Acute cellular and molecular responses and chronic adaptations to low-load blood flow restriction and high-load resistance exercise in trained individuals

Running head: Molecular responses to blood flow-restricted exercise

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ABSTRACT

Blood flow restriction (BFR) with low-load resistance exercise (RE) is often used as a surrogate to traditional high-load RE to stimulate muscular adaptations, such as hypertrophy and strength. However, it is not clear whether such adaptations are achieved through similar cellular and molecular processes. We compared changes in muscle function, morphology and signaling pathways between these differing training protocols. Twenty-one males and females (mean ± SD: 24.3 ± 3.1 years) experienced with resistance training (4.9 ± 2.6 years) performed nine weeks of resistance training (three times per week) with either high-loads (75–80% 1RM; HL-RT), or low-loads with BFR (30–40% 1RM; LL-BFR). Before and after the training intervention, resting muscle biopsies were collected, and quadricep cross-sectional area (CSA), muscular strength and power were measured. Approximately 5 days following the intervention, the same individuals performed an additional 'acute' exercise session under the same conditions, and serial muscle biopsies were collected to assess hypertrophic- and ribosomal-based signaling stimuli. Quadricep CSA increased with both LL-BFR (7.4 ± 4.3%) and HL-RT (4.6 ± 2.9%), with no significant differences between training groups (p=0.37). Muscular strength also increased in both training groups, but with superior gains in squat 1RM occurring with HL-RT (p<0.01). Acute phosphorylation of several key proteins involved in hypertrophy signaling pathways, and expression of ribosomal RNA transcription factors occurred to a similar degree with LL-BFR and HL-RT (all p>0.05 for between-group comparisons). Together, these findings validate low-load resistance training with continuous BFR as an effective alternative to traditional high-load resistance training for increasing muscle hypertrophy in trained individuals.

NEW & NOTEWORTHY

Low-load resistance exercise with blood flow restriction (LL-BFR) is an effective method for stimulating muscular adaptations, but phenotypical and mechanistic comparisons with *traditional* high-load training (HL-RT) in trained populations are scarce. The findings indicate that hypertrophy, but not strength, is comparable between LL-BFR and HL-RT, and the acute cellular and molecular processes for hypertrophy were similar, but not identical, between protocols. Thus, LL-BFR is an effective alternative to HL-RT for obtaining hypertrophy in trained populations.

Key Words: OCCLUSION, ISCHEMIA, HYPOXIA, KAATSU, HYPERTROPHY

INTRODUCTION

It is generally recommended that individuals perform resistance training with external loads of at least 70% of their one-repetition maximum (1RM) to maximize the positive outcomes of resistance training (e.g. muscle hypertrophy and increase strength) (1). Such loading conditions place high levels of mechanical tension on muscle tissue, which is believed to act as a primary mediator of intracellular anabolic signaling pathways, and consequently hypertrophic adaptations via mechanotransduction (2). However, in certain situations, it is often necessary to limit mechanical loading while facilitating muscular development; for example, when periodizing an athlete's total training stress (3), or during rehabilitation of musculoskeletal injury (4).

Emerging evidence continues to strengthen our understanding that skeletal muscle exhibits a hypertrophic response to both *mechanical* and *metabolic* stimuli (5, 6). Specifically, metabolic by-products induced by a high volume of muscle contractions, limited rest durations, or restricting blood flow (BFR) to the exercising muscles appear to compensate for reduced mechanical loading and achieve similar outcomes in skeletal muscle (5, 7, 8). Indeed, much lower loads e.g., 20–50% of 1RM with BFR (LL-BFR) have been used to elicit similar chronic hypertrophy to high-load non-restricted exercise (9-12), and similar rates of myofibrillar protein synthesis (13). This supports the belief that muscle fiber recruitment and the downstream anabolic processes responsible for hypertrophy may be achieved through means other than high loading conditions (6, 7, 14). Consequently, LL-BFR has been celebrated as a promising strategy to deliver muscular benefits to those populations who desire periodized exposure to high mechanical loads, or cannot tolerate such loads (4, 15).

Importantly, little is known regarding how LL-BFR compares with traditional high-load training (>70% 1RM, HL-RT), particularly at the intramuscular level. Although the

magnitude of hypertrophy achieved with training appears to be similar between these protocols (12), there is conflicting evidence on how such differences in loading conditions influence morphological adaptations at the fiber level (10, 13, 16, 17). There is some evidence suggesting that BFR provides a greater metabolic stimulus, with low loads preferentially stressing type I muscle fibers (18, 19). Some chronic studies align with these acute findings and report greater increases in type I fiber area with LL-BFR training (16, 17). Together these data suggest that type I fibers may be selectively targeted when using low loads with BFR.

It is crucial to consider whether such fiber-type specific adaptations influence characteristics of muscular performance. For example, if BFR with low loads targets type I fibers at the expense of type II fibers, this may result in inferior strength and especially power adaptations. Such outcomes would be highly undesirable for some athletic cohorts after resistance training (20). Although athletes are unlikely to use LL-BFR training exclusively in the long term, it is necessary to gauge whether this training mode has a comparable influence on the skeletal muscle phenotype compared with traditional HL-RT. This comparison of resistance training protocols will also help inform practitioners and coaches about how best to structure each training mode—and therefore the proportion of mechanical and metabolic stimuli—to develop the desired physical qualities for differing individuals and sports.

Many of the cellular mechanisms underpinning BFR exercise remain unclear. Following traditional resistance exercise, it is well recognized that key intracellular signaling pathways such as mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) are activated (21-23) to aid in the muscle remodeling process. More recently, the importance of ribosomal biogenesis to this process has also come to light (24) for its role in increasing ribosomal capacity (25) and subsequently contributing to muscle hypertrophy as a result of a greater translational ability. Indeed, both the transcription of rRNA genes (26)

and mTOR pathway activity through the ribosomal protein S6 kinase (27) are strongly correlated with muscle hypertrophy. However, it is not clear if these key cellular and molecular responses occur in the same fashion following LL-BFR exercise, and if so, how these responses compare in magnitude to traditional HL-RT. If divergent mechanistic pathways to muscle hypertrophy exist between protocols, this may suggest there are synergistic possibilities for muscular adaptations if both approaches are included within a program (6).

The aims of this study were to (i) compare how LL-BFR and HL-RT influence chronic morphological, molecular and functional responses in trained individuals, and (ii) examine acute cell signaling and gene expression responses associated with muscle hypertrophy following resistance exercise. It was hypothesized that (i) skeletal muscle cross-sectional area (CSA) would increase similarly between conditions following the training period, which would be corroborated by similar acute signaling and gene expression responses, and (ii) that despite similar increases in muscle CSA, LL-BFR would preferentially increase the size of type I fibers, whereas HL-RT would preferentially increase the size of type II fibers.

METHODS

An overview of the methods are detailed herein. Additional detail pertaining to the subsections can be found within the supplementary methods file specified in the endnote.

Experimental Design. The present study involved two chronologically sequential experimental parts, investigating firstly the chronic, and then secondly the acute responses to two resistance exercise protocols. The first part consisted of a randomized controlled trial in which participants performed 9 weeks of lower body strength training. Prior to the training

period, participants were stratified and block randomized into two groups based on absolute lean lower body mass (using dual energy x-ray absorptiometry), relative squat strength, and sex. Subsequently, the groups were randomly assigned to become a high-load resistance training group (HL-RT; *n*=10), or a low-load resistance training with blood flow restriction group (LL-BFR; *n*=11). Muscle mass and strength were assessed, and resting muscle biopsies were collected from *m. vastus lateralis* before and within 5–7 days after training. Following 4–5 days of rest after the completion of post-training assessments, the same participants returned to complete the second part of the study, which was an acute exercise trial. Muscle biopsies and performance-based assessments were obtained following a single exercise session that was representative of the sessions performed in the chronic training study. The post-training measures after the 9 weeks of training represented the baseline measures for the acute study. All experimental procedures adhered to the standards set by the *Declaration of Helsinki* and were approved by the ethical committees of the Norwegian School of Sport Science and the Norwegian Center for Research Data (protocol number 63-190618).

Subjects. Twenty-four healthy males and females were recruited to participate in the study. Sample size was determined from previous literature adopting similar experimental designs and outcome measures as those included in the present study (10, 28, 29). All participants performed regular resistance training for a minimum of 2 years leading up to the study (training history 4.9 ± 2.6 years; relative squat strength 1.7 ± 0.2 times body mass for males, 1.1 ± 0.2 times body mass for females). Before providing their written informed consent, the requirements and risks involved in the experiments were explained to participants, and participants were screened using the Australian Adult Pre-exercise Screening System (APSS) (30). Participants were considered eligible to participate if they had consistently performed

158 lower body resistance training at least once per week for the last 2 years, and were familiar
159 with the barbell back squat exercise. Further exclusion criteria included cardiovascular
160 disease, musculoskeletal injury, pregnancy, or those taking medications known to enhance
161 blood clotting risk. 21 participants completed the training study and the associated
162 assessments, after 3 participants withdrew citing personal reasons unrelated to the study. Pre-
163 and post- training muscle biopsies were not obtained from one participant in LL-BFR due to
164 personal preferences, however all other measures for this individual were completed. Another
165 participant from LL-BFR was excluded from immunohistochemical analyses because the
166 quality of their biopsy tissue was not adequate for analysis. For the acute study, two
167 participants in HL-RT and one participant in LL-BFR opted not to participate due to
168 availability or personal preferences. All acute measures and analyses were completed for the
169 remaining participants (HL-RT: n=8; LL-BFR: n=10). The participant characteristics for
170 each study component are outlined in Table 1.
171
172 **Training Study**
173 ***Resistance Training***. An overview of the resistance training program is displayed in Table 2.
174 The 9-week training block consisted of three supervised lower-body resistance sessions per
175 week, for a total of 26 sessions. Exercise volume and frequency were reduced during week 5
176 of training to promote recovery and adaptation (3). Resistance training was progressive,
177 including barbell back squat, leg press, knee extension and Bulgarian split squat exercises.
178 This combination of multi-joint and single-joint exercises was selected to provide our
179 previously strength-trained participants with sufficient volume to promote training
180 adaptations, and to reflect more the typical lower body resistance exercises adopted in athletic
181 programs. Loads were adjusted on a session-by-session basis, using repetitions in reserve
182 (RIR) to gauge intensity, and to attempt to standardise the proximity to muscular failure

between participants and training groups. Loads were progressed if RIR for consecutive sets exceeded two repetitions in HL-RT, and four repetitions in LL-BFR. Conversely, if the desired number of repetitions could not be completed, or muscular failure occurred during exercise, loads were reduced for the subsequent session. This method of progressive overload was chosen primarily to serve as a more ecologically valid comparison of HL-RT and LL-BFR training protocols, and to eliminate the inherent bias that occurs when attempting to match volume between low-load and high-load conditions (31). In addition, it allowed for investigation of the external loads that trained individuals can tolerate with BFR, and consequently, how much volume each group would perform.

Blood Flow Restriction. In the LL-BFR group, individualized BFR cuff pressures were determined using Doppler ultrasound (Phillips, HD15 PureWave). While seated in the upright position, the posterior tibial artery was imaged while a 10-cm nylon cuff (Sports Rehab Tourniquet, Brisbane, AU) was positioned around the most proximal region of the dominant thigh, and inflated incrementally. Arterial occlusion pressure (AOP) was recorded as the minimum pressure at which arterial pulse waves were no longer detected visually or aurally. During training, the same nylon cuffs were positioned around the most proximal region of both thighs. The cuffs were then inflated manually using a handheld sphygmomanometer to a pressure corresponding to 60% AOP. Cuffs were inflated immediately before the first set of each exercise, and remained inflated during the inter-set recovery periods. Following the final set of each exercise, the cuffs were deflated. Between exercises, cuffs remained positioned around the thighs, but were not inflated.

Assessments of Muscle Function. Muscular strength and power were measured four times over the course of the study. Prior to training, the series of assessments were performed

twice, each separated by a week. The first occasion was to familiarize participants with the testing protocols, before collecting the true baseline measures the following week. Midway through the training period, muscular strength tests were repeated. Following the training intervention, all muscle function assessments were performed between 5−7 days following the final training session. The details of each assessment involved are described below.

Isometric strength of the dominant knee extensors and flexors was assessed during unilateral maximal isometric contractions, using a dynamometer (HUMAC Norm, CSMi, Stoughton, MA) as previously described (28).Participants performed three 3-second maximal voluntary contractions (MVCs) of the knee extensors, each separated by 120 seconds. Subsequently, three MVCs of the knee flexors were performed at the same knee angle. For all MVCs, participants were instructed to apply force maximally and as rapidly as possible for the entire 3 secondsTest-retest CV values were 4.7% and 4.2% for peak torque during isometric knee extension and knee flexion contractions, respectively.Barbell back squat one-repetition maximum (1RM) was measured to assess dynamic strength, and to establish initial training loads during the studyFailure to complete the concentric (raising) portion of the movement, or not achieving the specified range of motion (90-degree knee angle); were deemed as an unsuccessful attempt. The highest load that was successfully completed was recorded as the 1RM score. Finally, countermovement and squat jumps were performed on a force plate (FP4, HUR Labs, Kokkola, Finland) to assess lower body power and velocity characteristics. Participants were then instructed to jump for maximal height, for three attempts for each style of jump.Test-retest CV values were 5.7% and 5.4% for jump height by takeoff velocity for countermovement and squat jumps, respectively. Test-retest CV values were 4.0% and 4.3% for peak power for countermovement and squat jumps, respectively.

Assessments of Muscle Mass. Muscle mass was quantified using two methods. First, the summated CSA of the quadriceps muscle group of the dominant leg was measured using magnetic resonance imaging (MRI; Magnetom Avanto 1.5T, Siemens AG, Munich, Germany). Second, lean muscle mass was measured using Dual Energy X-ray Absorptiometry (DEXA; Lunar iDXA, GE Healthcare, Buckinghamshire, UK). MRI scans were performed over days 12 and 13 before commencing training, and again 5 days following the final training session, before the post-training biopsy and acute component of the study commenced. Participants were instructed to abstain from any exercise in the 24 hours before each scan. Fifteen separate transverse section images were collected; these images were equally distributed relative to the participant's femur length between the greater trochanter and the lateral epicondyle. From these 15 images, images three to 12 (1 being most distal) were selected for each participant for analysis. Images one and two, and 13-15 were not used due to poor image contrast owing to placement of the coil. The 10 images were analyzed using ITK-SNAP software (University of PA, Philadelphia, USA; www.itksnap.org) to generate individual CSAs, which were then summated. The test-retest CV for total quadricep CSA from six participants' scans was 1.6%. Whole body DEXA imaging was conducted on participants in a fasted but hydrated state, after abstaining from exercise in the previous 24 hours. Lower body lean mass was determined from each scan to firstly assist with block randomization participants at baseline, as well as providing a secondary measure of muscle mass changes following training. The test-retest CV for lower body lean mass from DEXA was 1.7%.

Collection of Muscle Tissue. Resting muscle biopsies were obtained from the mid-portion of *m. vastus lateralis* from the dominant leg of each participant. Baseline biopsies were collected 9–10 days before the first training session. Post-training biopsies were collected

257 5–7 days after the final training session, to align with the acute component of the study. Post-
258 training biopsies were collected ~3 cm proximal to the pre-training site. All muscle biopsies
259 were collected under local anesthesia (10 $mg \cdot ml^{-1}$ xylocaine + 5 $\mu l \cdot ml^{-1}$ adrenaline,
260 AstraZeneca, London, UK) with a sterile 6-mm Bergstrom needle (Pelomi, Albertslund,
261 Denmark). Following collection, the biopsy sample was quickly cleaned for visual fat and
262 connective tissue. Samples intended for immunohistochemistry were aligned and covered in
263 O.C.T embedding matrix (CellPath, Newtown, UK) and frozen in dry ice cooled isopentane.
264 Samples for western blotting were gently cleaned for non-muscle tissue in cold physiological
265 saline before being snap frozen in liquid nitrogen. All samples were transferred to storage at
266 −80°C for subsequent analysis. Muscle tissue intended for RT-PCR was immersed in
267 RNAlater® solution (Ambion, TX, USA), stored for 24–48 hours at 4 °C, and thereafter
268 stored at −20 °C for later treatment and analysis.
269
270 ***Assessment of Muscle Fiber Type, Fiber Area, and Myonuclear Number.*** Mean CSA
271 analysis included 269 type I fibers (range, 115–590) and 408 type II fibers (range, 104–
272 1024). Nuclei positive for DAPI and PCM1 were counted and quantified as myonuclei and
273 related to respective fiber types. A random selection of 50 type I and 50 type II fibers were
274 included in the analysis. See supplementary file for additional detail.
275
276 ***Control Procedures.*** Before baseline and post-training testing sessions, participants were
277 instructed to refrain from consuming any stimulants or ergogenic aids that may influence
278 performance. During the 9 weeks of training, 24 h food diaries were collected each week,
279 including a mixture of training days, non-training days and weekend days. General
280 recommendations were made to participants to consume a diet rich in protein (e.g.
281 1.5–2.0$g \cdot kg^{-1} \cdot d^{-1}$), whilst 30 g servings of whey protein isolate were provided to participants

following each training session (100% Whey, ProteinFabrikken, Norway). Participants were also instructed to refrain from taking any performance supplements (e.g., creatine) during the study, as well as to refrain from any additional lower body strength training. Upper body resistance training and endurance training were permitted, and were recorded weekly in an exercise diary. Participants were also asked to limit high-intensity exercise (e.g., intervals-based training) to twice weekly at most, details of which were also diarized.

For all outcome measures, assessors were blinded to group assignment where possible. For laboratory and imaging assessments (e.g. Western blotting, magnetic resonance imaging etc.) assessors were blinded to group assignment. All baseline performance assessments (e.g. 1RM, isometric strength etc.) were collected prior to group assignment. Post-training performance assessments were not blinded due to financial constraints.

Adverse events. During the training study, one participant in the LL-BFR group reported an exercise-induced migraine which would return following any physical exertion. Following a two-week break from training, the participant could complete sessions free of pain. The participant completed 25 out of 26 sessions in the training block.

Acute Study

Experimental Design. The second experiment in the study involved performing an acute training session to evaluate the short-term molecular signaling and muscle function responses to each training approach. This was initiated approximately 5–7 days following the final training session, and ~3–5 days after post-training assessments were completed, where participants returned to complete a single lower body resistance exercise session. Then at 2, 24 and 48 hours after exercise, muscle biopsies were collected, and muscle function was

306 assessed to evaluate fatigue and subsequent recovery. The post-training measures were used
307 as the pre-exercise time point here.
308
309 ***Acute Exercise Session.*** The exercise session completed in the acute study was the same as
310 that in the training study (outlined in Table 2). Participants remained in the same groups to
311 which they were previously allocated; therefore, those individuals who had previously trained
312 for 9 weeks using BFR, performed the session with BFR, and vice versa. The exercise loads
313 used in the acute session were based on those used in the final training session, and were
314 adjusted if necessary, to maintain 1–2 RIR and 2–4 RIR for HL-RT and LL-BFR,
315 respectively. The RIR method was selected over other conventional methods (e.g. %1RM), to
316 mirror the stimulus provided by each session of the subsequent training period, and to
317 standardize perceived effort and proximity to failure across participants and exercise
318 protocols. Acute responses were investigated at the end of the training block to minimize any
319 exaggerated responses that occur when participants are unaccustomed to a particular training
320 protocol.
321
322 ***Collection of Muscle Tissue.*** The process for obtaining and processing muscle biopsies in the
323 acute study was the same as that previously described for the training study, aside from the
324 details below. The 2 h post-exercise biopsy was collected through the same incision as the
325 post-training/pre-exercise biopsy, but with alternate needle direction to ensure ~4 cm distance
326 between biopsy collection sites. Biopsies obtained at 24 and 48 h post-exercise were taken
327 from the non-dominant leg, from separate incisions, to reduce the number of samples taken
328 from one muscle. Two hours preceding each biopsy (with the exception of the 2 h post-
329 exercise biopsy), a standardized oatmeal meal (0.16 g protein·kg^{-1} body mass) was provided
330 to participants. A serving of whey protein isolate (0.4 g protein·kg^{-1} body mass) was provided

to participants upon completing the acute exercise session (i.e. two hours prior to the 2 h post-exercise biopsy).

Western Blotting. Please see supplementary methods file.

RNA extraction and cDNA synthesis.. Please see supplementary methods file.

Real-Time qPCR. Please see supplementary methods file.

Assessments of Muscle Function. Two performance tests were completed before, and after exercise to determine acute decrements in muscle function, as measures of the fatigue and recovery timelines for each exercise type. Performance assessments were completed following the collection of the pre-exercise biopsy, approximately 5 minutes post-exercise following ingestion of whey protein isolate, and following the 2, 24 and 48 h biopsy collections. Unilateral isometric peak torque was first measured from the non-dominant knee extensors, as described above in the training study methodology. Subsequently, three maximal countermovement jumps were performed (also as detailed previously in the training study methodology) to evaluate lower body power and velocity characteristics.

Assessments of Thigh Volume and Soreness. At the same time points as the muscle function assessments, the circumference of the non-dominant thigh of the participants was measured. They were subsequently used to estimate total thigh volume using the formula for segmental limb volume developed by Katch and Katch (32). Participants were also asked to rate their perceptions of soreness (CR-10+) while performing an isometric squat at 90 degrees, at each time-point.

Statistical Analyses. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS; v24, IBM, Armonk, NY, USA). Data were firstly checked for normality using the Shapiro-Wilk test. Protein phosphorylation data was log transformed to conform to normal distribution. Two-factor analysis of variance (ANOVA) with repeated-measures were performed to assess the effects of group (LL-BFR vs HL-RT) and time (PRE vs POST training) for the training study. For the acute study, two-factor ANOVA with repeated-measures were also performed to assess the effects of group (LL-BFR vs HL-RT) and time (pre, 0, 2, 24, 48 h post). Any significant main effects were followed with post-hoc Student's *t* tests (unpaired for significant group interactions and paired for significant time effects) to identify the origin of the significant difference. To account for multiplicity, the False Discovery Rate (FDR) method of Benjamini and Hochberg (33) was selected instead of Bonferroni's correction, to reduce the possibility of type II error (given the limited sample size, and small number of pair-wise comparisons made in the present study (34)). Effect sizes (*d*) for paired data (e.g. pre vs post) were calculated based on the identified mean differences and the standard deviation (SD) of these differences (35). For unpaired data (e.g. LL-BFR vs HL-RT), traditional Cohen's *d* effect sizes were computed using pooled SD. All effect sizes were interpreted as; $d<0.2$ = null effect, $d<0.5$ = small effect, $d<0.8$ = moderate effect, and $d>0.8$ = a large effect (36). Data are presented as mean ± SD with 95% confidence intervals (CI) unless stated otherwise. Statistical significance was accepted as $p \leq 0.05$.

RESULTS

Training Study

Adherence. All 21 participants completed the training study with high adherence. To be included in the final analyses, participants were required to complete at least 24 sessions during the training block. Participants undertaking LL-BFR completed 25.5 ± 0.8, and HL-RT completed 25.7 ± 0.5, out of 26 sessions, respectively.

No meaningful differences were observed between groups when analyzing participants' diet and exercise diaries across the training period.

Training Load Progression. The average volume-loads (sets × reps × load) performed during squat, leg press and leg extension exercises were calculated during the first and final weeks of training to evaluate load progressions between protocols. There was a time*group interaction (p=0.004) and time effect (p<0.001) for volume-load. Volume-load increased with HL-RT from 7,796 ± 3,027 kg to 10,279 ± 2,969 kg (p=0.001; ~38%), and in LL-BFR from 7,179 ± 3,463 kg to 12,317 ± 4,480 kg (p<0.001; ~79%). The volume-load increase was greater in LL-BFR (5,138 ± 2,020 kg [95% CI: 3,781 to 6,494]) than in HL-RT (2,483 ± 1502 kg [95% CI: 1,328 to 3,637], p=0.004). For example, HL-RT participants began training the back squat with a load of 73.7 ± 2.7 % of baseline 1RM, and finished training with a load of 91.4 ± 11.8 % of baseline 1RM. By contrast, LL-BFR participants began training with a load of 30.5 ± 3.3 % of baseline 1RM, and finished training with a load of 56.3 ± 9.8 % of baseline 1RM.

Changes in Muscle Morphology. Changes in muscle morphology are displayed in Figure 1. There were no time*group interactions for summated cross-sectional area (CSA; Figure 1A) of the quadriceps (p=0.369), nor DEXA fat-free mass (Figure 1B) (p=0.185). However, a

time effect (p<0.001) was evident. Quadriceps CSA increased after HL-RT (92.7 ± 22.1 cm^2 [95% CI: 36.4 to 149.0], d=1.52) and LL-BFR (118.8 ± 17.6 cm^2 [95% CI: 80.7 to 156.9], d=2.09). Lower body lean mass increased after HL-RT (194 ± 98 g [95% CI: 9 to 379], d=0.75) and LL-BFR (380 ± 93 g [95% CI: 146 to 614], d=1.09).

Muscle fiber CSA and myonuclei number changes are displayed in Figure 2. No time*group interactions existed for type I fiber CSA (p=0.331) and myonuclei number (p=0.139) or type II fiber CSA (p=0.677) and myonuclei number (p=0.113). However, a time effect existed for type II fiber CSA (p=0.010) and myonuclei accretion (p=0.029), but not type-I fibers (both p>0.05). Type II fiber CSA increased after HL-RT (998 ± 416 μm^2, [95% CI: 700 to 1296], d=0.55), and after LL-BFR (742 ± 438 μm^2 [95% CI: 405 to 1079], d=0.66). Myonuclei accretion increased after HL-RT for both type I (17 ± 23% [95% CI: 0 to 34], d=0.73) and type II fibers (24 ± 31% [95% CI: 2 to 46], d=0.77), whereas no change was observed after LL-BFR for type I fibers (3 ± 21% [95% CI: –13 to 19], d=0.05) or type II fibers (5 ± 15% [95% CI: –7 to 16], d=0.23).

Changes in Muscle Function. Changes in muscle function, confidence intervals and effect sizes are reported in Table 4. There was no time*group interaction (p=0.508) for maximal knee extensor isometric torque; however, a time effect was observed (p=0.003). Torque increased in HL-RT (9.7%, p=0.031), but not in LL-BFR (5.2%, p=0.253). Knee flexor torque showed a trend toward a time*group interaction (p=0.059), but a time effect was present (p=0.050). Maximal flexion torque increased in LL-BFR (15.0%, p=0.016), but not in HL-RT (1.9%, p=0.951).

A time*group interaction (p=0.008) and time effect (p<0.001) was evident for back squat 1RM. Squat strength increased both in HL-RT (17.5%, p<0.001) and LL-BFR (9.3%, p<0.001). The increase was greater in HL-RT than in LL-BFR (p=0.024, d=1.02).

No time*group interaction (p=0.058) or time effect (p=0.092) existed for squat jump (SJ) height. However, the data suggested a strong tendency for SJ height to increase with HL-RT (+7.2%, 2.3 cm [95% CI: 0.4 to 4.1], d=0.89), but not with LL-BFR (0.1%, –0.1 cm [95% CI: –1.9 to 1.6], d=0.0). There were no time effects or time*group interactions for counter-movement jump (CMJ) height or peak power (all p>0.05).

Acute Study

Anabolic Signaling. The acute protein signaling responses are displayed in Figure 3. There was no time*group interaction (p=0.088) for p70S6k^{Thr389} phosphorylation, however a time effect was observed (p<0.001). p70S6k^{Thr389} phosphorylation was greater at 2 h (33.0-fold change [95% CI: 2.3 to 63.7], p<0.001, d=1.44), 24 h (15.6-fold change [95% CI: –7.3 to 38.6], p<0.001, d=1.20) and 48 h (8.6-fold change [95% CI: 2.9 to 14.3], p<0.001, d=1.24) compared with pre-exercise after HL-RT. Following LL-BFR p70S6k^{Thr389} phosphorylation was greater at 2 h (9.7-fold change [95% CI: 0.1 to 19.3], p<0.001, d=1.60) compared with pre-exercise. p70S6k^{Thr389} phosphorylation may have been greater 24 to 48 h post-exercise after HL-RT compared with LL-BFR (d=0.8). p70S6k^{Thr389} phosphorylation area under the curve (AUC) from 2-48 h tended to be higher with HL-RT (825 ± 1017 A.U.) compared with LL-BFR (179 ± 177 A.U, p=0.064, d=0.89).

No time*group interaction (p=0.450) or time effect existed (p=0.186) for rpS6$^{Ser235/236}$ phosphorylation.

No time*group interaction existed for 4EBP-1$^{Thr37/46}$ phosphorylation (p=0.833), however a time effect was observed (p<0.001). 4EBP-1$^{Thr37/46}$ phosphorylation decreased 2 h following HL-RT (–0.6-fold change [95% CI: –0.8 to –0.4], p=0.008) and LL-BFR (–0.5-fold change [95% CI: –08 to –0.3], p<0.001), while it returned to pre-exercise levels in both groups at 24 h and 48 h (all p=0.728).

No time*group interaction (p=0.383) existed for ERK1/2$^{Thr202/Tyr204}$ phosphorylation, however a time effect was observed (p=0.003). ERK1/2$^{Thr202/Tyr204}$ phosphorylation was elevated at 2 h (1.7-fold change [95% CI: 1.3 to 2.1], p=0.004, d=1.22) and 48 h (2.0-fold change [95% CI: 1.2 to 2.8], p<0.001, d=1.12) following LL-BFR. However, no increases were observed following HL-RT.

mRNA and pre-rRNA Expression. Gene expression of transcriptional factors and pre-ribosomal RNA (rRNA) are illustrated in Figure 4. No time*group interaction or time effect (p=0.562) existed for RNA Polymerase I Subunit A (POLR1A) mRNA expression (p=0.687). However, a significant group difference was observed; POLR1A mRNA was greater in LL-BFR than in HL-RT, p=0.044). No time*group interaction existed for c-Myc mRNA expression (p=0.606), however a time effect was observed (p=0.001). c-Myc mRNA expression increased at 2 h (32.1-fold change [95% CI: 16.9 to 47.4] p<0.001), 24 h (2.2-fold change [95% CI:1.3 to 3.0] p<0.001), and 48 h (2.5-fold change [95% CI: 1.1 to 3.8] p<0.001) following LL-BFR. Following HL-RT, c-Myc mRNA expression increased at 2 h (27.5-fold change [95% CI: 23.8 to 31.2, p=0.010) and 24 h (3.0-fold change [95% CI: 2.1 to 4.0, p=0.003), but not 48 h post-exercise (3.9-fold change [95% CI: 0.1 to 7.7], p=0.124). No time*group interaction existed for transcription initiation factor 1A (TIF-1A) mRNA expression (p=0.606), however a time effect was observed (p=0.002). TIF-1A mRNA expression increased only at 48 h post-exercise for both LL-BFR (1.5-fold change [95% CI: 0.9 to 2.1], p=0.042) and HL-RT (2.1-fold change [95% CI: 1.3 to 2.9], p=0.009). No time*group interaction existed for TATA box-binding protein-associated factor 1A (TAF-1A) mRNA expression (p=0.830), however a time effect was observed (p<0.001). TAF-1A mRNA expression increased at 24 h (1.6-fold change [95% CI: 1.1 to 2.1], p=0.010) and 48 h (1.8-fold change [95% CI: 1.3 to 2.4], p=0.009) post-exercise for LL-BFR. Following HL-RT, TAF-1A mRNA expression increased only at 24 h post-exercise (2.0-fold change [95%

476 CI: 1.3 to 2.7], p=0.031). No time*group interaction existed for upstream binding
477 transcription factor (UBF) mRNA expression (p=0.970). UBF mRNA expression did not
478 increase following exercise (p=0.086), and there were no differences between groups
479 (p=0.498). 45S pre-rRNA expression did not increase following exercise in either group
480 (p=0.711). However, a significant group difference was observed (greater in LL-BFR than in
481 HL-RT, p=0.016).
482
483 ***Total RNA Content and rRNA Transcript Expression.*** Total RNA content and expression of
484 rRNA transcripts are illustrated in Figure 5. No effects of time, group or time*group
485 interactions were observed for total muscle RNA content, 5.8S rRNA, 18S rRNA, 28S rRNA
486 or 45S pre-rRNA (all p>0.05).
487
488 ***Muscle Function.*** Acute changes in muscle function are displayed in Figure 6. There was no
489 time*group interaction (p=0.866) for knee extensor maximal isometric torque, however a
490 time effect existed (p<0.001). Knee extensor torque decreased from pre- to post-exercise
491 after HL-RT (−27 ± 16 Nm [95% CI: –40 to –13], −12%, p=0.005) and LL-BFR (−27 ± 30
492 Nm [95% CI: –52 to –2], −12%, p<0.001). At 2 h post-exercise, torque remained reduced in
493 both HL-RT (−18 ± 9 Nm [95% CI: –26 to –11], −9%, p=0.045) and LL-BFR (−20 ± 31 Nm
494 [95% CI: –42 to 2], −7%, p=0.005). Torque recovered to pre-exercise levels in both groups
495 24 to 48 h post-exercise (all p>0.05).
496 No time*group interaction (p=0.933) existed for maximal jump height achieved
497 during the countermovement jump, however a significant time effect was observed
498 (p<0.001). Jump height was reduced from pre- to post-exercise after HL-RT (−5 ± 4 cm
499 [95% CI: –9 to –2], −11%, p<0.001) and LL-BFR (−4 ± 3 cm [95% CI: –6 to –2], −12%,
500 p<0.001). At 2 h post-exercise, jump height remained reduced in both HL-RT (–2 ± 1 cm

[95% CI: –3 to –1], –6%, p=0.010) but not LL-BFR (–1 ± 3 cm [95% CI: –3 to –1], –4%, p=0.099). Jump height recovered to pre-exercise levels in both groups 24 to 48 h post-exercise (all p>0.05).

Thigh volume and soreness perceptions. No time*group interaction existed for thigh volume (p=0.514), however time effects were observed (p<0.001). Thigh volume increased from pre- to post-exercise after HL-RT (320 ml [95% CI: 105 to 536], 4.0%, p<0.001) and LL-BFR (445 ml [95% CI: 288 to 600], 5.6%, p<0.001), but returned to pre-exercise levels 2 to 48 h post-exercise in both groups. A time*group interaction (p=0.008) and time effect (p<0.001) for perceived soreness existed. Soreness perceptions increased from pre to post exercise for HL-RT and LL-BFR (both p<0.001). Perceptions of soreness remained elevated 2 to 48 h after HL-RT (all p<0.001), and 2 to 24 h after LL-BFR (p<0.001 to 0.009) yet tended to decrease by 48 h (p=0.071). Soreness perceptions were greater at 24 h (p=0.008) and 48 h (p=0.022) following HL-RT, compared with LL-BFR.

DISCUSSION

This study compared the chronic adaptations and acute responses in skeletal muscle between resistance training with high loads, versus low loads combined with BFR. The key findings were that: (i) in strength-trained individuals, comparable skeletal muscle hypertrophy can be obtained with both LL-BFR, and HL-RT; and (ii) the activation of several signaling proteins involved in mTOR and MAPK pathways, and expression of transcriptional factors involved in initiating ribosome biogenesis, are increased similarly in the short term after LL-BFR and HL-RT, which may account (in part) for the comparable chronic adaptations. Most acute responses and chronic adaptations were similar between the two conditions; however, some important differences were observed. The increase in squat 1RM

was greater in HL-RT than in LL-BFR, with some evidence suggesting the same trend for maximal knee extension torque.

Chronic hypertrophic responses. Our findings support our primary hypothesis, and reinforce the current consensus that marked skeletal muscle hypertrophy can be achieved with LL-BFR (9, 10, 12, 28). Importantly, the magnitude of hypertrophy was comparable to that observed after resistance training with much higher loads. Although this has been demonstrated previously (9-11, 37), these studies have utilized untrained participants. Importantly, and a novelty of the present study, is that this evidence has been attained from a trained cohort, identifying that these individuals can also achieve robust hypertrophy with LL- BFR, akin to HL-RT.

Hitherto, it was unclear whether the acute processes within muscle responsible for these hypertrophic adaptations were similar between HL-RT versus LL-BFR. The only other studies to have investigated this to date, noted that myofibrillar protein synthesis (13) and expression of genes related to muscle function and plasticity (10) were similarly elevated after LL-BFR and HL-RT in untrained individuals. Although we did not directly assess myofibrillar protein synthesis, we found that key protein kinases involved in the mTOR and MAPK pathways (which are involved in myofibrillar protein synthesis (6, 21, 22)) responded similarly following LL-BFR and HL-RT exercise. Likewise, we demonstrated that expression of ribosomal RNA transcriptional factors increased following both LL-BFR and HL-RT, which may also enhance muscle protein synthesis rates. Importantly, these cellular and molecular responses to a single session were demonstrated in resistance trained individuals, and in trained skeletal muscle (i.e., *following* the chronic training period), which likely provides a truer reflection of the nature and magnitude of these anabolic processes than in a naïve state (i.e., before the chronic training period) (10).

Progression in volume-load. It is worth noting that the progression in volume-load across the training period was significantly greater with LL-BFR compared to HL-RT. A previous study involving BFR training observed volume-load to increase moreso with HL-RT compared to the LL-BFR conditions (38). However, in this study, sets were carried out until muscular failure and loads were not progressed during the training block. Instead, volume-load was increased by increasing the number of repetions only, which may explain the divergent findings. In a design similar to the present study, where set and repetition schemes were fixed and the magnitude of loading was progressed, Schoenfeld et al. (39) observed a greater increase in volume-load following LL-RT (without BFR) compared with HL-RT. Thus, it appears that compared to higher load protocols, lower load protocols (with or without BFR) inherently permit greater progression in loads (thereby enhancing volume-load) across a training period.

Interestingly, previous literature suggests that RT protocols that comprise of higher volume-loads stimulate greater muscle protein synthesis than lower volume-load protocols (40). The findings from our study do not align with these previous findings, because the acute cellular and molecular responses, and chronic hypertrophic responses were comparable between LL-BFR and HL-RT. In the present study, our HL-RT protocol included multiple exercises, and lower loading conditions (75% vs 90% 1RM) than that of Burd et al. (40). Therefore, volume-load with HL-RT was still substantial, despite being less than LL-BFR. It is likely that a threshold for volume-load exists, over which greater volume-loads do not confer greater acute protein synthetic responses, or chronic hypertrophy (38, 39, 40).

Acute anabolic signaling responses. Despite the previously reported similar net responses in protein synthesis (13) and chronic hypertrophy (9-11, 37) after these training modalities, the

contributions of distinct intracellular pathways (i.e., mTOR vs MAPK) to achieve these net responses have not been examined in this context. Herein, phosphorylation of p70S6K^{Thr389} appeared to be especially robust following HL-RT (33-fold increase at 2 h post-exercise), which has been strongly correlated to chronic increases in muscle CSA (27). A higher temporal response in p70S6K phosphorylation tended to occur after HL-RT over the 48 h period (p=0.064, d=0.89), from phosphorylation remaining above rest throughout. This may be expected, as phosphorylation of p70S6K at the Thr^{389} site is particularly responsive to mechanical stimuli (22, 23, 42). The different loading conditions imposed between groups in the present study may therefore explain the prolonged phosphorylation of p70S6K, and increase in total p70S6K protein content following HL-RT. Despite this nuance, the chronic changes in muscle CSA were similar between HL-RT and LL-BFR in the present study. Thus, stimuli other than purely mechanical tension may promote skeletal muscle hypertrophy in the long term (5-7), and metabolites themselves may possess anabolic properties instead of purely serving to enhance muscle fiber recruitment (and potentially operate through a mechanism independent of p70S6K). Indeed, both in vivo and in vitro studies have observed the application of lactate (in the absence of exercise) to stimulate ERK1/2 and mTOR signaling pathways acutely (43), and myotube formation with increases in fiber diameter and myonuclei content, respectively (44). Other stimuli that are associated with hypertrophy include hypoxia, metabolite accumulation, and cell swelling (7), all of which are induced by BFR exercise.

ERK1/2 phosphorylation significantly increased after LL-BFR, and although this response did not seem to be group-dependent (no interaction), a large effect was observed with LL-BFR (d=1.22) rather than HL-RT (d=0.25). ERK1 and -2 (ERK1/2) are components of the MAPK family, which is known to respond to stress-related external stimuli, and to be particularly responsive to lower load, higher volume protocols than higher load, lower

601 volume resistance training protocols (45). Recent work in mice suggests a role for ERK1/2 in
602 the regulation of fiber type-specific gene programs. Specifically, ERK1/2 appears to induce a
603 type I, oxidative fiber phenotype in developing mouse muscle (46). Therefore, it may be that
604 stress-related stimuli associated with exercise, such as tissue hypoxia and/or higher metabolic
605 demands (5, 8) act as stimuli for the ERK1/2 pathway. For example, injection of exogenous
606 lactate increases ERK1/2 phosphorylation 3.5-fold in mouse muscle (43). Despite these data,
607 the present study did not find a difference between LL-BFR and HL-RT in ERK1/2
608 phosphorylation, and may have been underpowered to detect a such interaction. Future work
609 should seek to expand on ERK1/2 (and other MAPK signalling pathways) responses to LL-
610 BFR and HL-RT protocols.
611
612 ***Fiber-type specific responses.*** In contrast to our hypothesis, we did not observe a preference
613 for type I fiber adaptation with LL-BFR, nor a preference for type II fiber adaptations with
614 HL-RT. Both training groups increased the CSA of type II fibers, whereas only HL-RT
615 increased myonuclear number. Previous studies report that type I fibers are subjected to
616 greater stress during LL-BFR exercise (18, 19), and this appears to translate chronically into
617 increased capillarisation and fiber area in type I fibers (16, 17). The lack of change in
618 myonuclear accretion with LL-BFR is also interesting, as this contrasts with previous
619 literature (16-18, 28). Although robust hypertrophy following LL-BFR has been reported in
620 the absence of myonuclear accretion (10). The lack of type I fiber adaptations following BFR
621 in the present study may relate to the BFR protocol and method of progressive overload that
622 we used. To allow for a more ecologically valid comparison of HL-RT and LL-BFR training
623 protocols, and to eliminate the inherent bias that occurs when attempting to match volume
624 between low-load and high-load conditions (31), we chose a BFR protocol that minimized
625 muscular failure (using three to four repetitions in reserve), and kept volume (sets × reps)

constant, while progressing the external loading. By the final week of training, some participants were exercising with loads that equated to ~50% of their baseline 1RM. Thus, the nature of the stimulus likely differed to that of previous studies (16-19), which used lower loading conditions (bodyweight to 20% 1RM), and/or completed some (or all) sets until muscular failure. Such conditions were likely associated with longer ischemic periods, which severely fatigues type II fibers and relies on the superior recovery rate of type I fibers to maintain force output (14). Despite this, our results indicate a similar hypertrophy of type I and type II fibers with LL-BFR (10% vs. 13%), and a more pronounced type II fiber hypertrophy with HL-RT (2% vs. 18%). Therefore, it may be advisable to use lower loading conditions (<20% 1RM) and complete some or all sets until muscular failure if the aim is to preferentially target type I fiber adaptations.

Acute ribosomal RNA responses. These molecular responses represent the initiation of ribosome biogenesis, which is requisite for the increased capacity for protein synthesis that occurs with muscle hypertrophy (25, 26). Following resistance exercise, increased transcription of rDNA occurs (24, 47), and this is responsible for the accumulation of rRNA, which is believed to precede hypertrophy (25). In the present study, we observed a comparable increase in expression of some of the key, early transcriptional factors including c-Myc, TIF-1A and TAF-1A following exercise, between LL-BFR and HL-RT. Interestingly, neither group demonstrated a significant increase in total RNA or expression of pre-rRNA or rRNA transcripts in the 48 h following exercise, despite the robust hypertrophy observed with training. This lack of change in rRNA components may be because (i) the participants in this study were trained, and (ii) the participants completed the acute study following 9 weeks of training. In support of this notion, Nader et al. (48) report that ribosomal gene expression is attenuated in trained skeletal muscle. Nonetheless, these gene responses are in alignment with

651 the previously discussed cellular signaling responses. Collectively, these data suggest that
652 acute molecular responses in muscle were similar between LL-BFR and HL-RT, which may
653 account for the similar muscle hypertrophy between these groups in the long term.
654
655 ***Chronic muscle function responses.*** From a practical standpoint, the maintenance and
656 increase in strength and power qualities in trained individuals, despite the exclusive
657 performance of BFR, is of importance. Previous studies assessing how BFR affects muscular
658 strength and power often include untrained participants (9-11, 13, 37). This choice of
659 untrained participants makes it difficult to translate findings to well-trained cohorts, who
660 possess a lower adaptive potential. The few studies that have included athletic or trained
661 cohorts have examined LL-BFR in conjunction with traditional HL-RT (instead of LL-BFR
662 exclusively). These studies found either small increases in strength (16, 49), or maintenance
663 of strength and power qualities (50) when LL-BFR was performed, which aligns with the
664 findings of the present study.
665 Direct comparisons of strength outcomes between LL-BFR and HL-RT is an
666 interesting area. There are consistent reports that HL-RT produces superior strength gains to
667 LL-BFR, particularly when strength is assessed using dynamic tests (e.g. 1RM) (12, 16, 38).
668 Even when seemingly non-specific tests (e.g. isometric MVC) are used, HL-RT may still be
669 more effective at eliciting strength gains (12). The findings of the present study appear to
670 support this, because isometric strength did not increase with LL-BFR, and squat 1RM
671 increased more with HL-RT. However, some researchers have postulated that such strength
672 differences may be influenced by the strength tests themselves, which inherently favor HL-
673 RT as external loads used in training are much greater than LL-BFR (9, 51). Indeed, there is
674 evidence to suggest that more opportunities to perform strength assessments (i.e., practice)
675 may attenuate these differences in strength (51). With that said, in the present study subjects

performed the strength assessments on four occasions (familiarization, baseline, mid-testing, post-testing), and notable differences in strength were still observed. Although it is likely that test specificity did contribute somewhat to these findings, we believe there is a stronger physiological contribution to the divergent strength adaptations observed in the present study, and many previous studies (12, 16, 38). For example, adaptations within the nervous system that influence central drive, including motor unit recruitment, firing rates, and synchronization, may have been inferior with LL-BFR due to the lower loading conditions used (14). In particular, the inhibitory influence of group III/IV afferents—which are thought to be strongly activated during ischemic exercise (8, 14, 16) —might limit the firing rates of the highest threshold motor units, thereby limiting maximal strength performance (14).

This neural adaptation theory may also reveal why studies including more strength assessments report similar increases in strength between LL-BFR and HL-RT. Frequent maximal strength assessments would stimulate maximal motor unit recruitment, firing rates and/or synchronization, and offset potential differences between LL-BFR and HL-RT protocols, when compared with isolated pre- and post-training assessments. Neural adaptations were not assessed in the present study, so we can only speculate if this contributed to the inferior strength adaptations with LL-BFR. Another suggested explanation for the observed differences in strength and power qualities may be related to mechanical or morphological properties of tendons (37). Although such differences were questioned in a recent study (9), we cannot exclude differences in mechanical properties in both muscle and tendons as possible causes for the distinct functional adaptations in this study. Future research should seek to investigate motor unit recruitment and voluntary activation adaptations as well as differences in tissue mechanical properties following chronic periods of LL-BFR and HL-RT.

Acute muscle function & recovery. Muscular performance was also evaluated following the acute session to gain insight into the fatigue and recovery timelines for each mode of exercise. We report similar significant impairments in both isometric strength and countermovement jump performance after LL-BFR and HL-RT, immediately following exercise, and at 2 h post-exercise. Performance in both assessments recovered to baseline levels at 24 h. While there are some accounts of prolonged impairments in muscle function extending past 24 h with both LL-BFR and HL-RT (52, 53), much of the previous literature suggest these decrements in performance are due to fatigue, given they subside in the hours following exercise (54-57). Discrepancies between outcomes are likely due to the exercise load and volume used, with higher volumes (e.g. 5 sets) or sets performed to muscular failure, resulting in prolonged strength impairments (52, 53). In the present study, although volume was relatively high (3 exercises, 3-4 sets), we used a submaximal repetition scheme (30-15-15-15). Moreover, we aimed to standardize proximity to failure using the RIR method. It is also pertinent to note in the present study that these assessments were completed after 9 weeks of exposure to an identical (albeit progressive) stimulus to that used in the preceding training period. Therefore, participants were well accustomed to the respective stimuli of high- or low-loads with BFR, unlike the cited example, which involved participants new to strength training (52, 53). We solely quantified the peak torque or jump height produced by the individual, which is a limitation of the present study. Ultimately, this provides an integrated measure of fatigue, but cannot distinguish between peripheral or central origins of such fatigue (58). Previous studies report severe peripheral fatigue with BFR which promptly recovers (<8 min) upon reperfusion to match the degree of impairment that occurs with HL-RT (55).

Alongside measures of muscle function, we also inferred muscle damage indirectly through thigh swelling and perceptions of soreness. Soreness ratings were greater at 24, and

48 h following HL-RT, which contrasts the findings of Brander et al. (59), who reported higher perceived soreness following both continuous and intermittent LL-BFR protocols compared with HL-RT. The discrepancy is likely due to the use of untrained participants in that study, whereas the 9-week training period in the present study presumably induced a repeated bout effect (52). Thigh swelling measurements were comparable between exercise protocols throughout the 48 h recovery period, which corroborates previous findings (52). Collectively, the muscle function, circumference and soreness data suggest that there were similar fatigue and recovery timelines between LL-BFR and HL-RT, which aligns with previous literature (52, 54). While muscular function and swelling may recover to baseline within 24 h following LL-BFR exercise, it is not clear if other processes within skeletal muscle are also restored within this time frame to support optimal performance in subsequent exercise sessions. Therefore, the recovery period between LL-BFR training sessions is an important consideration. Although a period of 24 h appears to be sufficient, further research on this topic is necessary before definitive conclusions can be drawn.

Considerations. A 'traditional' non-exercise control group was not included in the present study. Without this, it cannot be confirmed that the outcome measures displaying a time effect increased because of the training program *per se*. However, all variables included are known to increase with resistance training, and the magnitude of effects are commensurate with those previously reported (9, 10, 12, 13). The aim of the present study was not to assess the efficacy of LL-BFR. Instead, it was to compare LL-BFR to protocols with greater ecological validity, hence why the HL-RT group was included over a non-exercise or low-load non-BFR control.

Both male and female participants were recruited in the present study. We view this as both a strength and a limitation. Young female participants are underrepresented in BFR

literature (60), which makes it difficult to speculate if responses to BFR exercise may differ between sexes. In the present study, analyses of sex-specific responses were not conducted as this fall outside the scope of the investigation. Moreover, the inclusion of invasive measurement techniques, such as muscle biopsies, limited our sample size; thus, the study is insufficiently powered to evaluate training responses between sexes. Nevertheless, although training responses between males and females may differ in absolute terms, it appears these are similar when expressed relatively (61). For this reason, many outcome measures in the present study are expressed as a percentage change from baseline. Finally, we acknowledge it is possible that the menstrual cycle may exert some influence on training adaptations (60). To mitigate any potential impact, we stratified female participants into training groups based on contraceptive use, and method of contraceptive (e.g. hormonal, intrauterine etc.) to ensure an equal distribution across groups.

In conclusion, we compared the chronic and acute effects of LL-BFR and HL-RT, to evaluate the influence of resistance exercise protocols differing in contributions of mechanical versus metabolic stimuli on skeletal muscle characteristics. The findings supported our primary hypothesis, that the greater metabolic stimulus provided by LL-BFR can compensate for reduced mechanical loading and induce significant skeletal muscle hypertrophy, commensurate with that observed after HL-RT. The acute signaling and transcriptional responses within muscle that are responsible for such changes in hypertrophy also appear similar, yet particular signaling pathways may be more active than others, depending on the nature of the dominant external stimulus e.g., mechanical stretch versus tissue hypoxia, and should be further explored in future investigations. Examining these training protocols in isolation has permitted an understanding of which functional properties may develop/diminish after either training mode. Higher loading conditions appear necessary

776 to maximize muscular strength adaptations. Despite this, lower loads combined with BFR
777 permit the maintenance of muscular strength and power in trained individuals. Ultimately,
778 these findings encourage future studies to combine these protocols to distinguish whether
779 they pose a synergistic effect in skeletal muscle.
780
781 **ENDNOTE**
782 At the request of the author(s), readers are herein alerted to the fact that additional materials
783 related to this manuscript may be found at https://doi.org/10.6084/m9.figshare.15167739.v1.
784 These materials are not a part of this manuscript and have not undergone peer review by the
785 American Physiological Society (APS). APS and the journal editors take no responsibility for
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794
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796 The studies within this manuscript were performed at Department of Physical Performance at
797 the Norwegian School of Sport Science, in Oslo, Norway. CD was involved in the conception
798 and design of the work, acquisition, analysis, and interpretation of the data, and in the
799 preparation and drafting of the manuscript. TCN, MM, KTC, OH, NP, and BE were involved
800 in the acquisition, analysis and interpretation of the data, and in the preparation and drafting

of the manuscript. JSC, JP TR, and LAR were involved in the conception and design of the work, interpretation of the data, and in the drafting of the manuscript. All authors approved the final version of the manuscript and all agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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FIGURE LEGENDS

Figure 1. Absolute changes in total quadricep cross-sectional area (CSA) from MRI (A), and lower body lean mass from DXA (B). Solid bars indicate mean pre- vs post-training response in the LL-BFR (black) and HL-RT (white) group. Error bars indicate standard deviation of group responses. Horizontal lines indicate individual participant responses. ***indicates a significant change from baseline ($p<0.001$).

Figure 2. Changes in type I (A) and type II (B) fiber areas and myonuclear number per type I (C) and type II (D) fibers. Representative immunofluorescence images of muscle fiber membranes stained red for dystrophin (E), and myonuclei stained green for PCM1 (F) and blue for DAPI (G). Nuclei within skeletal muscle were classified as myonuclei if they were positive for PCM1 and DAPI, and the geometrical center was located within the dystrophin ring (H). Scale bar = 100μm. Error bars represent standard deviation. *indicates a significant change from baseline ($p<0.05$).

Figure 3. Fold changes in phosphorylation of (A) p70S6K at Thr^{389}, (B) rpS6 at $Ser^{235/236}$, (C) 4E-BP1 at $Thr^{37/46}$, and (D) ERK1/2 at Thr^{202}/Tyr^{204}. (E) Representative immunoblots are provided for each target protein with molecular mass information. ***indicates a significant change from baseline ($p<0.001$). **indicates a significant change from baseline ($p<0.01$). *indicates a significant change from baseline ($p<0.05$). †indicates a significant difference from LL-BFR ($p<0.05$). Error bars represent standard deviation

Figure 4. Fold changes in expression of (A) POLR1A mRNA, (B) c-Myc mRNA, (C) TIF-1A mRNA, (D) TAF-1A mRNA, (E) UBF mRNA and (F), 45S pre-rRNA relative to the to the geometric mean of the expression of two housekeeping genes (GAPDH and HPRT)**.** Error bars represent standard deviation. #indicates significantly different from HL-RT (main effect of group, $p<0.05$). *indicates a significant change from baseline ($p<0.05$)

Figure 5. Percentage change of (A) total muscle RNA content (μg RNA/mg tissue wet weight) and expression of rRNA transcripts (B) 5.8S rRNA, (C) 18S rRNA, (D) 28S rRNA and (E) 45S pre-rRNA relative to the geometric mean of the expression of two housekeeping genes (GAPDH and HPRT). Error bars represent standard deviation

Figure 6. Acute decrements and recovery in (A) knee extension peak torque and (B) countermovement jump height. Data are presented as percent change (%) from pre-exercise values. Perceived muscle soreness (C) across acute study is presented using CR-10 scale. Error bars indicate standard deviation. ***indicates a significant change from pre-exercise ($p<0.001$). **indicates a significant change from pre-exercise ($p<0.01$). *indicates a significant change from pre-exercise ($p<0.05$). †indicates a significant difference from LL-BFR ($p<0.05$).

Table 1. Participant Characteristics

	HL-RT	LL-BFR
Training Study		
Participants	n=10	n=11
Age (years)	24.3 ± 2.9	23.7 ± 3.1
Body Mass (kg)	77.4 ± 12.9	75.5 ± 10.3
Lower Body Lean Mass (kg)	20.3 ± 3.5	19.4 ± 3.9
Relative Squat Strength (1RM/BM)	1.4 ± 0.4	1.3 ± 0.3
Training History (years)	4.8 ± 2.8	5.2 ± 2.9
Acute Study		
Participants	n=8	n=10
Age (years)	23.8 ± 2.7	23.9 ± 3.2
Body Mass (kg)	78.1 ± 14.5	75.9 ± 10.7
Lower Body Lean Mass (kg)	20.3 ± 3.9	19.4 ± 4.1
Relative Squat Strength (1RM/BM)	1.3 ± 0.4	1.3 ± 0.3
Training History (years)	4.0 ± 2.3	5.5 ± 2.8

Data are presented as mean ± SD. Abbreviations: Training history (years of continuous previous strength training); HL-RT, high-load resistance training; LL-BFR, low-load blood flow restriction; 1RM, one-repetition maximum.

Table 2. Training Program Overview

	Low-Load Blood Flow Restriction (LL-BFR)		High-Load (HL-RT)	
	Repetitions (in set order)	**Intensity**	**Repetitions (in set order)**	**Intensity**
Session One and Three				
1. **Squat**	Weeks 1–4 (30, 15, 15, 15 reps) Week 5 (30, 15, 15 reps) Weeks 6–9 (30, 15, 15, 15 reps)	~30% 1RM (adjusted to maintain 1–4 RIR)	Weeks 1–4 (8, 8, 8, 8 reps) Week 5 (8, 8, 8 reps) Weeks 6–9 (8, 8, 8, 8 reps)	~75% 1RM (adjusted to maintain 1–2 RIR)
2. **Leg Press**	Weeks 1–4 (30, 15, 15, 15 reps) Week 5 (30, 15, 15 reps) Weeks 6–9 (30, 15, 15, 15 reps)	~30% 1RM (adjusted to maintain 1–4 RIR)	Weeks 1–4 (8, 8, 8, 8 reps) Week 5 (8, 8, 8 reps) Weeks 6–9 (8, 8, 8, 8 reps	~75% 1RM (adjusted to maintain 1–2 RIR)
3. **Leg Extension**	Weeks 1–4 (30, 15, 15, 15 reps) Week 5 (30, 15, 15 reps) Weeks 6–9 (30, 15, 15, 15 reps)	~30% 1RM (adjusted to maintain 1–4 RIR)	Weeks 1–4 (8, 8, 8, 8 reps) Week 5 (8, 8, 8 reps) Weeks 6–9 (8, 8, 8, 8 reps	~75% 1RM (adjusted to maintain 1–2 RIR)
Session Two				
1. **Bulgarian Split Squat**	Weeks 1–4 (15, 15, 15, 15 reps) Week 5 (15, 15, 15 reps) Weeks 6–9 (15, 15, 15, 15 reps)	~15% of PTBM (adjusted to maintain 1–4 RIR)	Weeks 1–4 (8, 8, 8, 8 reps) Week 5 (8, 8, 8 reps) Weeks 6–9 (8, 8, 8, 8 reps	~30% of PTBM (adjusted to maintain 1–2 RIR)
2. **Leg Extension**	Weeks 1–4 (30, 15, 15, 15 reps) Week 5 (30, 15, 15 reps) Weeks 6–9 (30, 15, 15, 15 reps)	~30% 1RM (adjusted to maintain 1–4 RIR)	Weeks 1–4 (8, 8, 8, 8 reps) Week 5 (8, 8, 8 reps) Weeks 6–9 (8, 8, 8, 8 reps	~75% 1RM (adjusted to maintain 1–2 RIR)

Exercise order is denoted by 1–3 (session one and three) and 1–2 (session two). Inter-set recovery periods were 120 seconds for HL-RT and 45 seconds for LL-BFR. Inter-exercise recovery periods for 3 minutes for both conditions. Prescribed tempo was 1 s for the concentric phase, and 1 s for the eccentric phase of repetitions, with no pause in between phases. PTBM, pre-training body mass; 1RM, one-repetition maximum; RIR, repetitions in reserve.

Table 3. RT-qPCR primer details.

Target:	Forward sequence:	Reverse sequence:
GAPDH	AACCTGCCAAATATGATGAC	TCATACCAGGAAATGAGCTT
HPRT	TGACACTGGCAAAACAATGCA	GGTCCTTTTCACCAGCAAGCT
5.8S ribosomal RNA	ACTCTTAGCGGTGGATCACTC	GTGTCGATGATCAATGTGTCCTG
28S ribosomal RNA	TGACGCGATGTGATTTCTGC	TAGATGACGAGGCATTTGGC
18S ribosomal RNA	TGCATGGCCGTTCTTAGTTG	AACGCCACTTGTCCCTCTAAG
45S pre-ribosomal RNA	GCCTTCTCTAGCGATCTGAGAG	CCATAACGGAGGCAGAGACA
c-MYC	GGTAGTGGAAAACCAGCAGCC	TCTCCTCCTCGTCGCAGTA
POL1RA	CCTCAAGGTATCGCCCAGTC	GGCAACTTCTGTTCTTGGGC
TAF1A	AGGTTTAGCGCCTGCTCATA	CTGAAATCACTCATACCCGCCT
TIF1A	CATTTTGTGCCTCCCCGAGT	GTATTGGCATGAGAAACCACGG
UBF	AAGAAGCCTCCCATGAACGG	CGGCCAGCTTTTTGTAGTGC

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HPRT, hypoxanthine guanine phosphoribosyl transferase; POL1RA, RNA polymerase 1 subunit A; TAF1A, TATA box-binding protein-associated factor 1A; TIF1A, transcription initiation factor 1A; UBF, upstream binding transcription factor.

Table 4. Assessments of muscular function measured before (Pre) and after (Post) training in the chronic study

	Low-Load Blood Flow Restriction (LL-BFR)				High-Load (HL-RT)			
	Pre	**Post**	**Mean difference [95% CI]**	***d***	**Pre**	**Post**	**Mean difference [95% CI]**	***d***
Strength								
Knee Extension Peak Torque (Nm)	258 ± 67	269 ± 77	12 [-11 to 34]	0.4	269 ± 45	292 ± 54	26* [3 to 48]	0.8
Knee Flexion Peak Torque (Nm)	100 ± 39	114 ± 41	14* [5 to 23]	1.0	93 ± 14	93 ± 15	0.3 [-12 to 12]	0.0
Squat 1RM (kg)	105 ± 38	114 ± 40 *	9 [6 to 12]	1.6	107 ± 36	120 ± 32 *#	19 [10 to 28]	1.5
Power								
CMJ Height (cm)	35.5 ± 8.7	36.8 ± 8.0	1.4 [-0.1 to 2.8]	0.7	35.2 ± 8.4	36.9 ± 7.7	1.7 [0.0 to 3.5]	0.7
CMJ Peak Power (W)	3,409 ± 984	3,440 ± 906	31 [-96 to 158]	0.2	3,704 ± 1,235	3,772 ± 1,193	68 [-46 to 181]	0.4
SJ Height (cm)	35.1 ± 8.3	35.0 ± 7.7	-0.1 [-1.9 to 1.6]	0.0	32.9 ± 6.4	35.1 ± 6.6*	2.3 [0.4 to 4.1]	0.9
SJ Peak Power (W)	3,474 ± 1,037	3,497 ± 924	21 [-127 to 170]	0.1	3,598 ± 1,099	3,699 ± 1,064	101 [-51 to 253]	0.5

All data are presented as mean ± SD values. *d*, Cohen's effect size. Nm, Newton-meter. 1RM, one-repetition maximum. CMJ, countermovement jump. SJ, squat jump. W, watts. *indicates a significant change from baseline ($p<0.05$). #indicates a significant difference from LL-BFR ($p<0.05$).

Acute cellular and molecular responses and chronic adaptations to low-load blood flow restriction and high-load resistance exercise in trained individuals

Low-load BFR

30-40% 1RM

VS

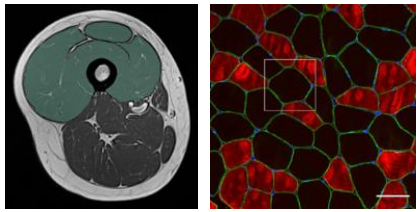
High-load RT

75-80% 1RM

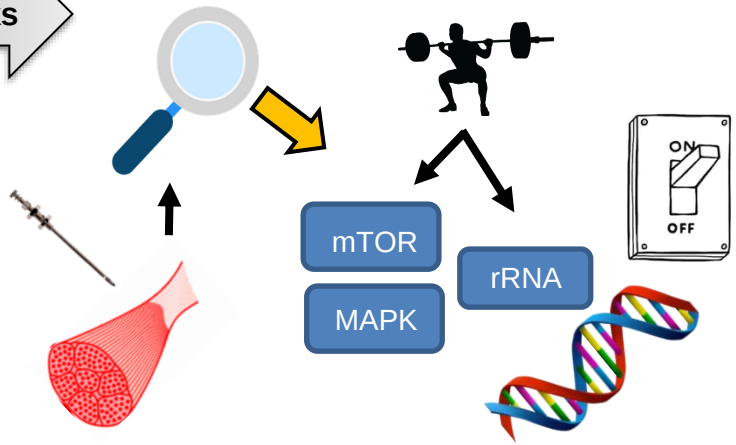
9 weeks

Chronic

Squat 1RM



Acute



OUTCOMES

	LL-BFR	HL-RT
Quad CSA	↑	↑
Squat 1RM	↑	↑↑
Type II Fiber Area	↑	↑
Type II Fiber Myonuclei	↑	↑
p70S6K	↑	↑
ERK1/2	↑	↑
C-Myc	↑	↑
TIF-1A	↑	↑
TAF-1A	↑	↑

CONCLUSION Muscle mass, but not strength, increased similarly between training groups. Acute phosphorylation of key proteins involved in hypertrophy signaling pathways, and expression of ribosomal RNA transcription factors occurred to a similar degree with LL-BFR and HL-RT. Thus, low-load BFR is an effective alternative to traditional high-load resistance training for increasing muscle hypertrophy in trained individuals.