EFFECTS OF PROXIMAL LIMB BLOOD FLOW RESTRICTION TRAINING ON DISTAL LIMB PERFORMANCE AND RECOVERY

by

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ABSTRACT

Purpose: To determine if proximal limb blood flow restriction (BFR) influences muscular performance and recovery of the distal limb musculature (i.e., gastrocnemius, soleus, and tibialis anterior). **Methods:** Participants (N = 20; M±SD: 23.0 ± 3.8 years; 174.1 ± 9.0 cm; 77.9 ± 13.0 kg; 23.8 ± 8.6%body fat) completed a baseline visit and two experimental conditions consisting of exercise only (control; CON) and exercise with BFR. CON and BFR were performed in a counter-balanced order. Personal tourniquet pressure (PTP) was determined in each leg using the Delfi PTS II system at the BFR session only. Participants underwent the following assessments before and after each experimental visit: anatomical cross-sectional area (CSA) of the gastrocnemius, toe tap test, average stride length test, and counter-movement jump. The exercise protocol consisted of 3 sets of 15 repetitions of ankle plantarflexion (PF) and dorsiflexion (DF at 60 and 500 degrees per second, respectively, using an isokinetic dynamometer. Average force per repetition and total work performed were measured by the isokinetic dynamometer for both PF and DF during exercise. The only difference between BFR and CON was the application of BFR during the exercise protocol (unilateral occlusion at 80% of PTP, applied 30 s before initiating exercise on each leg). Two-way repeated measures analysis of variance (ANOVA) was performed to determine if changes in CSA, and measures of muscular strength and performance differed by BFR application (condition × time). Significance was set as $p<0.05$. **Results:** Average force per repetition and total work performed was lower during BFR compared to CON for both PF and DF ($p<0.05$ for both). CSA was increased post- versus pre-exercise following BFR compared to CON ($p<0.05$). Likewise, toe taps and stride length performance was

decreased post- versus pre-exercise following BFR compared to CON(both $p<0.05$). Jump height decreased post- compared to pre-exercise with no difference between conditions ($p<0.05$).

Conclusion: The addition of BFR to exercise elicited higher levels of muscular fatigue and decreased muscular performance compared to CON. These impairments were far greater than expected, producing significant reductions in force production, neuromuscular activation, and recovery rate.

LIST OF ABBREVIATIONS AND SYMBOLS

1RM	one-repetition maximum
ANOVA	analysis of variance
BFR	blood flow restriction
BP	blood pressure (mmHg)
Ca2+	calcium
cm	centimeter
CMJ	countermovement jump
CON	control condition
CSA	cross-sectional area
EMG	electromyography
EPO	erythropoietin
ft/lbs	foot pounds
GLUT4	glucose transporter type 4
h	hours
iNOS	inducible nitric oxide synthase
IRB	institutional review board
kg	kilogram
LOP	limb occlusion pressure
m	meter

M	mean
mTOR	mammalian target of rapamycin
mV	millivolts
MVC	maximal voluntary contraction
PAR-Q+	physical activity readiness questionnaire+
Pi	inorganic phosphate
PTS II	personalized tourniquet system II
ROS	reactive oxygen species
RPE	ratings of perceived exertion
RT	resistance training
s	seconds
SE	standard error
SENIAM	surface electromyography for the non-invasive assessment of muscles
VEGF	vascular endothelial growth factor
VO_{2max}	maximal oxygen uptake (ml/kg/min)
ηp2	partial eta squared

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INTRODUCTION

Resistance training (RT) is the most effective training modality used to induce muscular hypertrophy (1). RT also causes adaptations to occur in connective tissues such as tendons and ligaments, as well as bone growth (2). RT has been shown to increase muscular strength, muscular endurance, power, and hypertrophy (3). Initial increases in strength and power primarily come from acute neuromuscular adaptations, these neuromuscular adaptations can be caused by manipulating variables such as intensity, volume and frequency of training (3). Manipulating these variables can alter the number of motor units, size of motor units that are being recruited, and the rate of force production (4). Typically, higher intensity training with loads ≥ 65% are recommended for gains in strength, power, and hypertrophy (5). The main factor in RT that induces hypertrophy is total load, as long as intensity is controlled for (3). Gains in hypertrophy typically take twelve weeks or longer to develop (2).

RT generates large amounts of mechanical tension within muscle tissues, connective tissues, and bones. The mechanical tension on bones from both mechanical load and forces generated from contracting muscle tissue cause the process of osteogenesis to begin in order to strengthen the bones so that they can support the application of force (2). Chronic RT causes an increase in bone mineral density (BMD), which helps to prevent diseases such as osteoporosis and osteopenia. Chronic RT also enhances growth and density of the tendons and ligaments of the exercising tissue, decreasing the risk of connective tissue tears (2). Within the muscle tissue, hypertrophy is induced by stimulating and recruiting larger motor units, which are typically

comprised of higher force producing, type II muscle fibers, increasing the mechanical tension on the whole tissue. This increases muscular strength and power which increases tension production on bones, further promoting osteogenesis and allowing for BMD maintenance (2). RT also slows the pace of sarcopenia due to its effects on protein synthesis and protein breakdown (6). Increases in lean body mass, such as bone and muscle tissue, help to develop a healthy body composition, which decreases risk of developing diseases associated with metabolic syndrome (2, 7). This has been demonstrated through maintenance of blood glucose levels within a healthy range as a result of regular RT (8).

There are many pathways in which RT induces muscular hypertrophy, two of which are through myotrauma and metabolic stress signaling (1). Microtears which form within the sarcomere and stimulate sarcolemmal integrin receptors, resulting in cell signaling cascades that promote satellite cell proliferation and mobilization into the myofiber (1). This typically results in the formation of a new myonucleus and greater myonuclear domain, enhancing protein synthesis capacity and fiber hypertrophy. Metabolic stress is caused by a buildup of metabolites, such as lactic acid, inorganic phosphate, and creatine, that are induced by type II fiber recruitment and hypoxia within the tissue (1, 9). This happens when metabolites accumulate in the tissue faster than they can be cleared by the blood. Support for the role of metabolic stress in muscular hypertrophy comes from studying training routines of bodybuilders who perform exercise set and rep schemes that heighten metabolite build up at the expense of higher intensity training, whereas powerlifters perform training with larger mechanical loads and see less hypertrophic adaptations (1).

Blood flow restriction (BFR) training is growing in popularity as an exercise training modality for both recreational and professional athletes alike, due to its ability to induce

muscular hypertrophy at loads as low as 20% of the individuals one-rep maximum (1RM) (10). Several studies have demonstrated that low-load training with BFR induces gains in strength and muscular hypertrophy (11-13). BFR training is also a prominent research topic among researchers who seek to delineate the physiological mechanisms behind its use. BFR typically requires the use of inflatable cuffs placed on the proximal portion of a limb to occlude blood flow (10). The goal of BFR is to reduce arterial blood flow and eliminate venous return, leading to venous pooling and swelling of the tissue. Reducing arterial blood flow to the tissue creates an oxygen-poor environment and induces hypoxia (10).

Reduced blood flow results in the accumulation of metabolites such as lactate, ADP, and inorganic phosphate, some of which are upstream inducers of the mechanistic target of rapamycin (mTOR) signaling pathway leading to enhanced protein synthesis and muscular hypertrophy (10, 14). The increase of metabolites also results in cell swelling as a result of osmotic draw of water into the cell (1, 9, 10, 15). After completion of the exercise the increase in swelling and accumulation of metabolites causes macrophages to activate and infiltrate the tissue (15). Macrophages play a major role in promoting growth and regeneration after tissue damage, thus, promoting hypertrophy (16).

BFR has shown great promise in its ability to induce muscular hypertrophy and strength gains, although specific mechanisms have yet to be fully elucidated. BFR has also shown to be useful in a clinical rehabilitation settings, allowing patients to see greater effects from low load training when paired with BFR (17). Direct compression to the musculature has also shown to increase the amount of oxygen in the tissue, possibly due to the release of endothelium vasoactive factors (18). Research has shown that compressive pressure as low as 20mmHg applied directly to the calf musculature increased oxygenation of the tissue at rest in both seated

and standing positions (19, 20). Compression garments applying pressures around 20mmHg to the thigh has also shown to decrease muscle activation of the rectus femoris, but not affect muscle activation in the gastrocnemius or tibialis anterior (21). However, research on the effects of traditional BFR application on the performance and recovery of the distal limb musculature is very limited. It is assumed that the same physiological responses are seen throughout the whole limb. Variables such as muscle fiber type differences and lack of compression could change the way the distal limb responds to proximal limb BFR.

SPECIFIC AIMS

The specific aims of this study are as follows:

1) To determine if BFR application around the proximal thigh in healthy adults during exercise affects the amplitude of neuromuscular stimulation in the soleus and gastrocnemius, causing an increase in the recruitment of larger motor units compared to control (CON; exercise without BFR).
2) To determine if BFR application around the proximal thigh in healthy adults during exercise affects maximal force production and total work performed in the tibialis anterior and gastrocnemius compared to CON (exercise without BFR).
3) To determine if BFR application around the proximal thigh in healthy adults during exercise affects the rate of recovery in the tibialis anterior and gastrocnemius post-exercise compared to CON (exercise without BFR).

Based on similar research examining the effects of BFR training on the quadriceps, it was hypothesized that BFR would increase the amplitude of neuromuscular stimulation and cause recruitment of larger motor units at a lower load compared to CON. Furthermore, it was hypothesized that BFR would initially increase force output but ultimately decrease total work performed during the exercise protocol due to higher levels of fatigue compared to CON. Additionally, it was hypothesized that BFR would recover more slowly compared to CON post-exercise as a result of enhanced metabolic stress-induced fatigue. The results from this study will add to the knowledge of how BFR training affects distal musculature and better practitioner's decisions on when to use BFR in their training or rehabilitation settings.

LITERATURE REVIEW

Skeletal Muscle Anatomy and Physiology

Skeletal muscle is an organ that is made up of muscle fibers and connective tissue, rich in blood supply and nerve innervation (2). Individual, whole skeletal muscles are covered in a layer of fibrous connective tissue called the epimysium. The epimysium is contiguous with the origin and insertion tendons for each muscle and is made of collagen (2, 22). The tendons connect to a connective tissue called bone periosteum, which is made of layers of collagen fibers, mesenchymal progenitor cells, differentiated osteogenic progenitor cells, osteoblasts, and fibroblasts (23). The periosteum is connected to and encapsulates the bones. When the muscle contracts it transfers force through the tendon, to the bone, eliciting joint movement (2).

Encapsulated inside of the epimysium there are groups of muscle fibers, called fascicles, that are bundled together by the perimysium (2). The perimysium continues as the endomysium between the muscle fibers (24). Muscle fibers are long cylindrical cells that sometimes run the whole length of the muscle. These fibers are the individual cells of the skeletal muscle and are comprised of a fluid medium, called sarcoplasm, typical cellular organelles, and filamentous contractile proteins called myofibrils. The myofibrils contain interdigitated myofilaments; actin, known as the thin filament, and myosin, known as the thick filament. The myofibrils are composed of sarcomeres, which are the contractile unit of the cell. The sarcomeres are organized in a parallel series that runs throughout the entire myofibril. The structure of the sarcomere spans from Z-line to Z-line. Anchored to each Z-line are strands of actin filament wound with troponin

and tropomyosin; proteins that regulate muscle contraction (25). The M-line is in the center of the sarcomere, branching out from each side are strands of myosin filaments which are covered in branch-like globular heads able to bind and pull on the interdigitated actin filaments during contraction (25). The portion of the sarcomere that is made up of actin with no myosin overlap is called the I-band, these are located next to the Z-lines. The portion of the sarcomere where myosin spans on either side of the M-line is called the A-band. The portion of the sarcomere that is made up of lonely myosin is called the H-zone. Organization of A-bands and I-bands along the muscle fiber give the myofibril a striated look (25). During contraction, myosin pulls on actin causing the I-bands and H-zone to shrink and the Z-lines to move toward the M-line (25).

Muscular contraction starts when a motor neuron carries an action potential from the motor cortex in brain or spinal cord through thousands of branches to the muscle that it innervates releases acetylcholine (Ach), a neurotransmitter, into the neuromuscular junction (26). Ach then binds to nicotinic Ach receptors on the sarcolemma which causes a conformational change of the receptor, opening a pore into the cell (27). This allows sodium and potassium to move along their gradient and depolarize the cell. The new AP travels down and along the cell membrane and through T-tubules which traverse deep into the internal structures of the muscle fiber (28). This causes voltage gated channels in the sarcoplasmic reticulum (SR) to release calcium into the sarcoplasm.

Calcium binds to a subunit of troponin called troponin C which causes a conformational change, this pulls on the troponin subunit troponin T which is bound to tropomyosin, moving tropomyosin out of the way. With tropomyosin out of the way, the actin-myosin binding site is exposed (27). ATP binds to the globular myosin heads where it is then split by ATPase. This causes the myosin head to shift and grab the actin strand (28). With the release of an inorganic

phosphate molecule, myosin then pulls actin, shifting it toward the M-line of the sarcomere. ADP is then released from the myosin head and it releases the actin strand. ATP then binds to the myosin head and the cycle repeats to elicit a full muscular contraction (28).

Muscle Mechanics

Human skeletal muscle is heterogenous (29). Motor units, the functional unit of the motor system, are made of a single motor neuron and group muscle fibers of the same structural and functional properties. Motor units grouped together form muscles, with each containing different fiber type isoforms (29). The skeletal fiber isoforms have different physiological and morphological characteristics, particularly twitch time (2). The commonly identified fibers are Type I (slow-twitch), Type IIa (fast-twitch), and Type IIx (fast-twitch). Additionally, there is an observable difference in the fiber types' ability to demand and supply energy for contraction (2). Selective recruitment of the various fiber types allows for diverse functionality from fine motor skills to gross motor skills, as well as functions of strength and endurance (30).

Type I fibers have high capacity for aerobic energy supply, and a high capacity to utilize free fatty acids in the blood stream (2, 31). Type I fibers have high mitochondrial density and rely on oxidative metabolism for energy. The high yield of energy from oxidative metabolism allows type I fibers to specialize in long duration contractile activities (32). Type I fibers smaller in diameter and are associated with lower force output (2). Type I fibers are found in abundance in muscle groups that are used frequently for low intensity functions, such as in the calf musculature, which is active when standing, walking, and running (33).

Type IIa and IIx muscle fibers have low capacities for aerobic energy supply, lower mitochondrial density, and rely heavily on glycolytic metabolism (2). Type II fibers are relatively large in diameter and are associated with high force production. Type IIx fibers are

associated with the largest diameter and highest force output (2). Due to the reliance on glycolytic metabolism, type II fibers are fatigable, with type IIx fibers fatiguing the quickest. Endurance training can increase the oxidative capacity of type II fibers, but strength and power training decreases the oxidative capacity of type II fibers (32).

The physical and morphological differences affect the order in which the muscle fiber types and motor units that contain them are activated (2). In order to activate a motor unit the stimulus must be strong enough to cause a contraction, if the stimulus is strong enough then all of the fibers in the motor unit are activated. If the stimulus is insufficient, none of the fibers in the motor unit are activated (2). Motor units are recruited in order of size, from smallest to largest, from type I to type IIx (34). The larger motor units are activated when strong stimuli are applied, along with smaller motor units (34). As muscular fatigue progresses, larger motor units are activated to compensate for the fatigue (34).

Repetitive contraction of the muscle causes a decrease in calcium release from the sarcoplasmic reticulum. With less calcium able to bind to troponin, the muscles' ability to contract is limited as myosin is unable to bind to actin (35). Repetitive contraction also causes structural damage to the sarcolemma that results in a decreased ability to perform muscle contraction, resulting in fatigue (36). Elevated fatigue increases neural activation to recruit larger motor units so that contractions can be maintained (34).

Resistance Training

RT is known to cause muscular strength, size and power adaptations, as well changes to various other body systems such as endocrine and neurological adaptations (2). Increases in strength and power generally come from neuromuscular adaptations (3). Manipulating RT variables such as frequency, volume, and intensity helps to induce neuromuscular adaptations by

increasing the stimulus and causing more and larger motor units to become recruited, increasing force production (4). Loads greater than or equal to 70% of 1-Rep max (1RM) are typically required in order to induce muscular hypertrophy as well as gains in strength (5). Hypertrophy can be induced by stimulating and recruiting larger motor units, which are typically comprised of higher force producing, type II muscle fibers, which increases the mechanical tension on the whole tissue (2). RT increases muscular size, or CSA, by inducing metabolic stress and/or myotrauma (1).

Hypoxia and Metabolites

Hypoxia is an important driver of exercise-based adaptations due to its' ability to alter gene expression and various other factors that control oxygen homeostasis. Some of the main proteins that are up-regulated as a result of hypoxic signaling include HIF-1, GLUT4, iNOS, VEGF, and EPO, all of which are associated with processes of exercise-induced adaptation (37, 38).

Hypoxia also promotes the accumulation of metabolites such as lactate, some of which are upstream inducers of the mammalian target of rapamycin (mTOR) signaling pathway, leading to enhanced protein synthesis and muscular hypertrophy (10, 14). The increase of metabolites also results in cell swelling as a result of osmotic draw of water into the cell (1, 9, 10, 15). After completion of exercise the increase in swelling and accumulation of metabolites causes M1 macrophages to activate and infiltrate the tissue (15). M1 macrophages propagate the inflammatory response by causing further damage to the tissue and releasing proinflammatory cytokines (16). This causes M2 macrophages to invade and release anti-inflammatory cytokines. M2 macrophages play a major role in promoting growth and regeneration after tissue damage, thus, promoting hypertrophy (16).

Hypoxia causes the tissue to rely more heavily on anaerobic metabolism, such as fast glycolysis. The by-product of rapid fast glycolysis is lactate. Pooling of lactate causes a drop in regional local pH. The lower pH alters mitochondrial metabolism, this is caused by an increase in hydrogen ions within the inter membrane space of the mitochondria. Hypoxia drastically reduces the oxidative phosphorylation capacity of the mitochondria, therefore the hydrogen ions cannot be cleared (39).

Another metabolite associated with RT is inorganic phosphate (P_i). As muscles contract repeatedly and ATP is used, ADP and inorganic phosphate accumulate. This increase in ADP drives glycolysis, but the build-up of P_i can cause other reactions, such as a decrease in the amount of calcium that the SR can release for contraction, promoting fatigue (40).

Blood Flow Restriction

Blood flow restriction (BFR) training originally called "KAATSU" training was developed in Japan. BFR training is growing in popularity as an exercise training modality for both recreational and professional athletes alike, due to its ability to induce muscular hypertrophy at loads as low as 20% of 1RM (10). Several studies have demonstrated that low-load training with BFR induces gains in strength and muscular hypertrophy. BFR typically requires the use of inflatable cuffs placed on the proximal portion of a limb to occlude blood flow (10).

The goal of BFR is to reduce arterial blood flow and eliminate venous return, leading to venous pooling and swelling of the tissue. Cell swelling is able to prevent catabolism within the muscle, shifting the balance of protein metabolism towards anabolism (41). Increasing fluid content within cells also prevents the down-regulation of mTOR signaling associated with cellular dehydration (42). As volume increases in the cell, mechanical tension on the cell

membrane activates integrin-associated volume osmosensors, causing activation of the c-JUN NH2-terminal kinase (JNK) signaling pathway, which has been shown to modulate growth within cells (43).

Reducing arterial blood flow to the tissue creates an oxygen-poor environment and induces hypoxia (10). Along with the pooling of blood and other fluid, metabolites, such as lactate, pool within the muscle, inducing metabolic greater stress (44). Reactive oxygen species (ROS), more specifically, superoxide anion, is produced enzymatically within contracting muscle fibers via xanthine oxidase and NADPH oxidase (NOX2) (39). With the use of BFR, mitochondrial production of ROS is minimal due to the hypoxic environment and the limited oxidative capabilities of the mitochondria. Therefore, xanthine oxidase and NOX2 are the main sources of ROS produced with BFR (45, 46). ROS are also accumulated at a higher rate due to BFR-induced hypoxia, due to impaired clearance (44).

The accumulation of metabolites and ROS increase the rate of fatigue within the muscle. BFR has shown to increase the rate of fatigue within working muscle when compared to non-BFR exercise performance and fatigue (47). The increased fatigue due to BFR increases muscle activation and EMG amplitude due to recruitment of greater motor unit recruitment and force production at lower intensities (13, 18, 48, 49). When BFR-induced fatigue was significant enough to induce central fatigue, muscle activation and EMG amplitude is decreased (21). BFR has shown to increase the rate of fatigue to an extent that decreases recovery rate (47).

BFR training has been studied almost exclusively regarding its effects on the quadriceps. For example, Lauber et al. studied muscle activation, muscle swelling and muscle force in the vastus lateralis and found that BFR induced muscle swelling, increased muscle activation, and decreased muscle torque, all to a significant degree, after three sets of 90s isometric knee

extension with 50% limb occlusion pressure (50). Husmann et al. conducted a study on the effects of BFR on force output and recovery in the quadriceps and found that up to two-mins post-exercise, the BFR group had significant decreases in force output and rates of recovery (47). A study conducted by Sousa et al. found that there was an increase in isometric force in the quadriceps following their BFR intervention which consisted of four sets of knee extensions at 30% of 1RM with 80% of limb occlusion pressure (51). To date, little research has been conducted on the effects of BFR in relation to the lower limb musculature such as the soleus, gastrocnemius, and tibialis anterior. It is plausible that without direct compression to the tissue, we may see a more enhanced hypoxic effect and greater rate of fatigue, without decreased muscle activation. Thus, the purpose of this study is to determine if proximal limb BFR-induced hypoxia without direct compression has an effect on muscular performance and recovery of the distal limb musculature, such as the gastrocnemius, soleus, and tibialis anterior.

METHODS

Experimental Design

Participants completed three study visits, on separate days, with the two experimental exercise sessions assigned in a counter-balanced order (Figure 1). Session one (familiarization visit) and session two were separated by a minimum of 24 h and sessions two and three were separated by a minimum of 72 h. Participants were asked not to perform any strenuous exercise, defined as a volume of exercise that would induce delayed-onset muscle soreness, within 72 h of each study session and to refrain from consuming more than 40 mg of caffeine within 5 h of the study visits. Additionally, participants were asked to come to the laboratory having refrained from eating or drinking anything other than water 3 h prior to their arrival for each visit.

Participants

Using data from Bowman et al. (52), an *a priori* power analysis was performed (G*Power, version 3.1.9.6, Universität Kiel, Germany) following the recommendations of Beck (2013) (53). Using the pre-post isometric torque values, an effect size of 0.5 was determined based on the data from Bowman et al (52). Using a repeated-measures ANOVA (within-between interaction) statistical test, an effect size of 0.5, alpha (α) level of 0.05, and desired power ($1-\beta$) of 0.80, a sample size of 12 participants would be required to detect an effect, if one exists.

Inclusion criteria for the study required all participants to be free from known cardiovascular, metabolic and renal disease, and signs or symptoms suggestive of these diseases

as outlined in the ACSM pre-participation health screening process (54), participants had to be non-smokers, and recreationally resistance trained (i.e., having engaged in RT for at least 3 sessions per week over the last 6 months) to ensure that the exercise protocol did not cause any significant fatigue that could act as a confounding variable. During the pre-testing screening, potential participants were asked to list any current medications and address their RT experience prior to involvement in the research study.

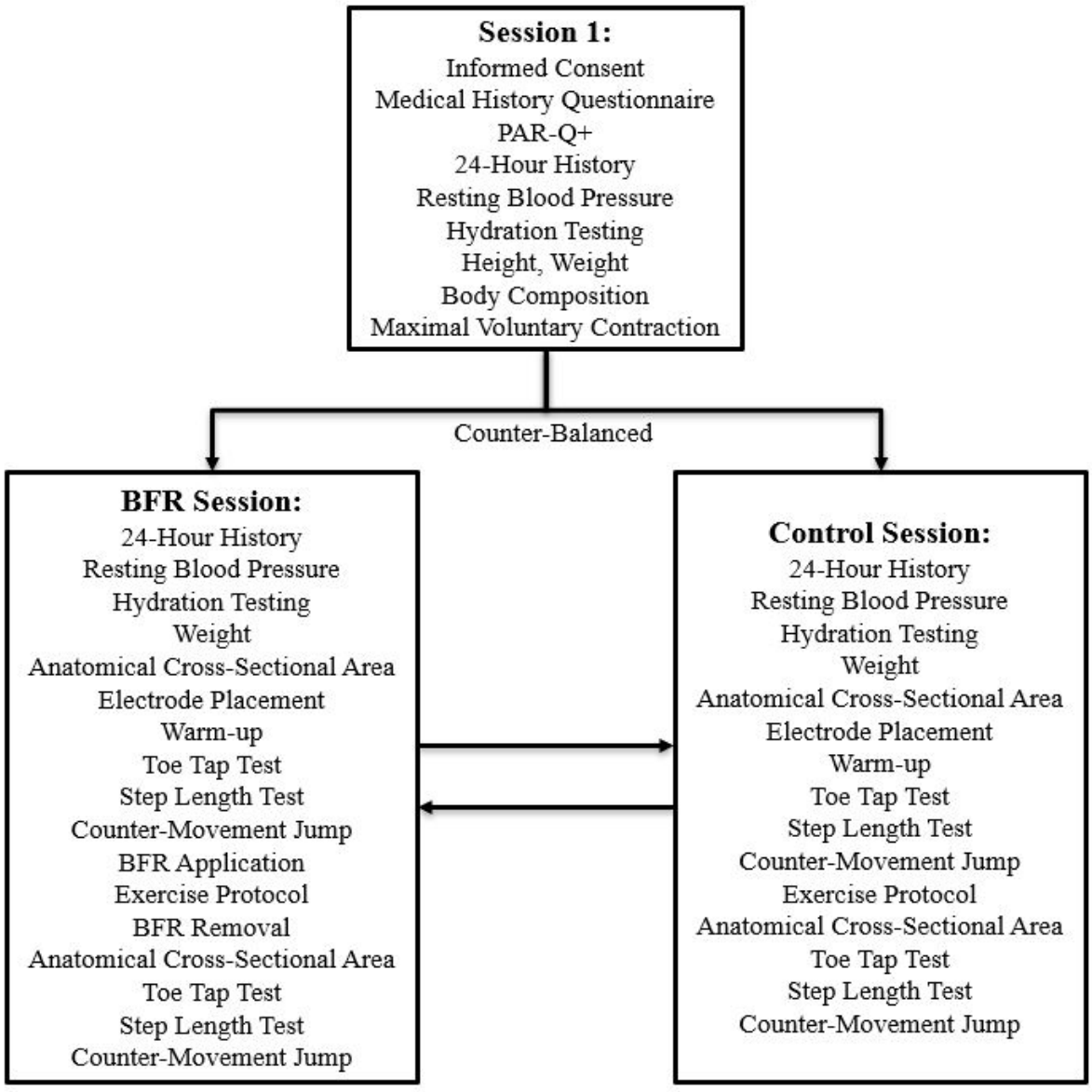

Figure 1. Experimental design.

Outcomes of Interest

The order of physiological and performance tests completed pre- and post-experimental sessions were determined during pilot testing. The tests were ordered from most to least time sensitive in terms of the duration of their effect post-exercise. Although all the outcome measures were time sensitive, the anatomical cross-sectional area (CSA) and toe tap test had the shortest effect duration post-exercise and therefore were tested first. The step length test involves relatively short testing procedures, so it was performed before the counter-movement jump (which involves a longer testing process).

Anatomical Cross-Sectional Area

Anatomical CSA of the dominant-leg gastrocnemius was determined using an ultrasound imaging device (Phillips Ultrasound, iU22, Bothwell, WA). Participants laid prone on a medical procedure chair, the location of the widest part of the calf was measured using the crease of the popliteal fossa for reference. A line was drawn using a straight edge and skin-marker to ensure the same straight path was taken for both pre- and post-measure. The distance from the crease was recorded for accuracy between trials. The CSA measurement was taken with a panoramic view starting from the medial portion of the tibia and measured along the marked line to the lateral portion of the tibia. The CSA was measured in cm^2 using ImageJ software (Version 1.53, U.S. National Institutes of Health, Bethesda, Maryland, USA).

Toe Tap Test

Following the warm up, participants completed a toe tap test using a device constructed by the research team (Figure 2). This test is sensitive to changes in fatigue of the tibialis anterior and the device was modeled after other testing equipment that demonstrated good validity and reliability (55). Participants were instructed to sit in a chair with their knees flexed at

approximately 90 degrees and to rest their feet on the toe tap device. The participant tapped their toes as many times as possible within 10 s. The number of toe taps over the 10 s time period was recorded using a slow-motion camera and counted after the session.

Figure 2. Toe Tap Test Device.

Average Step Length Test

Following the toe tap test, participants completed an average step length test. The methods for this test were adopted from the NL1000 pedometer (New Lifestyles Inc., Lee's Summit, Missouri, USA) user manual and was originally used to program the pedometer step length (56). In the current study, this test was used to evaluate fatigue of the calf musculature using step length reduction as an indicator of fatigue. The average step length test was measured by having the participant take 10 steps along a measuring tape (adhered along the floor) at their preferred walking speed. They started at the "0" mark and their stopping point was recorded. This distance was then divided by 10 to determine average step length.

Counter-movement Jump

Following the step length test, participants completed the counter-movement jump testing. This test is sensitive to changes in fatigue within the calf musculature. Counter-movement jump was measured using portable force plates (Kistler 9286ba 10kn, Switzerland). Participants performed three jumps with one min of rest between each jump. Participants were instructed to stand at the center of the force plates and jump as high as possible with their hands

on their hips throughout the jump. The counter-movement jump metric analyzed was flight height, which was averaged from the three separate jumps. These procedures were performed both pre- and post-exercise to assess post-exercise fatigue differences.

BFR System and Operation

The Delfi PTS II is an FDA approved Blood Flow Restriction/tourniquet system that is commonly used in physical therapy, athletic training, and in research. This BFR system uses an inflatable tourniquet, much like a blood pressure (BP) cuff, to induce vascular occlusion in a limb. The system first determines the pressure needed for 100% arterial occlusion (limb occlusion pressure) and uses this information to calculate user-specific occlusion pressure based on the desired percentage to be used during exercise, relative to their personalized limb occlusion pressure. This specific system has a sensor that regulates pressure during muscular movement to maintain a constant pressure, rather than allow a spike in pressure during exercise. For the BFR session, the cuff was inflated to 80% of the participants' limb occlusion pressure, which was maintained for the entirety of the exercise protocol.

Electromyography

Electrodes were placed on the dominant-leg gastrocnemius medialis (GM) and soleus in accordance to surface electromyography for the non-invasive assessment of muscles (SENIAM) recommendations (57). GM electrodes were placed on the most prominent bulge of the muscle during plantarflexion. Soleus electrodes were placed at 2/3 of the line between the medial condyles of the femur to the medial malleolus. The lateral malleolus served as the reference electrode location. The electrode sites were shaved and cleaned with alcohol wipes prior to placing electrodes. Electrodes were spaced 20 mm apart in parallel to muscle fiber direction. EMG signals were collected with an electronic signal acquisition system (Biopac MP150

Physiograph), which was connected to a laptop computer. Before beginning the ankle plantar/dorsi-flexion protocol, the participant was asked to briefly perform weight-free plantar/dorsi-flexion to ensure signal detection. EMG signals were recorded during each set and repetition during the ankle plantar/dorsi-flexion to measure muscle activation and fatigue during the exercise protocol. Alterations to mean amplitude was analyzed to assess neuromuscular activation and fatigue.

Perceived Exertion

Rating of perceived exertion (RPE) has been shown to be a valid and reliable subjective marker of internal training load (58). In the current study, RPE was assessed using the Borg CR-10 scale (59), where 0 =no effort and 10 = complete muscular fatigue. Ten seconds after the completion of each set of the exercise protocol, participants were asked to rate the level of effort, on a scale of 0 to 10, needed to complete the set.

Study Visits

Session 1

Prior to the first visit, participants were emailed a copy of the informed consent form for them to review 24 h prior to arriving at the laboratory. Upon arriving to the laboratory, participants were formally consented. If the participant had no questions or concerns, the consent form was signed prior to engaging in the research protocol. Once consented, participants then completed a medical history questionnaire, physical activity readiness questionnaire (2020, PAR-Q+) (60), and 24-h history form. The purpose of the paperwork was to screen the potential subjects for inclusion, and those that were deemed eligible to participate, to confirm they were healthy and physically fit enough to perform the proposed procedures of the study.

After completion of the informed consent and paperwork, resting BP was measured after 5 min of seated rest using the BPM-100 BpTRU automated monitor (BpTRU Medical Devices; Coquitlam, BC, Canada). A total of 6 readings, taken 1-min apart in the dominant arm, were used to determine average BP calculated from 3 readings that agreed within 5 mmHg for both systolic and diastolic BP. Participants were excluded if they reported taking antihypertensive medications or if their average systolic or diastolic BP was greater than ≥130 or ≥80 mmHg, respectively. Participants excluded due to high BP (i.e., hypertension according to the ACC/AHA 2017 BP classification scheme) (61) would have their average resting BP confirmed on a second occasion 24 h later.

Next, participants were walked through all testing procedures including hydration testing, body composition assessment, toe-tap test, average step length test, counter-movement jump, the maximum voluntary muscular contraction test, and the resistance exercise protocol.

To ensure participant hydration, participants were asked to urinate into an 8-fluid ounce cup, filling it with at least 100 mL of urine. After completion, the investigator measured the specific gravity of the urine to identify hydration status by extracting a small amount of the urine with a disposable pipette and placing a few droplets on an analog refractometer. Urine specific gravity values less than 1.020 was required for the participant to be considered adequately hydrated. Participants whose values were greater than 1.020 were given ½ pint of water, then waited 30 min before re-evaluation.

After hydration determination, baseline data for basic anthropometric measurements (height, weight, and body composition using skinfold calipers) were obtained. Subjects had height taken using a stadiometer (SECA 67310, SECA©, Chino, CA) and weight was measured on a digital scale (Tanita BWB-800, Tanita©, Arlington Heights, IL). Participants remained

clothed with shoes off for the weight measurement. Participants were asked to wear similar clothing for each visit to help maintain measurement consistency. Next, 7-site Jackson-Pollock skinfold measures were taken to assess body composition. Skinfold thicknesses were measured from each subject with calibrated skinfold calipers. Skinfolds were measured from 7 sites on the right side of the body on every participant as follows: 1) a diagonal fold on the chest half way between the nipple and front of the shoulder for men, or 1/3 of the distance between the nipple and shoulder for women; 2) a vertical fold on the triceps halfway between the back of the shoulder joint and elbow, with the arm held freely to this side of the body; 3) a diagonal fold on the shoulder blade; 4) a vertical fold directly on the side the body in between the arm pit and hip bone; 5) a vertical fold on the abdomen 2 cm to the right of the belly button; 6) a diagonal fold 2 cm above the hip bone; 7) a vertical fold on the front portion of the thigh halfway between the hip bone and knee cap. Each site was measured in rotating order and the measurement was taken at least two times. The average value for each site, calculated from two measures that agreed within 2 mm, was utilized for body density calculation using the sum of seven skin folds formula (54).

Participants then laid supine on the isokinetic dynamometer (Humac Norm, CSMiSolutions, Stoughton, MA). The dynamometer was set so that the foot angle was 90 degrees from the anterior tibial surface. Ankle plantarflexion and dorsiflexion was performed at maximal effort for 5 seconds for the maximum voluntary contraction (MVC). MVC was taken 3 times, with 1 min of rest between trials for plantarflexion and then the isokinetic dynamometer was adjusted to approximately 110 degrees from the anterior tibial surface and the MVC protocol was repeated with maximal isometric ankle dorsiflexion.

Control and BFR Sessions

Participants completed the second and third sessions in a counter-balanced order such that they performed either the CON or BFR session at visit two. Upon arrival, hydration testing took place in the same manner as the first session. BP and body mass were recorded after hydration testing. If the participant met the hydration requirements to participate, then pre-exercise muscle CSA was taken via ultrasound imaging. Participants completed a warm-up consisting of 5 min of self-selected speed on a cycle ergometer which was followed by the evaluation of average stride length, toe tap test, and counter-movement jump, in that order. The individual testing procedures are described below.

Exercise Protocol: Participants laid supine on a Humac Norm isokinetic dynamometer and were tested for maximum degree of ankle plantarflexion and dorsiflexion with no resistance on the dynamometer, each a single time. The degree angle for each movement is recorded and set as the total degree of movement parameter on the Humac Norm software. To ensure participant safety and prevent hyperextension of the ankle, physical clamps (as part of the isokinetic dynamometer) were placed at the point of maximal plantarflexion and dorsiflexion to prevent further movement of the dynamometer arm. Next, electrodes were placed on the dominant-leg gastrocnemius medialis and soleus. The dominant limb was determined by asking the participant which leg they use to kick a ball. The electrode sites were shaved and cleaned with alcohol wipes prior to placing electrodes. Electrodes were spaced 20 mm apart in parallel to muscle fiber direction. Participants were then asked to perform 3 sets of 15 repetitions (90 seconds of rest between each set) of ankle plantarflexions and dorsiflexions performed at their voluntary maximum contraction load, either with or without BFR, depending on the experimental condition. The dynamometer speed was set at 60 degrees per second for

plantarflexion and 500 degrees per second for dorsiflexion. Both limbs were exercised, with the non-dominant leg being exercised first, to ensure similar fatigue between legs. During the BFR session, the BFR cuff was first applied as proximal as possible on the thigh of the dominant limb, but distal to the gluteal fold, while the participant laid supine on the isokinetic dynamometer with their legs bent, similar to the body position required during the exercise protocol. Participants were asked to lay still for a 5min stabilization period. After the stabilization period, the PTS II system was used to determine 100% arterial occlusion (i.e., 100% limb occlusion pressure [LOP]). The BFR cuff was then inflated to 80% of the pre-determined LOP and remained inflated for the remainder of the exercise protocol described above. Exercise was initiated approximately 30 s after cuff inflation and the cuff remained inflated for 30 s after the completion of the third set. The cuff was inflated for approximately 6 min in total, depending on how long it took the participant to complete each set.

Statistical Analysis

All analyses were performed using SPSS software (Version 25, IBM Corp., New York, NY, USA). Data are summarized as mean ± standard error (M ± SD), unless stated otherwise. Two-way repeated measures analysis of variance (ANOVA) was used to determine significant differences between time, condition, and the time×condition interaction for each outcome variable of interest. When appropriate, *post-hoc* pairwise comparisons incorporating the Bonferroni correction were used to determine where the significant differences occurred. Table 4, included in the Appendix, specifies the Alpha levels were modified alpha level for each study outcome after incorporating for the Bonferroni correction factor (Table 4). Sphericity was calculated, in any case that sphericity was violated, the Greenhouse-Geisser correction was applied. For any cases that sphericity was not calculated due to insufficient levels with the

repeated factor, the Greenhouse-Geisser and Huynh-Feldt sphericity estimates were used and were close to 1.00 ($\hat{\varepsilon}$=1.00; $\tilde{\varepsilon}$= 1.00, respectively). Each analysis included a sample size of 20 participants unless otherwise stated. An alpha level of $p<0.05$ was used to determine statistical significance.

RESULTS

Twenty-three young, healthy adults were recruited for the study. One participant declined to participate, and two participants did not finish the study due to equipment issues during data collection (Figure 2). Of the 20 participants that completed the study, one was removed from the average force and total work performed analyses due to a system malfunction that resulted in the loss of data. Participant descriptives are summarized in Table 1.

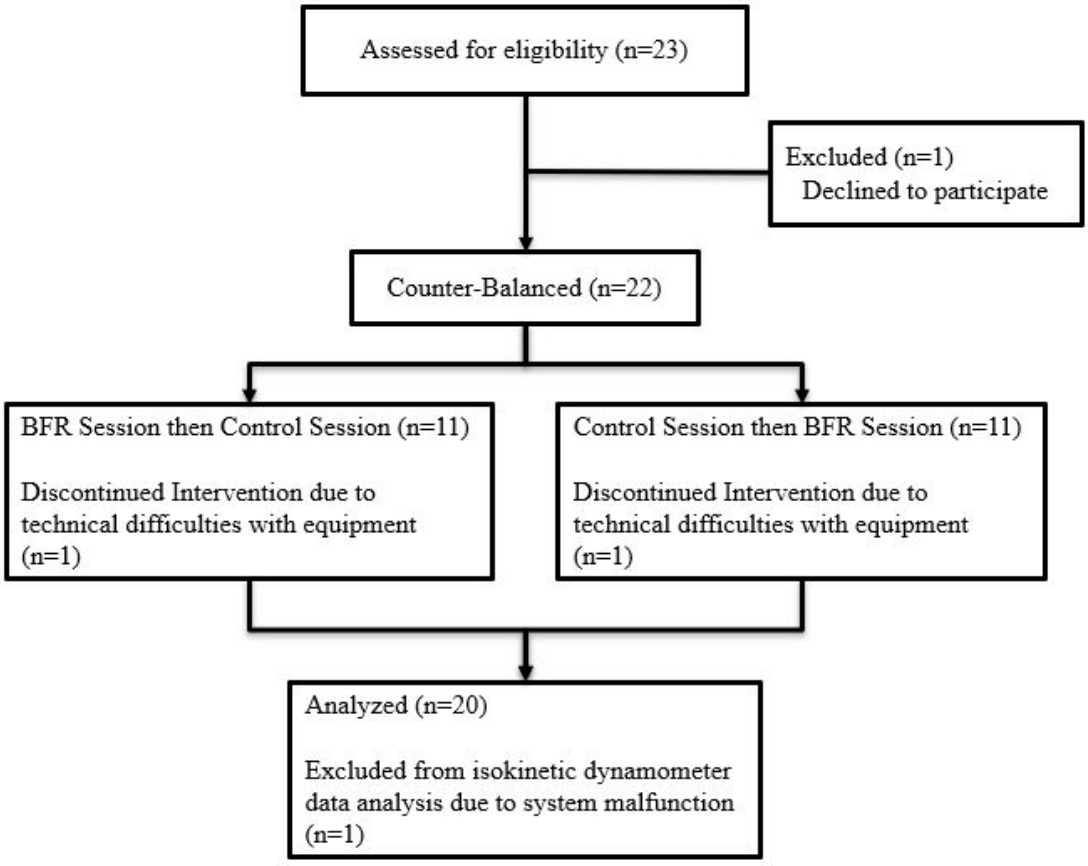

Figure 3. Participant Flow Chart

Table 1. Sample Descriptives (*M*±*SD*).

Variables	All (*N* = 20)	Female (*N* = 12)	Male (*N* = 8)
Age (y)	23.0 ± 3.8	22.4 ± 2.4	24.8 ± 5.2
Height (cm)	174.1 ± 9.0	169.5 ± 5.6	181.0 ± 8.2
Mass (kg)	77.9 ± 13.0	70.5 ± 9.4	89.0 ± 11.1
BMI (kg/m^2)	25.6 ± 3.5	24.6 ± 3.6	27.1 ± 2.6
BF (%)	23.8 ± 8.6	27.9 ± 7.2	17.0 ± 6.8
LOP (mmHg)	172.3 ± 26.5	172.9 ± 30.5	171.3 ± 21.0
80% LOP (mmHg)	137.5 ± 26.5	137.8 ± 24.4	137.0 ± 16.7

BMI, Body mass index. BF (%), Body fat percent. LOP, Limb occlusion pressure.

Anatomical Cross-Sectional Area:

The main effects of time ($F(1,19)=13.281$) and condition ($F(1,19)=7.916$) on the CSA of the gastrocnemius were large and significant (Table 2 and Figure 4) These main effects were qualified by a large and significant time×condition interaction ($F(1,19)=8.524$). Post-hoc tests revealed that the post-BFR CSA was significantly greater than pre-BFR (M_{diff} = 1.126±0.262cm^2), pre-CON (M_{diff} = 1.098±0.259cm^2), and post-CON (M_{diff} = 1.121±0.357cm^2) (all $p<0.05$).

Sphericity estimates were not calculated for the repeated factor (time), because it only had two levels. However, the Greenhouse-Geisser and Huynh-Feldt sphericity estimates for the rater factor were close to 1.00 ($\hat{\varepsilon}$=1.00; $\tilde{\varepsilon}$= 1.00, respectively). As a result, no correction was made to the degrees of freedom used to evaluate the significance of the F ratio for the repeated factor. The Levene test indicated no significant violation of the homogeneity of variance assumption for either time point. The assumption of normality was checked using the Shapiro-Wilk test of normality, no violations were found (all $p>0.05$).

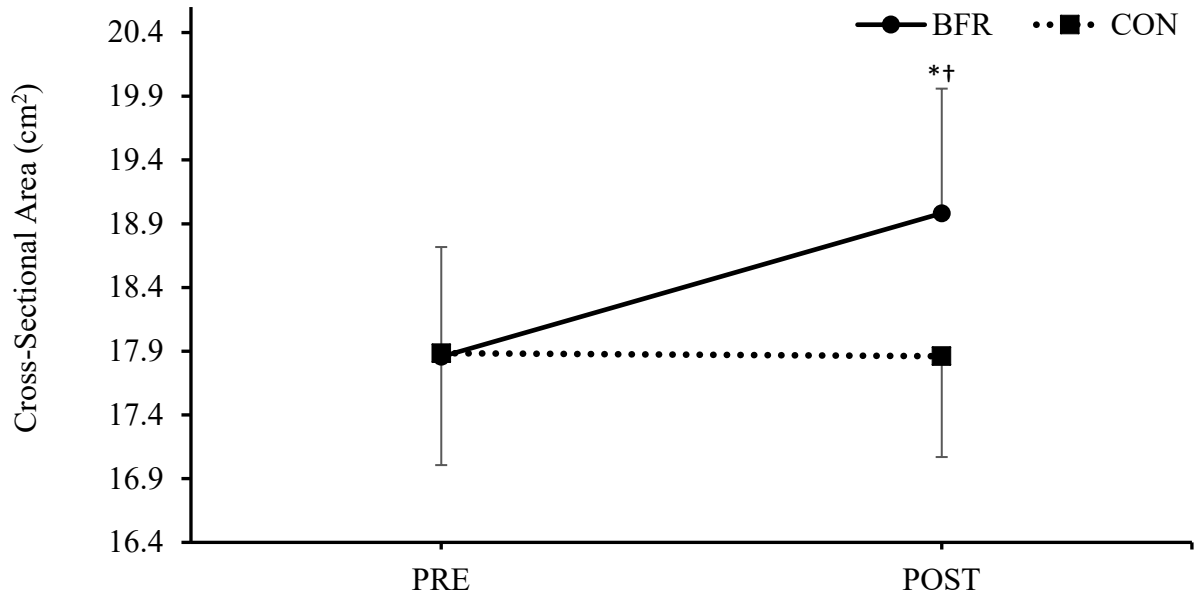

Figure 4. Anatomical cross-sectional area pre and post-exercise. * = Significantly different than set 1 ($p<0.05$). † = Significantly different between conditions ($p<0.05$).

Toe Tap:

The main effects of time ($F(1,19)=51.98$) and condition ($F(1,19)=53.238$) on toe tap test performance were large and significant (Table 2 and Figure 5). These main effects were qualified by a large and significant time×condition interaction ($F(1, 19) = 77.493$). Post-hoc tests revealed that the number of toe taps performed at post-BFR was lower than pre-BFR (M_{diff} = -19.20±2.09), pre-CON (M_{diff} = -18.40±2.06), and post-CON (M_{diff} = -13.70±1.61) (all $p<0.05$).

Sphericity estimates were not calculated for the repeated factor (time), because it only had two levels. However, the Greenhouse-Geisser and Huynh-Feldt sphericity estimates for the rater factor were close to 1.00 ($\hat{\varepsilon}$=1.00; $\tilde{\varepsilon}$= 1.00, respectively). As a result, no correction was made to the degrees of freedom used to evaluate the significance of the F-ratio for the repeated factor. The Levene test indicated no significant violation of the homogeneity of variance assumption for either time point. The assumption of normality was checked using the Shapiro-

Wilk test of normality, no violations were found (all p>0.05).

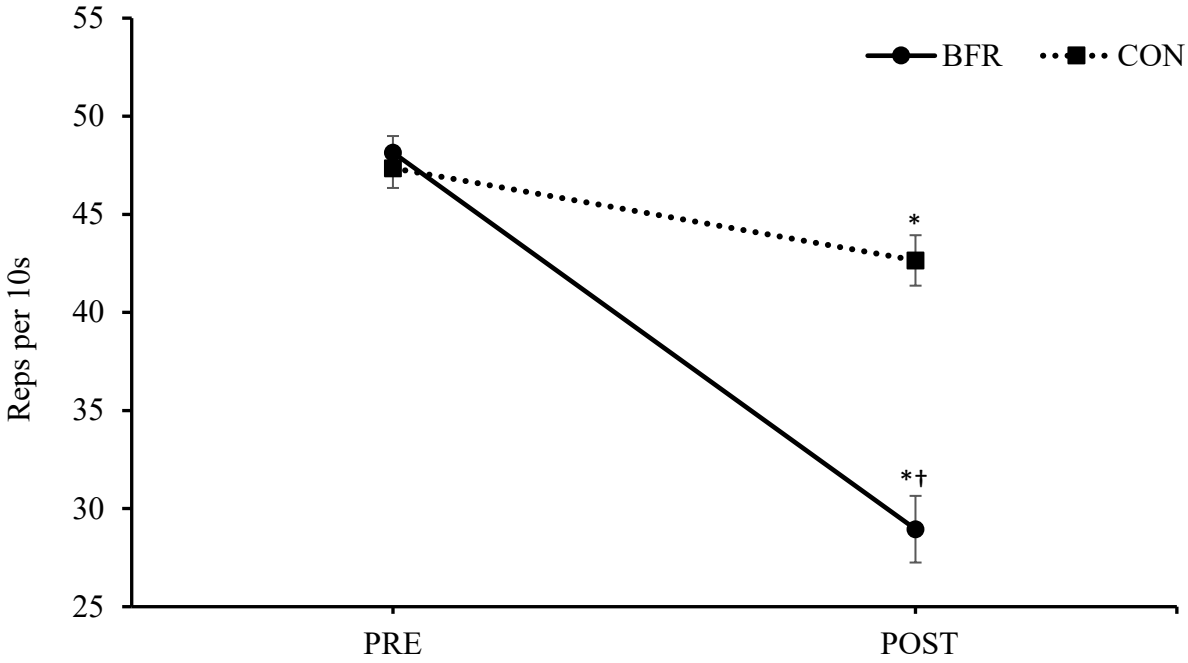

Figure 5. Number of toe taps pre and post exercise. * = Significantly different than set 1 (p<0.05). † = Significantly different between conditions (p<0.05).

Table 2. Physiological and performance outcomes measured pre- and post-exercise conditions (*N*=20).

				Time effect		Condition effect		Interaction	
Variable	**Condition**	**Pre-Exercise**	**Post-Exercise**	***P***	**$\eta 2_{partial}$**	***P***	**$\eta 2_{partial}$**	***P***	**$\eta 2_{partial}$**
ACSA (cm^2)	BFR	17.86 ± 0.86	18.98 ± 0.98*†	0.002	0.411	0.011	0.294	0.009	0.310
	CON	17.88 ± 0.88*	17.86 ± 0.79						
Toe Taps (number)	BFR	48.15 ± 0.84*	28.95 ± 1.70*†	<0.001	0.732	<0.001	0.737	<0.001	0.803
	CON	47.35 ± 1.01*	42.65 ± 1.29*						
Step Length (m)	BFR	2.32 ± 0.07	2.23 ± 0.07*	0.018	0.262	0.712	0.007	0.020	0.252
	CON	2.29 ± 0.07	2.27 ± 0.07						
CMJ (m)	BFR	0.32 ± 0.02	0.29 ± 0.02*	<0.001	0.070	0.665	0.010	0.103	0.134
	CON	0.31 ± 0.02	0.30 ± 0.02*						

BFR, Blood flow restriction. CON, Control. CMJ, Countermovement jump. ACSA, Anatomical cross-sectional area. $\eta 2_{partial}$, Partial eta effect size. * significantly different than pre-exercise, $p<0.05$. † significantly different between conditions, $p<0.05$.

Step Length:

The main effect of time ($F(1,19)=6.757$) on step length was large and significant (Table 2 and Figure 6). There was a large and significant interaction effect ($F(1,19)=6.417$) but no significant condition effect ($F(1,19)= 0.14$). Post-hoc tests revealed that the post-BFR step length was significantly lower than pre-BFR ($M_{diff}= 0.87\pm0.03$ m), but was not significantly different from pre-CON ($M_{diff}= 0.63\pm0.03$ m), or post-CON ($M_{diff}= 0.42\pm0.03$ m) (all $p<0.05$).

Sphericity estimates were not calculated for the repeated factor (time), because it only had two levels. However, the Greenhouse-Geisser and Huynh-Feldt sphericity estimates for the rater factor were close to 1.00 ($\hat{\varepsilon}=1.00$; $\tilde{\varepsilon}= 1.00$, respectively). As a result, no correction was made to the degrees of freedom used to evaluate the significance of the F ratio for the repeated factor. The Levene test indicated no significant violation of the homogeneity of variance assumption for either time point. The assumption of normality was checked using the Shapiro-Wilk test of normality, no violations were found (all $p>0.05$).

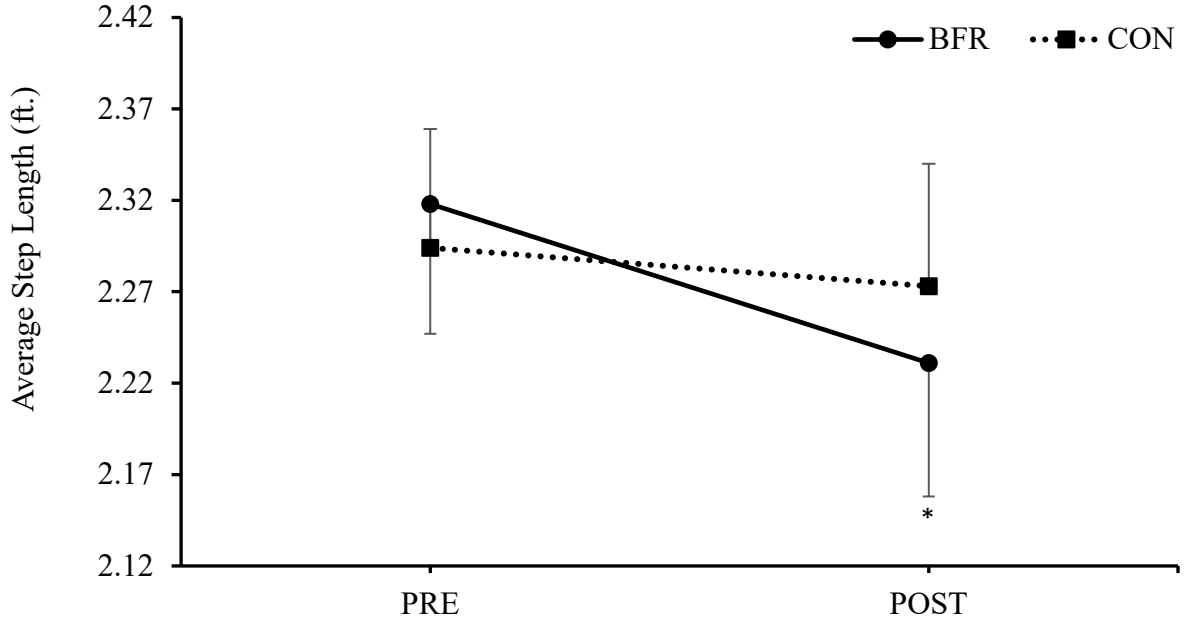

Figure 6. Average step length pre and post-exercise. * = Significantly different than set 1 ($p<0.05$).

Jump Flight Height:

The main effect of time ($F(1,19)=44.444$) on jump height was large and significant (Table 2 and Figure 7) The jump height post-exercise was lower than pre-exercise (M_{diff} = -0.020±0.003 m). There was no main effect of condition ($F(1,19)= 0.193$) or time×condition interaction effect ($F(1,19)=2.930$) on jump height.

Sphericity estimates were not calculated for the repeated factor (time), because it only had two levels. However, the Greenhouse-Geisser and Huynh-Feldt sphericity estimates for the rater factor were close to 1.00 ($\hat{\varepsilon}$=1.00; $\tilde{\varepsilon}$= 1.00, respectively). As a result, no correction was made to the degrees of freedom used to evaluate the significance of the F ratio for the repeated factor. The Levene test indicated no significant violation of the homogeneity of variance assumption for either time point. The assumption of normality was checked using the Shapiro-Wilk test of normality, no violations were found (all p>0.05).

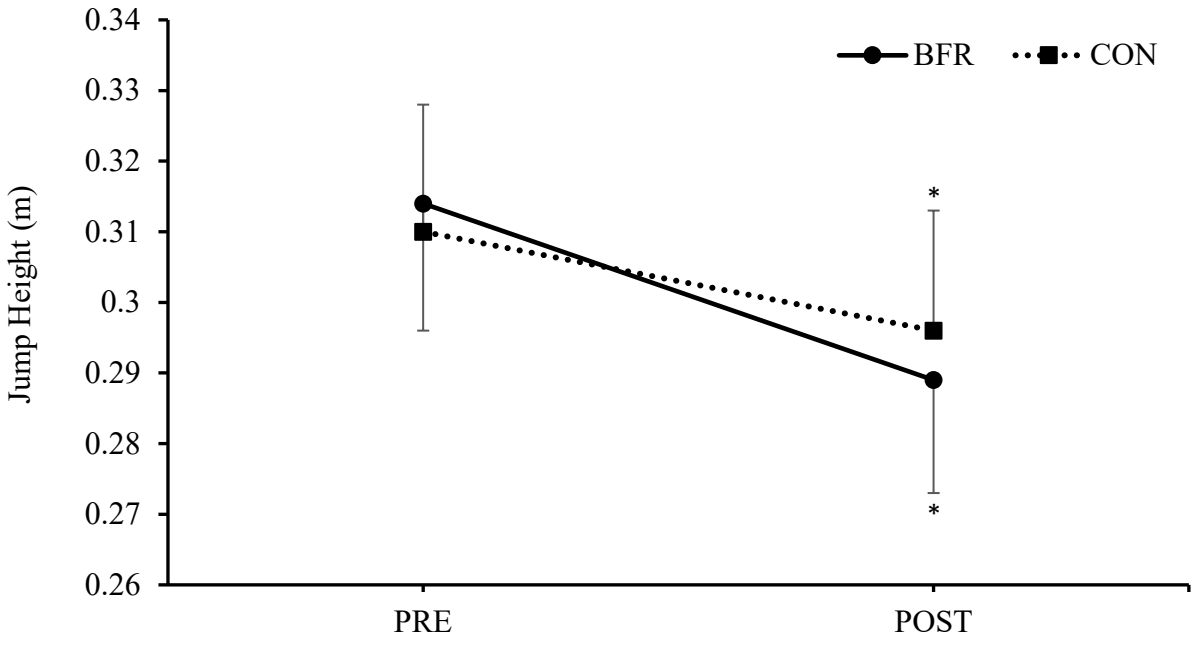

Figure 7. Average jump height pre and post exercise. * = Significantly different than set 1 (p< 0.05).

RPE:

The main effects of time (F(1.47,48)=130.586) and condition (F(1,19)=20.162) had a large and significant effect on RPE values (Table 3 and Figure 8). These main effects were qualified by a large and significant time×condition interaction (F(2, 38) = 11.984). Post-hoc tests revealed that there was an increase in RPE values within both conditions from set 1 to set 2, and from sets 2 to 3, with higher RPE values for sets 2 and 3 in the BFR compared to CON session (M_{diff} = 1.750±0.422 and M_{diff}=2.150±0.393, respectively) (all $p<0.05$).

Mauchly's test indicated the assumption of sphericity was violated (p=0.018) for the repeated factor (time). Therefore, the Greenhouse-Geisser correction was applied. The assumption of normality was checked using the Shapiro-Wilk test of normality. The Shapiro-Wilk test indicated that the assumption of normality was violated for the third set of BFR.

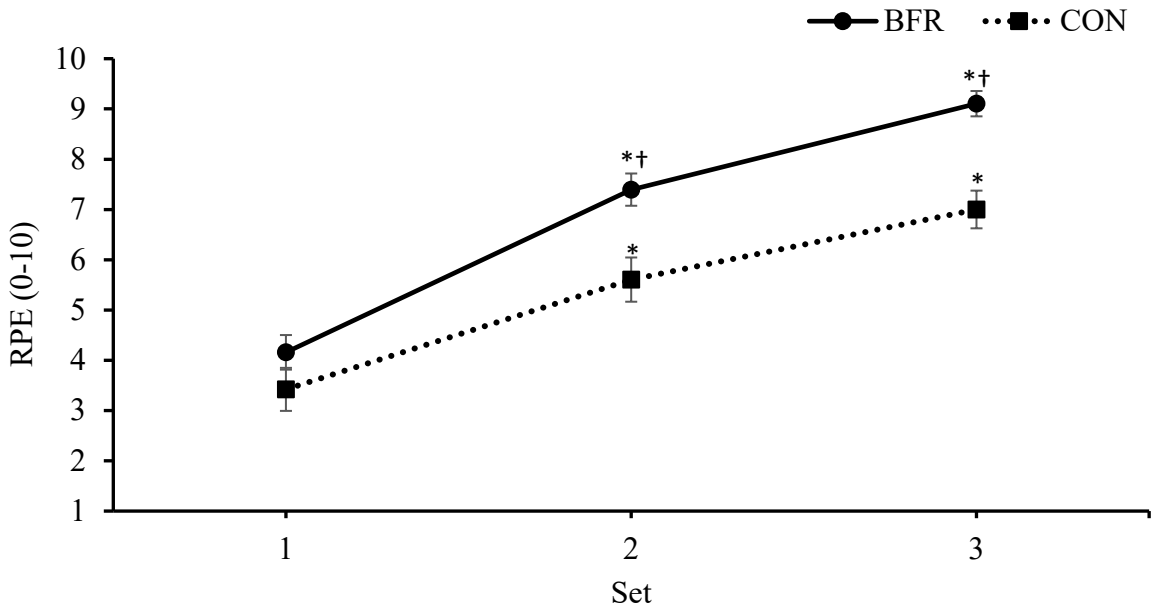

Figure 8. Average RPE between sets. * = Significantly different than set 1 ($p<0.05$). † = Significantly different between conditions ($p<0.05$).

Electromyography:

Gastrocnemius:

The main effects of time (F(1.508,38)=24.530) and condition (F(1,19)=10.204, p=0.005) on EMG amplitude in the gastrocnemius were large and significant (Table 3 and Figure 9). Set 2 EMG activation was lower than set 1 (M_{diff} = -0.034±0.006 mV) and set 3 was lower than set 2 (M_{diff} = 0.025±0.008 mV). However the interaction between time and condition (F(2,38)=2.633) was not significant.

Mauchly's test indicated the assumption of sphericity was violated (p=0.029) for the repeated factor (time). Therefore, the Greenhouse-Geisser correction was applied. The assumption of normality was checked using the Shapiro-Wilk test of normality. The Shapiro-Wilk test indicated that the assumption of normality was violated for sets two and three of CON (both p>0.05).

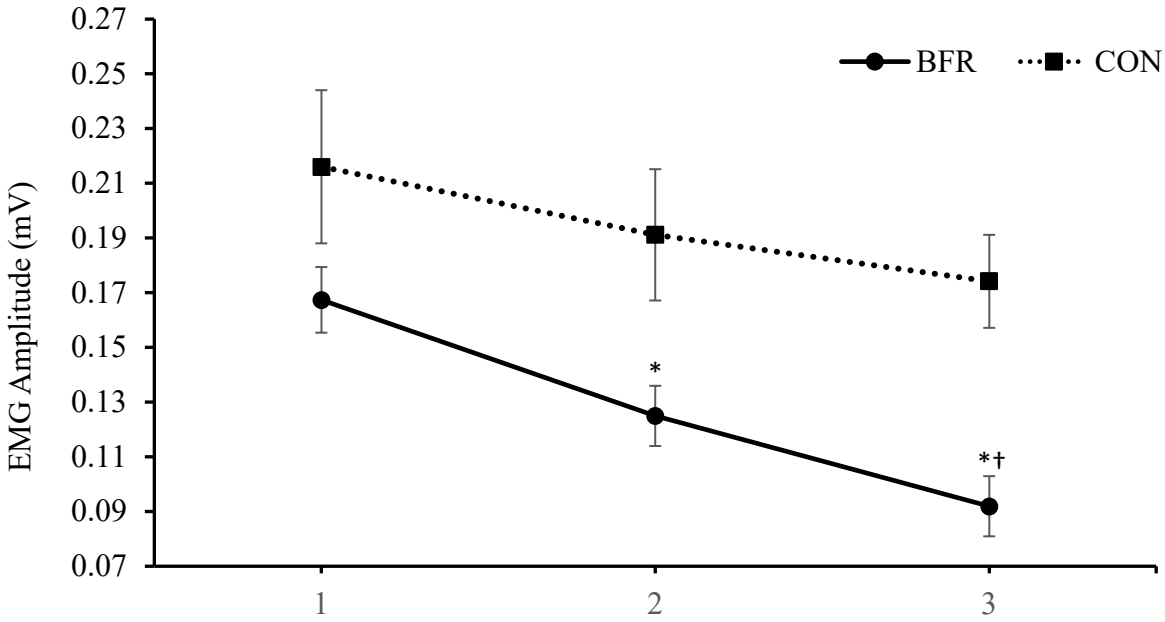

Figure 9. Gastrocnemius EMG amplitude per set. * = Significantly different than set 1 (p<0.05). † = Significantly different between conditions (p<0.05).

Soleus:

The main effect of time (F(1.358,38)=19.914) on the EMG amplitude in the soleus was large and significant (Table 3 and Figure 10). The interaction between time and condition (F(1.528,38)=11.907) was also large and significant while the main effect of condition was not significant. Post-hoc tests revealed that there was a decrease in EMG amplitude within the soleus during BFR from set 1 to set 2 (M_{diff} = 0.034±0.006 mV) and from set 2 to set 3 (M_{diff}= 0.037±0.007 mV) (all $p<0.05$).

Mauchly's test indicated the assumption of sphericity was violated ($p=0.003$) for the repeated factor (time). Therefore, the Greenhouse-Geisser correction was applied. The assumption of normality was checked using the Shapiro-Wilk test of normality. The Shapiro-Wilk test indicated that the assumption of normality was violated for all sets except the first and third sets of CON.

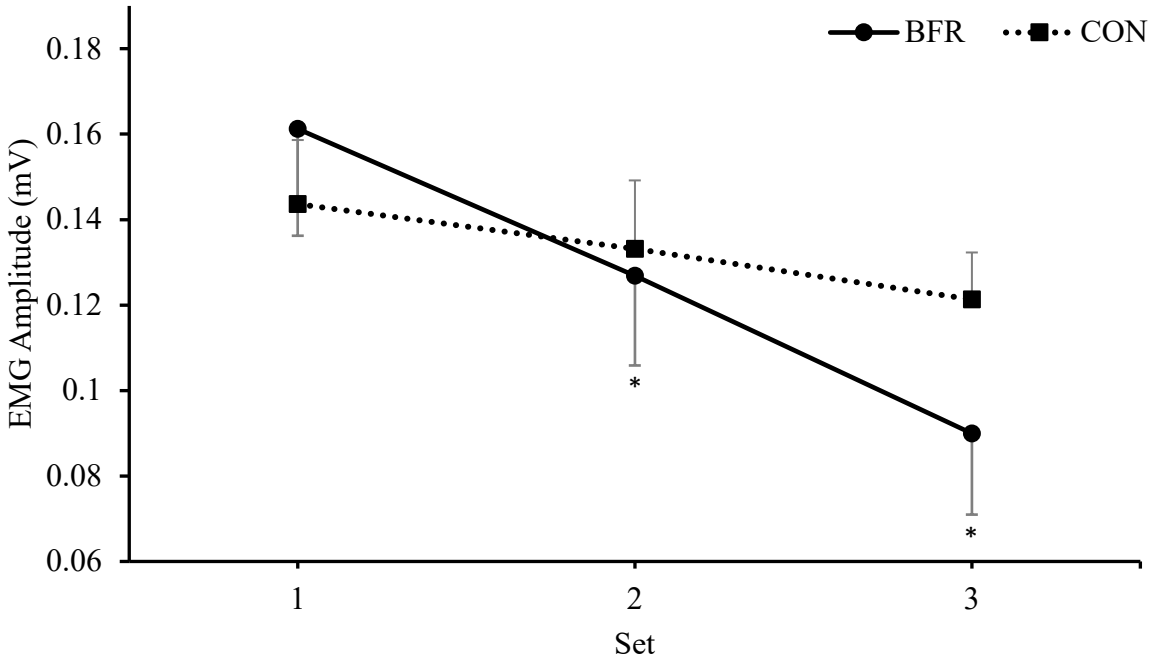

Figure 10. Soleus EMG amplitude per set. * = Significantly different than set 1 ($p<0.05$).

Average Force Production:

Plantarflexion:

The main effects of time ($F(1.294,36)=27.037$) and condition ($F(1,18)=33.566$) on average force during plantarflexion were large and significant (Table 3 and Figure 11). These main effects were qualified by a large and significant time×condition interaction ($F(2,36)=28.332$). Post-hoc tests revealed that there was a decrease in force during the BFR condition between sets 1 and 2 (M_{diff} = -8.579±1.319 ft/lbs) and between sets 2 and 3 (M_{diff} = -9.263±1.548 ft/lbs). The average force of set 2 during the BFR condition was lower than the average force during the second set of the CON condition (M_{diff} = -10.684±2.110 ft/lbs), and the average force of set 3 during the BFR condition was lower than the average force during the third set of the CON condition (M_{diff} = -17.947±2.454 ft/lbs) (all $p<0.05$).

Mauchly's test indicated the assumption of sphericity was violated ($p=0.001$) for the repeated factor (time). Therefore, the Greenhouse-Geisser correction was applied. The assumption of normality was checked using the Shapiro-Wilk test of normality. The Shapiro-Wilk test indicated that the assumption of normality, no violations were found (all $p>0.05$).

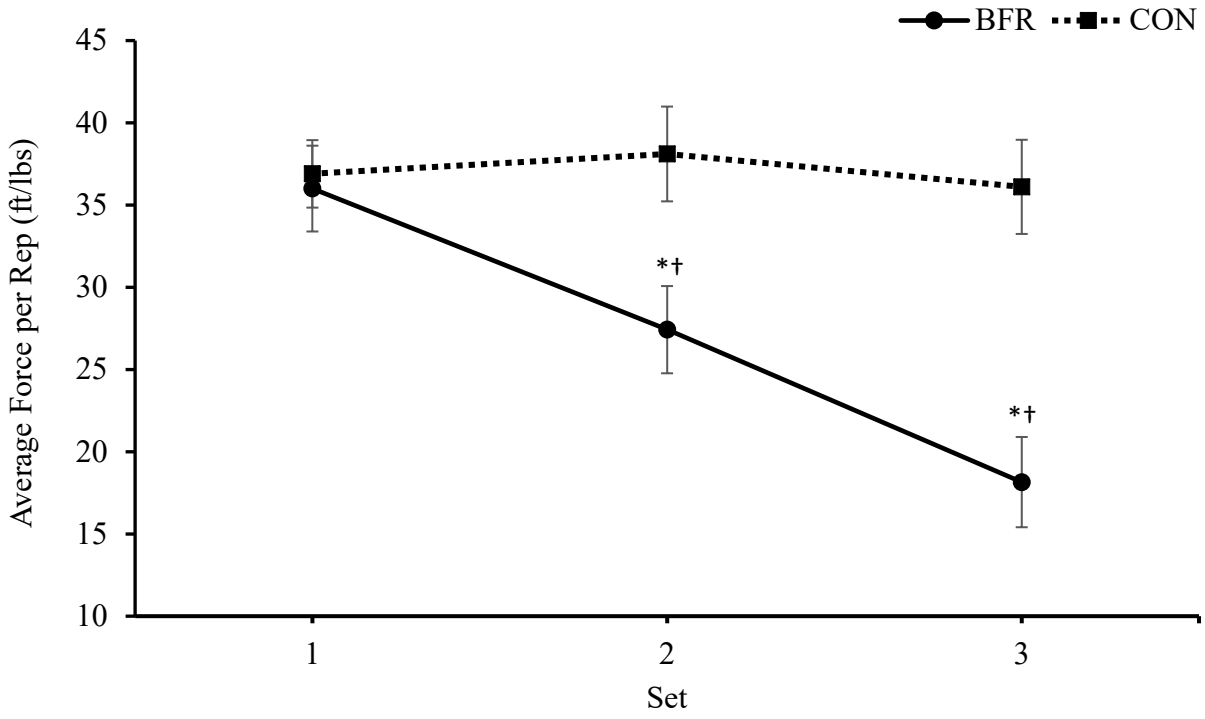

Figure 11. Plantarflexion average force per repetition per set. * = Significantly different than set 1 ($p<0.05$). † = Significantly different between conditions ($p<0.05$).

Dorsiflexion:

The main effects of time ($F(1.294,36)=27.037$) on average force during dorsiflexion was large and significant (Table 3 and Figure 12). The interaction between time and condition ($F(2,36)=28.332$) also had a large and significant effect, while the main effect of condition ($F(1,18)=33.566$) had no significant effect. Post-hoc tests revealed that there was a decrease in force during both conditions between sets 1 and 2 and between sets 2 and 3. The average force during set 3 was lower in the BFR compared to CON session (M_{diff} = -2.947±0.993 ft/lbs) ($p<0.05$).

Mauchly's test indicated the assumption of sphericity was violated ($p<0.001$) for the repeated factor (time). Therefore, the Greenhouse-Geisser correction was applied. The assumption of normality was checked using the Shapiro-Wilk test of normality. The Shapiro-Wilk test indicated that the assumption of normality was violated for all sets except the first set of BFR.

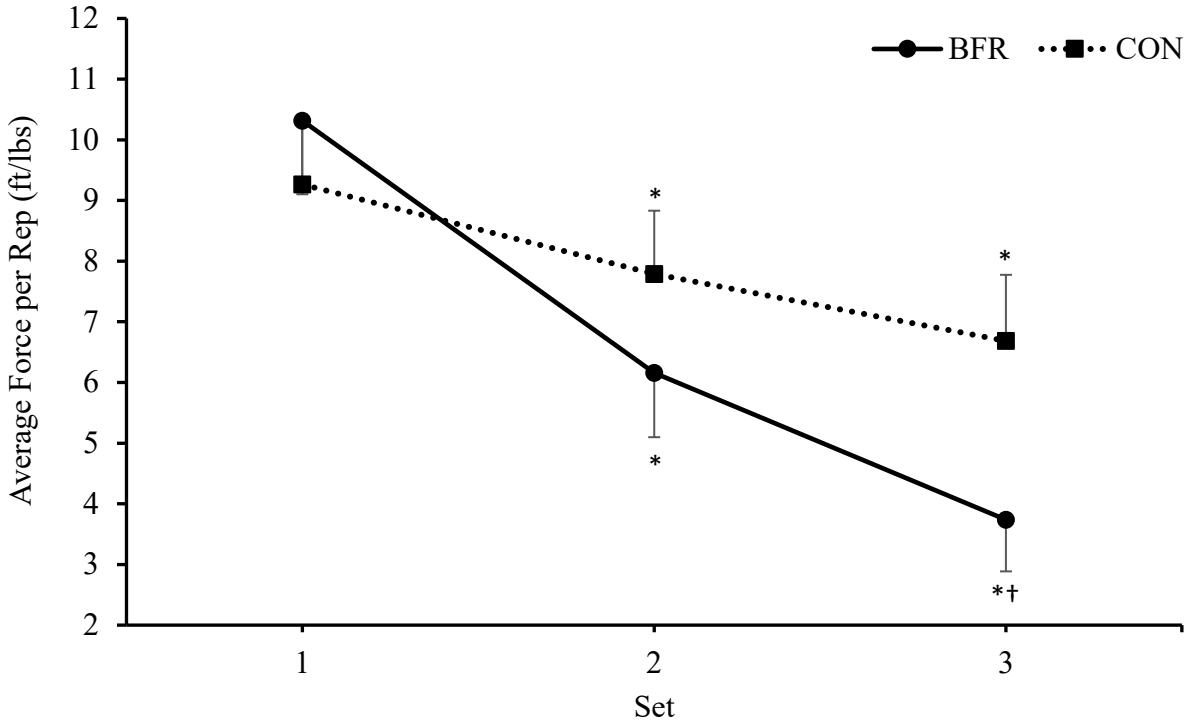

Figure 12. Dorsiflexion average force per rep per set. * = Significantly different than set 1 ($p<0.05$). † = Significantly different between conditions ($p<0.05$).

Total Work:

Plantarflexion:

The main effects of time ($F(1.246,36)=27.013$) and condition ($F(1,18)=23.343$) on total work performed during plantarflexion were large and significant (Table 3 and Figure 13). These main effects were qualified by a large and significant time×condition interaction ($F(2,36)=24.512$). Post-hoc tests revealed that there was a decrease in force during the BFR

condition between sets 1 and 2 (M_{diff} = -108.526±18.755 watts) and between sets 2 and 3 (M_{diff} = -124.632±21.535 watts). The total work of set 2 during the BFR condition was lower than the total work during the second set of the CON condition (M_{diff} = -114.579±27.228 watts), and the total work of set 3 during the BFR condition was lower than the total work during the third set of the CON condition (M_{diff} = -212.158±32.633 watts) (all $p<0.05$).

Mauchly's test indicated the assumption of sphericity was violated ($p<0.001$) for the repeated factor (time). Therefore, the Greenhouse-Geisser correction was applied. The assumption of normality was checked using the Shapiro-Wilk test of normality. No violations were found.

Dorsiflexion:

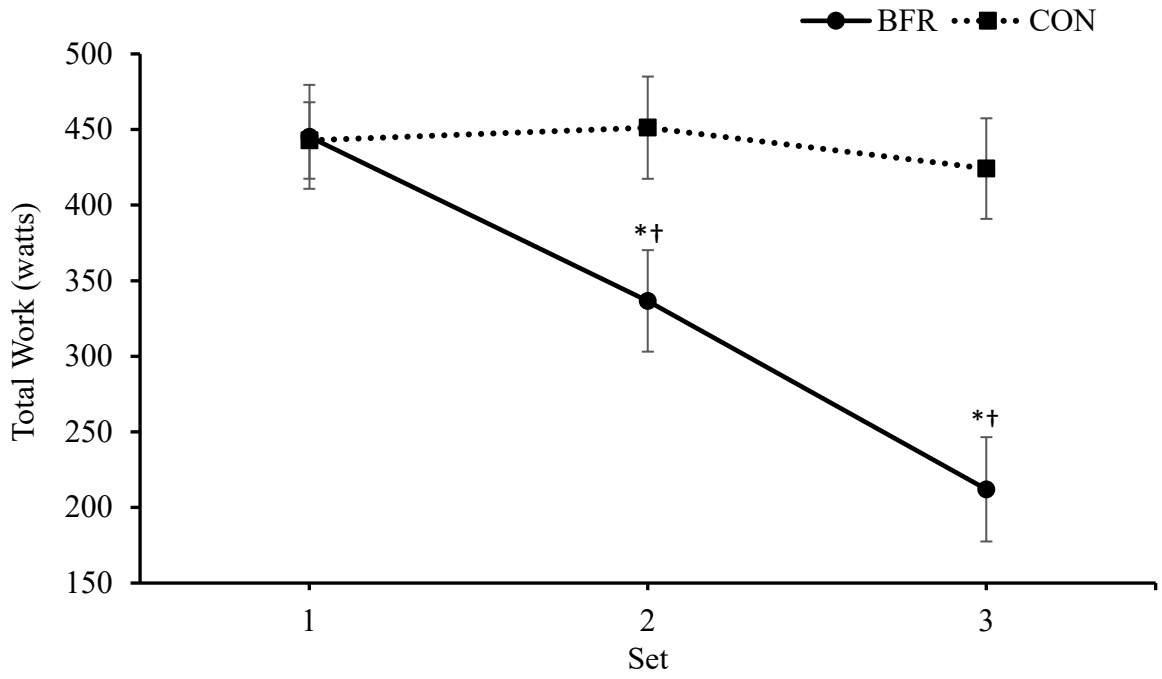

Figure 13. Plantarflexion total work performed per set. * = Significantly different than set 1 ($p<0.05$). † = Significantly different between conditions ($p<0.05$).

The main effects of time (F(1.235,36)=92.484) on total work performed during dorsiflexion was large and significant (Table 3 and Figure 14). The interaction between time and condition (F(1.429,36)=58.202) also had a large and significant effect, while the main effect of condition (F(1,18)=2.329) had no significant effect. Post-hoc tests revealed that there was a decrease in total work during both conditions between sets 1 and 2 and between sets 2 and 3. The average force of set 3 during the BFR condition was lower than the total work performed during the third set of the CON condition (M_{diff} = -16.526±4.732 watts) ($p<0.05$).

Mauchly's test indicated the assumption of sphericity was violated ($p<0.001$) for the repeated factor (time). Therefore, the Greenhouse-Geisser correction was applied. The assumption of normality was checked using the Shapiro-Wilk test of normality. All assumptions of normality were met except during set three of BFR ($p<0.05$).

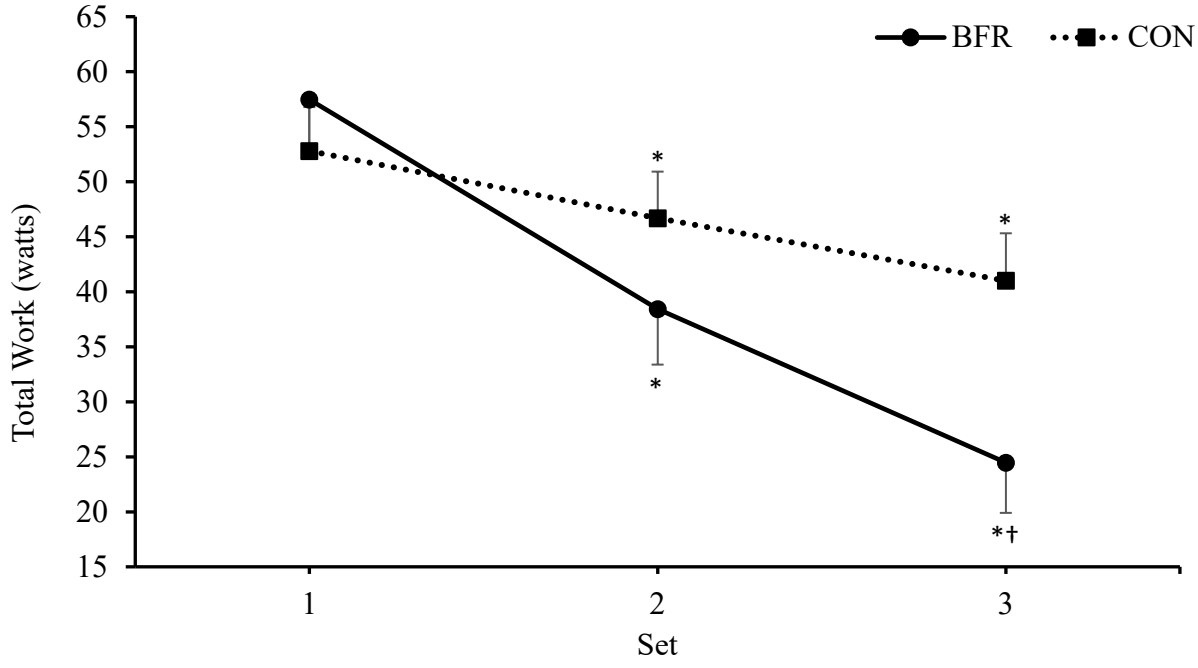

Figure 14. Dorsiflexion total work performed per set. * = Significantly different from set 1 ($p<0.05$). † = Significantly different between conditions ($p<0.05$).

Table 3. Physiological and performance outcomes measured during exercise conditions.

Variable	Condition	Set 1	Set 2	Set 3	Time effect		Condition effect		Interaction	
					P	$\eta 2_{partial}$	*P*	$\eta 2_{partial}$	*P*	$\eta 2_{partial}$
RPE (0-10)	BFR	4.15±0.33	7.33±.031*†	9.05±0.25*†	<0.00	0.873	<0.001	0.515	<0.001	0.387
	CON	3.45±0.41	5.58±.042*	6.90±.037*						
EMG – G (mV)	BFR	0.17±0.01	0.13±0.01*	0.09±0.01*†	<0.001	0.564	0.005	0.349	0.085	0.122
	CON	0.22±0.03	0.19±0.02	0.17±0.02						
EMG – S (mV)	BFR	0.16±0.03	0.13±0.02*	0.09±0.02*	<0.001	0.627	0.768	0.005	0.002	0.496
	CON	0.14±0.02	0.13±0.02	0.12±0.01						
Force/rep – PF (ft/lbs)	BFR	35.56±3.72	26.72±2.70*†	17.22±2.72*†	<0.001	0.600	<0.001	0.651	<0.001	0.612
	CON	36.56±2.14	37.78±2.96	35.83±3.01						
Force/rep – DF (ft/lbs)	BFR	9.94±1.22	5.72±1.02*	3.22±0.72*†	<0.001	0.836	0.481	0.030	<0.001	0.614
	CON	9.22±1.04	7.67±1.09*	6.56±1.14*						
Total work – PF (W)	BFR	445.2±34.4	336.6±33.6*†	212.0±34.5*†	<0.001	.0600	<0.001	0.565	<0.001	0.588
	CON	442.7±25.3	451.2±33.8	424.2±33.3						
Total work – DF (W)	BFR	55.6±4.9	36.2±4.8*	21.8±3.9*†	<0.001	0.837	0.768	0.005	<0.001	0.764
	CON	52.4±4.2	45.9±4.4*	40.3±4.5*						

BFR, Blood flow restriction. CON, Control. DF, Dorsiflexion. EMG, Electromyography. Force/rep, Average force production per repetition. G, Gastrocnemius. $\eta 2_{partial}$, Partial eta effect size. PF, Plantar flexion. RPE, Rating of perceived exertion. S, Soleus. W, Watts.

* significantly different than Set 1, $p<0.05$. † significantly different between conditions, $p<0.05$.

DISCUSSION

The purpose of this study was to determine if BFR had an effect on performance and recovery of the calf musculature which was distal to the placement of the BFR cuff. The main findings of this study indicate that BFR significantly affects performance and recovery of the calf musculature. The BFR condition caused an increase in anatomical CSA indicating that cell swelling occurred following exercise. BFR caused a significant reduction in EMG amplitude in the soleus and gastrocnemius compared to CON. The tibialis anterior fatigue and recovery was significantly greater in the BFR group as evidenced by the reduction in average force per rep, total work performed, and the total reduction in toe taps performed post-exercise compared to pre-exercise measures. The BFR group also saw a significant decrease in average step length post-exercise compare to pre-exercise measures. Along with enhanced fatigue, the BFR group saw significantly higher RPE values during the exercise.

In the current study, BFR significantly reduced EMG amplitude, which is contrary to the hypothesis derived from BFR studies performed in the quadriceps at 80% of LOP conducted by Fatela et al. and further supported by Loenneke et al. at lower pressures (48, 62). The EMG amplitude during the BFR condition of the current study decreased over time, which could be the result of rapid onset of fatigue. This was similar to the findings by Hsu et al. where direct compression to the quadriceps using compression garments was applied during 40-min treadmill running trials at 75% VO_2max and muscle activation was measured in the rectus femoris, tibialis anterior and gastrocnemius. Hsu et al. found that muscle activation was decreased in all of the

muscles measured (21). BFR did also reduce the average force production and total work performed over the course of the exercise protocol, the CONcondition saw no change in force output or total work performed. These findings are contrary to a previous report by Sousa et al. that suggests BFR increases torque production during maximal isometric knee extensions (51). This could be explained by the differences in muscle fiber type distribution between the calf musculature and the quadricep musculature. The gastrocnemius and soleus are predominantly type-I oxidative muscle fiber types, whereas the quadriceps have a higher distribution of type-II glycolytic fiber types, indicating that the calf musculature is more dependent on oxidative metabolism (33). The lack of oxygen from BFR could have induced fatigue to a greater degree than what was expected and what is observed during isometric exercise in the quadriceps.

BFR also acutely increased the CSA of the gastrocnemius, whereas CON did not. This is evidence that the mechanism of osmotic draw of fluid in the muscle resultant from the accumulation of metabolites from BFR is still happening in the distal musculature when the cuff is placed proximally. A study conducted by Biazon et al. demonstrated that there was an increase in CSA after knee extensions in a CON group using 3 sets of 10 repetitions at 80% of 1-RM as the stimulus, a BFR group coupled with high load exercise using 3 sets of 10 repetitions at 80% of 1-RM, and a BFR group coupled with low load exercise using 3 sets of 20 repetitions at 20% of 1-RM (63). The fiber type differences between the calf and thigh musculature would likely play a role in the rate of metabolite accumulation, as type I fibers rely more heavily on oxidative metabolism. This could explain why we see a significant difference in osmotic draw within the calf musculature with BFR, since metabolites accumulate in a hypoxic environment, whereas they may not accumulate as quickly in oxidative fiber types when oxygen is available.

BFR also decreased recovery rate within the calf musculature, including the tibialis anterior compared to CON. This indicated that the level of fatigue from the exercise with BFR was significant enough that the post-exercise measures still displayed signs of fatigue. Participants' stride length was reduced after BFR, indicating that the propulsive forces of the calf musculature were decreased after the BFR exercise protocol, while this effect was not observed without BFR. The participants' ability to activate their tibialis anterior in a toe tap test was also significantly decreased during the BFR condition, whereas we saw little decrease in the CON condition, indicating that the speed of recovery was slower after the BFR exercise condition. However, changes in jump height between conditions was not significantly different after exercise for either condition. This is supported by a study conducted by Husmann et al. where it was determined that BFR induced fatigue quickly during exercise and this increased fatigue remained after exercise, but 2-min post-exercise, the effects of BFR on recovery metrics were diminished (47). The jump height test during the current study was the last outcome measure tested and was tested past the 2-min post exercise mark. Thus, it is plausible that this extended delay in the testing procedure allowed for additional recovery, preventing an observable change.

The rating of perceived exertion was higher during the BFR trial, although RPE increased with CON, the increase in RPE during BFR was significantly higher. This contradicts a study conducted by Lixandrão et al. where it was found that RPE increased more in CON (64). This difference can be explained by differences in exercise protocols. The current study had volume matched exercise of 3 sets of 15 reps for both conditions, while the study by Lixandrão et al. had three training conditions, a high load non-BFR which conducted 4 sets to failure with 80% 1RM, a low load non-BFR which conducted 4 sets to failure with 30% 1RM, and the BFR condition which conducted 4 sets of 15 reps at 30% 1RM. The CON groups exercised to failure whereas

the BFR group did not. Another study by Santos et al. found that RPE values are higher when training to failure, suggesting that results from the Lixandrão et al. study may not accurately reflect the RPE associated with BFR utility (65).

Limitations

Although all efforts were made to maintain consistency, timing of post exercise outcome measures may not have all been measured at the same time point after the end of exercise. It is plausible that fatigue in the calf after this exercise protocol may have been reduced within 2 min post exercise. The counter-movement jump test was conducted last, which in some cases may have placed the measurement after the 2-min post-exercise mark. Thus, discrepancies in timing of measurement could have affected data outcomes. Another limitation was the sample size, although large enough to meet power requirements, it was not large enough to complete a sex comparison to determine if the effects seen are gender dependent. The current study also used a population of young healthy adults that were physically active, therefore, the findings may not be generalizable to an older or injured population. Another limitation was the violations of assumptions during the statistical analysis. Although there were some violations of the normality assumption, ANOVA is robust to violations of normality.

CONCLUSION

Overall BFR induced fatigue, causing significant reductions in force production, neuromuscular activation, and recovery rate. Cell swelling was observed in the BFR condition whereas no significant change in anatomical CSA was overserved in the CON condition. In some outcomes, BFR elicited a major decline in performance where no change was seen in the CON condition. In the current study, BFR placed proximally on the limb has shown to induce fatigue in distal musculature. The high rate of fatigue along with the evidence of cell swelling suggests that BFR training could cause similar hypertrophic responses observed in the more proximal limb musculature such as the quadriceps, but further research should be done to verify. Future research projects should investigate the differences in response to BFR between different fiber types, and also delineate response differences between cuff placements; proximal or distal.

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APPENDIX

Table 4. Modified α for post-hoc Bonferroni correction

Test	Modified α
CSA	0.0125
Toe Tap	0.0125
Step Length	0.0125
Jump Height	0.0125
RPE	0.0083
EMG-G	0.0083
EMG-S	0.0083
Force/rep-PF	0.0083
Force/rep-DF	0.0083
Total work-PF	0.0083
Total work-DF	0.0083

CSA, Cross-sectional area. DF, Dorsiflexion. EMG, Electromyography. Force/rep, Average force production per repetition. G, Gastrocnemius. PF, Plantar flexion. RPE, Rating of perceived exertion. S, Soleus.

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

November 9, 2020

Keith Saffold
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 20-015-ME "Effects of Proximal Limb Blood Flow Restriction Training on Distal Limb Performance and Recovery"

Mr. Saffold:

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 November 4, 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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THE UNIVERSITY OF ALABAMA®
Office of the Vice President for
Research & Economic Development
Office for Research Compliance

April 1, 2021

Keith Saffold
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 20-015-ME-A "Effects of Proximal Limb Blood Flow Restriction Training on Distal Limb Performance and Recovery"

Mr. Saffold:

The University of Alabama Medical Institutional Review Board has reviewed the revision to your previously approved full board protocol. The board has approved the minor change in your protocol.

Please remember that your approval will expire on November 4, 2021.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants.

Good luck with your research.

Sincerely,

[redacted]

Director & Research Compliance Officer

[redacted]

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