, 1996.

17. Freitas, EDS, Poole, C, Miller, RM, et al. Time Course Change in Muscle Swelling: High-Intensity vs. Blood Flow Restriction Exercise. *Int J Sports Med* 38: 1009-1016, 2017.

18. Loenneke, JP, Fahs, CA, Rossow, LM, Abe, T, Bemben, MG. The anabolic benefits of venous blood flow restriction training may be induced by muscle cell swelling. *Med Hypotheses* 78: 151-154, 2012.

19. Lang, F, Busch, GL, Ritter, M, et al. Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 78: 247-306, 1998.

20. Loenneke, JP, Fahs, CA, Thiebaud, RS, et al. The acute muscle swelling effects of blood flow restriction. *Acta Physiol Hung* 99: 400-410, 2012.

21. Teixeira, EL, Barroso, R, Silva-Batista, C, et al. Blood flow restriction increases metabolic stress but decreases muscle activation during high-load resistance exercise. *Muscle Nerve* 57: 107-111, 2018.

22. Winchester, LJ, Morris, CE, Badinger, J, Wiczynski, TL, VanWye, WR. Blood Flow Restriction at High Resistance Loads Increases the Rate of Muscular Fatigue, but Does Not Increase Plasma Markers of Myotrauma or Inflammation. *J Strength Cond Res*, 2020.

23. Beck, TW. The importance of a priori sample size estimation in strength and conditioning research. *J Strength Cond Res* 27: 2323-2337, 2013.

24. Friedman, K. Essentials of Strength Training and Conditioning, 4th Edition. *Med Sci Sports Exerc* 48: 2073-2073, 2016.

25. American College of Sports, M, Riebe, D, Ehrman, JK, Liguori, G, Magal, M. *ACSM's guidelines for exercise testing and prescription.* 2018.

26. Pickering, TG, Hall, JE, Appel, LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation* 111: 697-716, 2005.

27. Whelton, PK, Carey, RM, Aronow, WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. *J Am Coll Cardiol* 71: e127, 2017.

28. Laurent, CM, Green, JM, Bishop, PA, et al. A practical approach to monitoring recovery: development of a perceived recovery status scale. *J Strength Cond Res* 25: 620-628, 2011.

29. Waterfield, MJ, Sim, J. Clinical assessment of pain by the visual analogue scale. *British Journal of Therapy and Rehabilitation* 3: 94-97, 1996.

30. Armstrong, LE. Hydration assessment techniques. *Nutr Rev* 63: S40-54, 2005.

31. McDermott, BP, Anderson, SA, Armstrong, LE, et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. *J Athl Train* 52: 877-895, 2017.

32. Brožek, J, Grande, F, Anderson, JT, Keys, A. Densitometric analysis of body composition: revision of some quantitative assumptions. *Ann N Y Acad Sci* 110: 113-140, 1963.

33. Haff, GG, Triplett, NT. *Essentials of strength training and conditioning 4th edition.* Human kinetics, 2015.

34. Mota, JA, Giuliani, HK, Gerstner, GR, Ryan, ED. The rate of velocity development associates with muscle echo intensity, but not muscle cross-sectional area in older men. *Aging Clin Exp Res* 30: 861-865, 2018.

35. Mota, JA, Stock, MS. Rectus Femoris Echo Intensity Correlates with Muscle Strength, but Not Endurance, in Younger and Older Men. *Ultrasound Med Biol* 43: 1651-1657, 2017.

36. Mota, JA, Stock, MS, Thompson, BJ. Vastus lateralis and rectus femoris echo intensity fail to reflect knee extensor specific tension in middle-school boys. *Physiol Meas* 38: 1529-1541, 2017.

37. Young, HJ, Jenkins, NT, Zhao, Q, McCully, KK. Measurement of intramuscular fat by muscle echo intensity. *Muscle Nerve* 52: 963-971, 2015.

38. Laurentino, G, Ugrinowitsch, C, Aihara, AY, et al. Effects of Strength Training and Vascular Occlusion. *Int J Sports Med* 29: 664-667, 2008.

39. Fitschen, PJ, Kistler, BM, Jeong, JH, et al. Perceptual effects and efficacy of intermittent or continuous blood flow restriction resistance training. *Clin Physiol Funct Imaging* 34: 356-363, 2014.

40. Takarada, Y, Nakamura, Y, Aruga, S, et al. Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J Appl Physiol (1985)* 88: 61-65, 2000.

41. Peake, JM, Suzuki, K, Wilson, G, et al. Exercise-Induced Muscle Damage, Plasma Cytokines, and Markers of Neutrophil Activation. *Med Sci Sports Exerc* 37, 2005.

42. Amaral, SL, Papanek, PE, Greene, AS. Angiotensin II and VEGF are involved in angiogenesis induced by short-term exercise training. *Am J Physiol Heart Circ Physiol* 281: H1163-1169, 2001.

43. Dor, Y, Porat, R, Keshet, E. Vascular endothelial growth factor and vascular adjustments to perturbations in oxygen homeostasis. *Am J Physiol Cell Physiol* 280: C1367-1374, 2001.

44. Breen, EC, Johnson, EC, Wagner, H, et al. Angiogenic growth factor mRNA responses in muscle to a single bout of exercise. *J Appl Physiol (1985)* 81: 355-361, 1996.

45. Patterson, SD, Leggate, M, Nimmo, MA, Ferguson, RA. Circulating hormone and cytokine response to low-load resistance training with blood flow restriction in older men. *Eur J Appl Physiol* 113: 713-719, 2013.

46. Takano, H, Morita, T, Iida, H, et al. Hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with the reduction of muscle blood flow. *Eur J Appl Physiol* 95: 65-73, 2005.

47. Minchenko, A, Bauer, T, Salceda, S, Caro, J. Hypoxic stimulation of vascular endothelial growth factor expression in vitro and in vivo. *Laboratory investigation; a journal of technical methods and pathology* 71: 374-379, 1994.

48. Yasuda, T, Fukumura, K, Iida, H, Nakajima, T. Effect of low-load resistance exercise with and without blood flow restriction to volitional fatigue on muscle swelling. *Eur J Appl Physiol* 115: 919-926, 2015.

49. Jenkins, ND, Housh, TJ, Bergstrom, HC, et al. Muscle activation during three sets to failure at 80 vs. 30% 1RM resistance exercise. *Eur J Appl Physiol* 115: 2335-2347, 2015.

50. Alvarez, IF, Damas, F, Biazon, TMPd, et al. Muscle damage responses to resistance exercise performed with high-load versus low-load associated with partial blood flow restriction in young women. *European Journal of Sport Science* 20: 125-134, 2020.

Table 4.1. Descriptive Characteristics of Study Participants

	All (n=13)	**Female (n=4)**	**Male (n=9)**
Age (yrs)	24.8 ± 4.7	23.8 ± 4.6	25.2 ± 4.9
Height (cm)	177.8 ± 11.8	164.1 ± 5.5	184.0 ± 7.6
Weight (kg)	84.3 ± 16.7	65.6 ± 3.6	92.5 ± 12.9
BMI ($kg \cdot m^{-2}$)	26.4 ± 3.1	24.5 ± 2.0	27.3 ± 3.2
BF%	16.6 ± 8.2	24.0 ± 6.7	13.2 ± 6.6
Right LOP (mmHg)	219.6 ± 18.2	214.0 ± 10.7	222.1 ± 20.7
Right 80% LOP (mmHg)	175.6 ± 14.6	171.3 ± 8.6	177.6 ± 16.7
Left LOP (mmHg)	206.8 ± 25.8	204.8 ± 28.3	207.8 ± 21.1
Left 80% LOP (mmHg)	165.4 ± 20.7	163.8 ± 22.8	166.1 ± 21.1
1RM (kg)	143.7 ± 50.4	85.8 ± 15.2	169.4 ± 36.1

Notes: Data are presented as mean ± standard deviation; BMI - body mass index; cm - centimeters; BF% - body fat percentage; kg - kilograms; yrs - years; LOP - limb occlusion pressure; mmHg - millimeter of mercury; 1RM - one repetition maximum.

Table 4.2. Pre- and Post-Exercise Measures

Variable	Condition	Pre	Post	Time Effect		Condition Effect		Interaction	
				P	ηp^2	*P*	ηp^2	*P*	ηp^2
ACSA (cm^2)	BFR	9.96 ± 2.74	11.15 ± 2.74*	< .001	.801	.473	.044	.287	.094
	CTRL	10.46 ± 3.64	11.30 ± 4.01*						
Thigh Circumference (cm)	BFR	58.30 ± 5.85	59.43 ± 5.89*	< .001	.870	.659	.020	.578	.032
	CTRL	58.05 ± 5.77	59.32 ± 6.03*						
Echo Intensity (a.u.)	BFR	99.09 ± 36.18	95.69 ± 35.67	-	-	-	-	-	-
	CTRL	110.66 ± 30.08	107.60 ± 26.50						
IL-6 (pg·ml^{-1})	BFR	11.56 ± 6.77	14.08 ± 6.19*†	.009	.546	.286	.125	.097	.275
	CTRL	11.69 ± 7.23	12.06 ± 6.67						
VEGF (pg·ml^{-1})	BFR	61.39 ± 42.60	59.13 ± 38.10	.173	.196	.527	.046	.189	.184
	CTRL	57.41 ± 32.28	68.15 ± 53.86						

Values are Mean ± SD; BFR - blood flow restriction; CTRL - control; cm - centimeters; IL-6 - interleukin 6; VEGF - vascular endothelial growth factor; pg·ml^{-1} - picograms per milliliter; * significant difference from pre within condition at $p < .05$. † significant difference from control within time point at $p < .05$. # sphericity assumption violated (Greenhouse-Geisser correction).

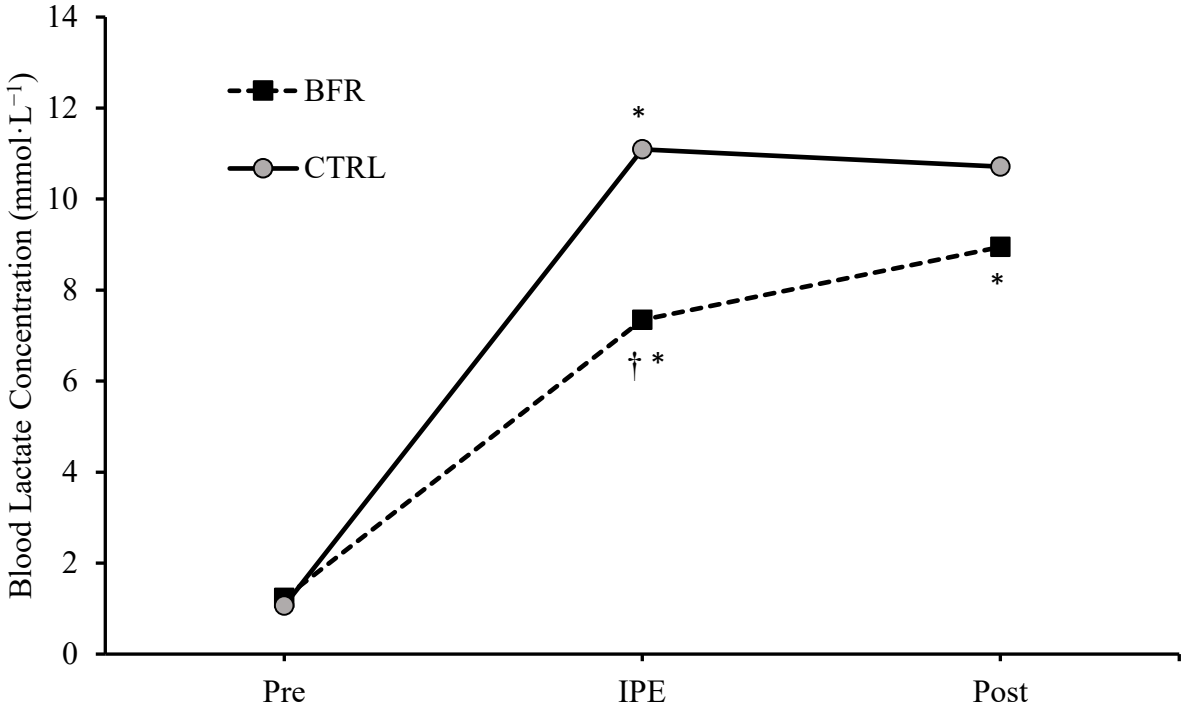

Figure 4.1. Changes in blood lactate concentrations (mmol·L^{-1}) over time from Pre (baseline) to immediately post exercise (IPE) and 4 minutes post (Post) exercise. BFR - blood flow restriction. CTRL - control. † significant difference ($p < .05$) between conditions. * significant difference ($p < .05$) within condition compared to previous time point. Error bars not included for clarity.

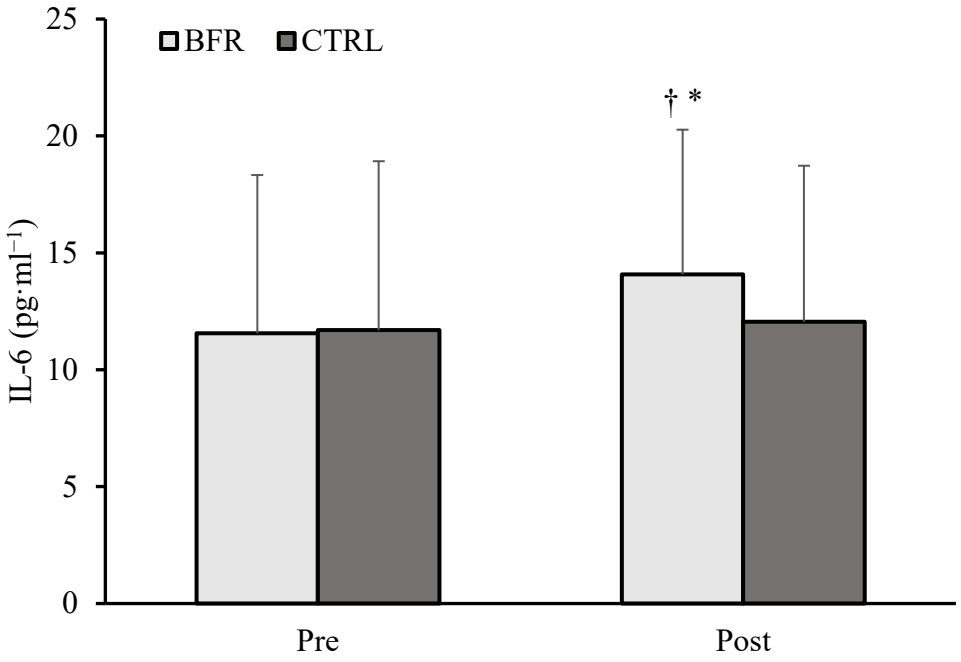

Figure 4.2. Changes in IL-6 ($pg \cdot ml^{-1}$) over time from pre- to post-exercise. † significant difference ($p < .05$) between conditions at same time-point. BFR - blood flow restriction. CTRL - control. * significant difference ($p < .05$) within condition compared to pre-exercise. Error bars represent standard deviation.

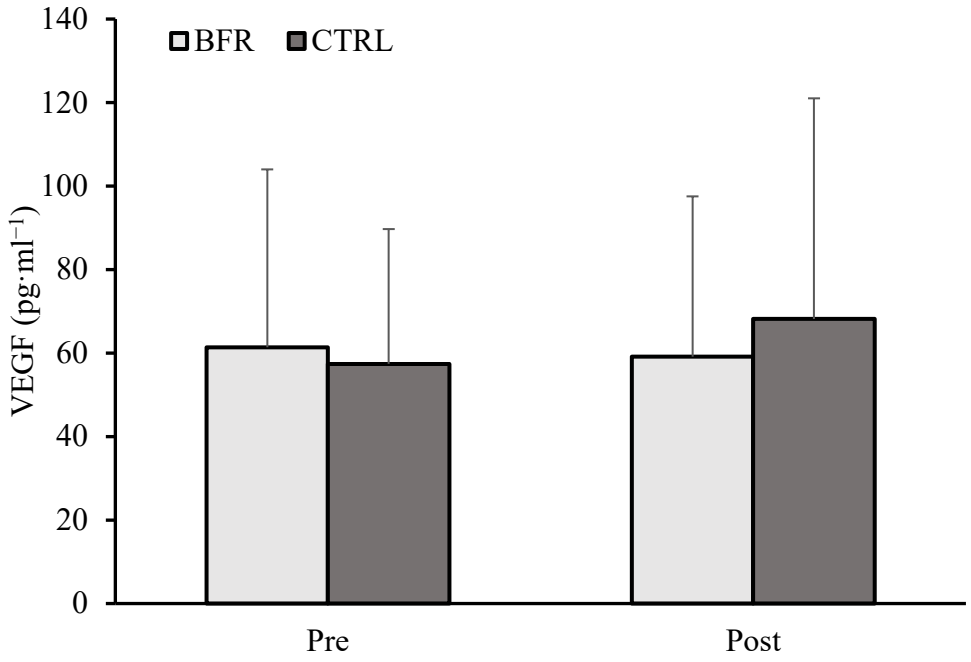

Figure 4.3. Changes in Vascular Endothelial Growth Factor (VEGF) ($pg \cdot ml^{-1}$) over time from pre- to post-exercise. BFR - blood flow restriction. CTRL - control. Error bars represent standard deviation.

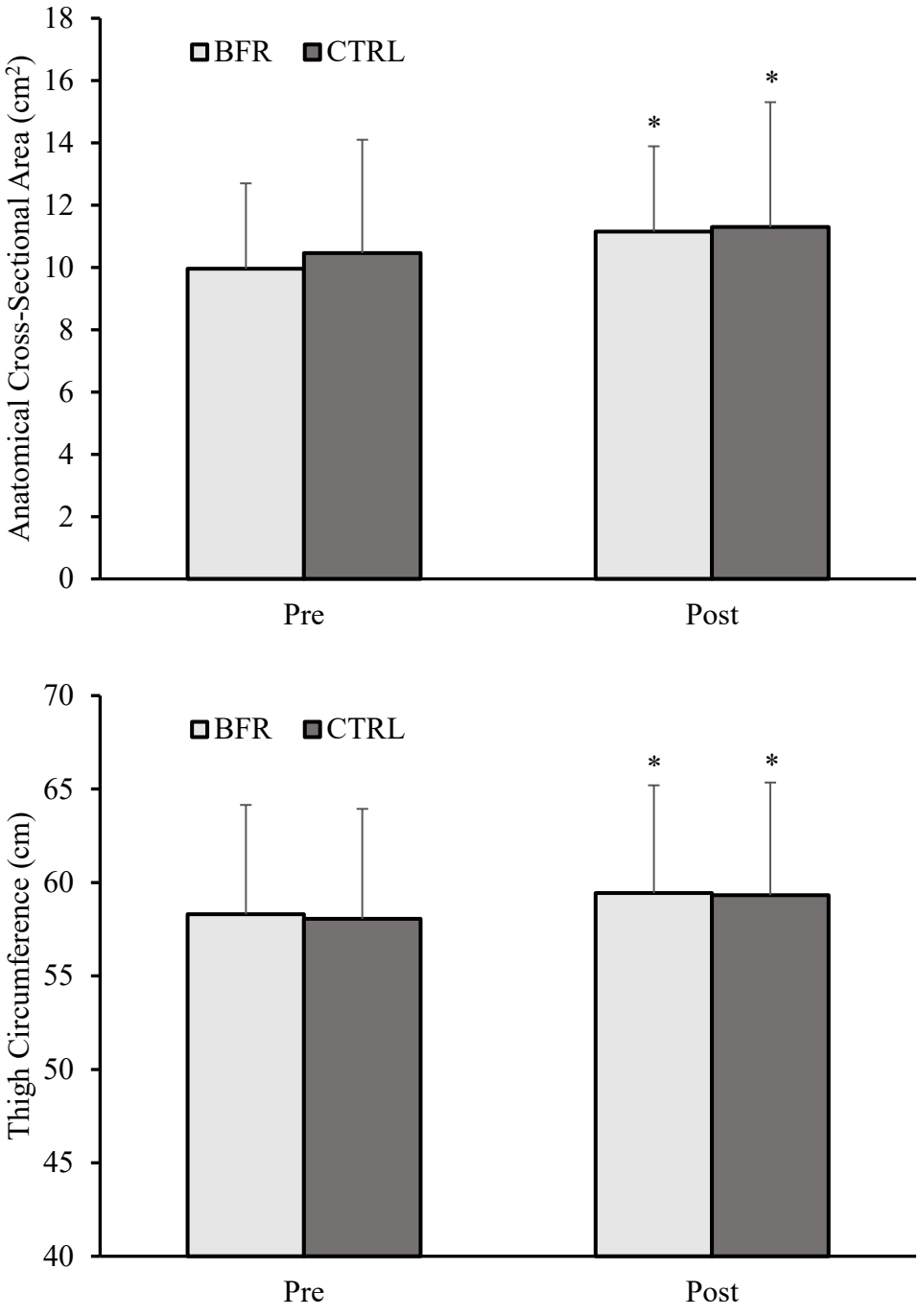

Figure 4.4. Changes in measures of muscle swelling measured by Thigh Circumference (cm) and Anatomical Cross-Sectional Area (ACSA) (cm^2) over time from pre- to post-exercise. BFR - blood flow restriction. CTRL - control. * significant difference ($p < .05$) within condition compared to pre-exercise. Error bars represent standard deviation.

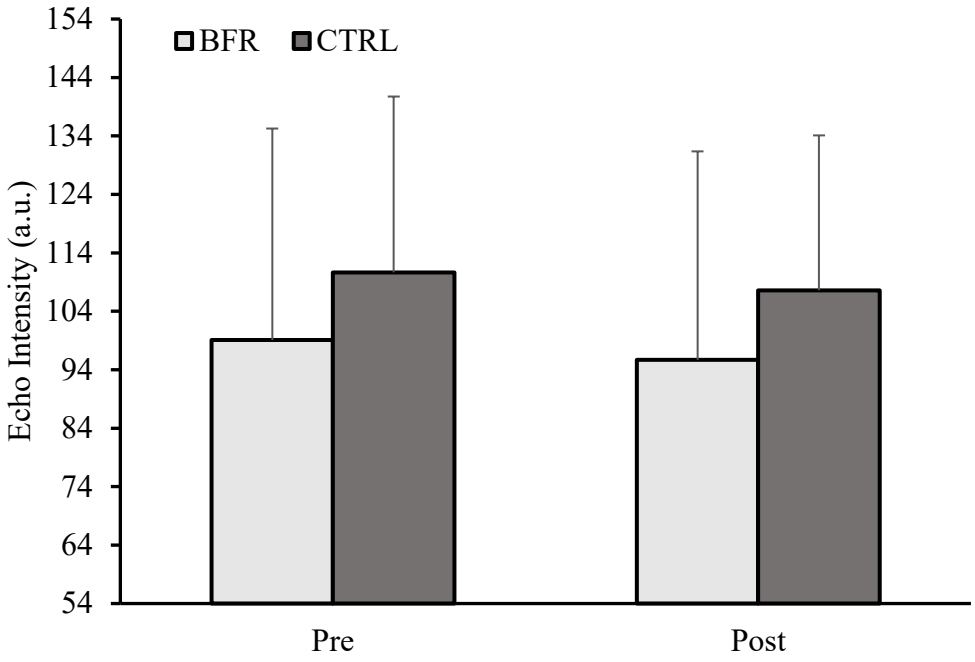

Figure 4.5. Changes in Echo Intensity (a.u.) over time from pre- to post-exercise. BFR - blood flow restriction. CTRL - control. Error bars represent standard deviation.

CHAPTER 5

CONCLUSION

Contemporary muscular hypertrophy recommendations focus on high-intensity resistance training (HI-RT) (>65% of 1-repetition-max (1RM)) for 8-12 repetitions for 1 to 3 sets (1, 2). Three primary factors are believed to be responsible for the hypertrophic response to HI-RT: mechanical tension, muscle damage, and metabolic stress. Induction of these mechanisms is greatly reliant on the exercise load, which is why a load of >65% 1RM is recommended to cause hypertrophy (1, 2). However, more recently, low-intensity (20-30% 1RM) blood flow restriction (LI-BFR) training has demonstrated usefulness as an alternative method for producing increases in muscle hypertrophy similar to HI-RT (3). Some of the proposed underlying mechanisms for these BFR training adaptations include metabolite accumulation, increased fiber type activation, hormonal responses, and cell swelling (4, 5). Both HI-RT and LI-BFR are effective RT modalities for enhancing muscular growth, however, the underlying mechanisms for hypertrophy differ due to differences in training load, with HI-RT promoting mechanotransduction and muscle damage, while LI-BFR promotes greater metabolic stress and myofiber swelling. Therefore, it may be possible that combining the two modalities, for high-intensity BFR (HI-BFR), may maximize the hypertrophic capabilities by enhancing the metabolic stress and cell swelling in the muscle, while simultaneously stimulating the muscle through mechanotransduction.

To better understand the effects of HI-BFR, we conducted three studies. In study 1, the aim was to compare the effects of varying BFR pressures based on individualized limb occlusion

pressures (LOP) on blood flow in the leg. We hypothesized that blood flow would decrease in a non-linear fashion with increases in occlusion pressure. One-way repeated-measures ANOVAs were used to examine potential differences in blood flow volume relative to rest (%Rel) and volumetric blood flow (cc/min) between relative pressures ranging from 10-90% LOP in 10% increments. Findings indicate a significant effect of relative cuff pressure on volumetric flow (cc/min) and blood flow relative to rest (%Rel). Significant differences were observed in volumetric flow compared to rest for the 50%, 60%, 70%, 80%, and 90% relative pressures (all $p < .05$). Volumetric flow at 80% occlusion pressure, a commonly used occlusion pressure in the lower limbs, was significantly different from all other pressures ($p < .05$) except 70% ($p = .052$). Similarly, significant reductions in relative blood flow (%Rel) were observed at 40%, 50%, 60%, 70%, 80%, and 90% relative pressures (all $p < .05$) compared to rest. Overall, findings confirm our hypothesis that blood flow reductions would be non-linear with increasing occlusion pressures. Lower occlusion pressures (i.e. ≤ 40% LOP) minimally occlude arterial blood flow and may not be sufficient for creating a hypoxic environment in the working muscle. 50% LOP may represent a minimum threshold pressure required to elicit significant decreases in arterial blood flow during BFR exercise.

In study 2, the purpose was two-fold, firstly, to compare the effect of BFR during high-intensity resistance exercise on inter-set fatigue, ratings of perceived exertion (RPE), and Pain, and secondly to determine if HI-BFR causes greater neuromuscular fatigue/impairment compared to HI-RT. The number of set repetitions, RPE, and Pain were recorded for each set during four sets of barbell back-squats (75%1RM) to failure under two conditions; BFR and CTRL. Pre-post neuromuscular performance measures (MVIC, CMJ, IHG, and MVP) were assessed pre-exercise and 10-min post-exercise. We hypothesized that the addition of BFR to the

back-squat protocol would cause greater impairment in muscle function and force production during performance measures following the exercise. Significantly greater number of repetitions were performed during the CTRL condition during sets 1, 2, and 4 ($p < .05$) compared to BFR. Although RPE between conditions was similar across all sets ($p \geq .05$), perceived pain was significantly greater in BFR across all sets ($p < .05$). Significant reductions in post-exercise neuromuscular measures were observed in MVIC-Flexion, CMJ, and MPV which was also associated with a significant reduction in MPV-RMS (all $p < .05$). Changes in post-exercise measures were consistent across exercise conditions.

In study 3, the aim was to examine if the underlying mechanisms of BFR-induced hypertrophy (metabolic stress, cell swelling, markers of inflammation) are further enhanced in response to a high load back-squat protocol. We hypothesized that the continuous occlusion during HI-BFR in the proposed study will enhance metabolic stress and cause an increase in acute cell swelling, without an increase in markers of muscle damage or inflammation compared to HI-RT following the exercise protocol. Significantly elevated blood lactate concentrations were measures immediately and 4-minutes post-exercise in both BFR and CTRL ($p < .001$). Lactate immediately post-exercise was significantly greater in CTRL compared to BFR ($p = .001$), but not four minutes post ($p = .063$). BFR post-exercise IL-6 was significantly elevated from baseline ($p = .011$) and significantly greater than CTRL ($p = .007$). No significant changes were observed post-exercise in VEGF or EI in either condition. Thigh circumference and ACSA were significantly greater post-exercise, but consistent across conditions.

Collectively, this dissertation demonstrates the utility of BFR in conjunction with high-intensity resistance exercise. Although significantly fewer total repetitions are performed during HI-BFR, with greater perceived pain, effects were similar to the CTRL condition for inducing

neuromuscular fatigue post-exercise and inducing cell swelling. However, total metabolic stress was lower in the BFR condition while causing elevated IL-6 compared to CTRL. While the current squat stressor study design was not without limitations, our findings demonstrate the potential for using lower occlusion pressures for similar blood flow reductions in the legs which may allow for closer matched total workload in future studies, allowing for better comparison between conditions.

REFERENCES

1. Campos, GE, Luecke, TJ, Wendeln, HK, et al. Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. *Eur J Appl Physiol* 88: 50-60, 2002.

2. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 41: 687-708, 2009.

3. Lixandrão, ME, Ugrinowitsch, C, Berton, R, et al. Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med* 48: 361-378, 2018.

4. Loenneke, JP, Wilson, GJ, Wilson, JM. A mechanistic approach to blood flow occlusion. *Int J Sports Med* 31: 1-4, 2010.

5. Pearson, S, Hussain, S. A Review on the Mechanisms of Blood-Flow Restriction Resistance Training-Induced Muscle Hypertrophy. *Sports Med* 45: 187-200, 2015.

APPENDIX

THE UNIVERSITY OF ALABAMA | Office of the Vice President for Research & Economic Development
Office for Research Compliance

July 16, 2020

Bjoern Hornikel
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 20-010-ME
"Agreement between Two Blood Flow Restriction Devices: KAATSU Master and Delfi PTS"

Mr. Hornikel:

The University of Alabama Medical Institutional Review Board has granted approval for your proposed research. Your application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on June 3, 2021. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

Please use reproductions of the IRB approved stamped consent form to obtain consent from your participants.

Good luck with your research.

Sincerely,

[redacted]
Medical IRB Chair

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205-348-8461 | Fax 205-348-7189 | Toll Free 1-877-820-3066

THE UNIVERSITY OF ALABAMA | Research & Economic Development
Office for Research Compliance

September 28, 2020

Bjoern Hornikel
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 20-013-ME "Effects of High Intensity Resistance Training with Blood Flow Restriction on Muscular Activation, Fatigue, and Hypertrophy"

Mr. Hornikel:

The University of Alabama Medical Institutional Review Board has granted approval for your proposed research. Your application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on August 5, 2021. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

Please use reproductions of the IRB approved stamped consent form to obtain consent from your participants.

Good luck with your research.

[redacted]
Medical IRB Chair

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