NORWEGIAN SCHOOL
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Low Load Blood Flow Restricted Exercise Augment Anabolic Signalling Comparable to High Load Resistance and Translates Into Similar Muscle Growth During 9 Weeks of Training

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Abstract

Purpose:

It is well established that high-load resistance training (HL-RT, >70%/1RM) activates mTORC1 and elevates muscle protein synthesis. In addition, evidence from the last decade point to the direction that lower mechanical loads (20-40% 1RM) combined with blood flow restriction (BFR-RT) can augment protein synthesis to a similar extent (Fry et al., 2010). Corresponding signalling pathways have been identified, however, no studies have directly compared the anabolic signalling succeeding a bout of BFR-RT vs. HL-RT. Thus, we aim to elucidate some of the anabolic signalling regulating muscle hypertrophy induced by BFR-RT compared to HL-RT.

Methods:

Twenty-one strength trained males and females (24±3y) performed 9 weeks of lower body strength training (3/week) with either BFR-RT or HL-RT. Before and after the intervention, muscle mass was quantified with DEXA and MRI, and muscle function was assessed with MVIC of knee extensors. Biopsies were obtained from m. vastus lateralis before and 2, 24 and 48-h after the last exercise session.

Results:

Quadriceps CSA increased after BFR-RT (7.4±4.3%, p<.001) and HL-RT (4.6±2.9%, p=.007) with no differences between groups (p=.152). HL-RT increased MVIC (9.7±12.2%, p=.030) whereas no significant changes were observed after BFR-RT (5.2±12.9%, p=.28); no group interaction (p=.416). Phosphorylation of p70S6K^{Thr389} was elevated from baseline at 2-h (31-fold increase, p=.008) and 24-h (14.6-fold increase, p=.018) in HL-RT and elevated at 2-h (9.8-fold increase, p=.001) in BFR-RT, with no differences between groups (p=.826). Phosphorylation of rpS6$^{Ser235/23}$ (p=.564) and 4E-BP1$^{Thr37/46}$ (p=.474) was not different between HL-RT and BFR-RT at any time point. Phosphorylation of ERK1/2$^{Thr202/Tyr204}$ increased from baseline at 24-h (65±53%, p=.009) in HL-RT and increased from baseline at 2-h (77±56%, p=.002) and 24-h (140±144%, p=.011) in BFR-RT, with no differences between groups (p=.563).

Conclusion:

Overall, these data support the application of BFR-RT to induce hypertrophic adaptations comparable to HL-RT. In addition, our data suggest that the key anabolic signalling proteins driving the hypertrophic responses from BFR-RT and HL-RT are activated following an exercise bout of either training modalities. This suggests that the activation of the mTORC1 pathway along with ERK1/2 to be important as a potential underlying signalling pathway of BFR-RT induced muscle hypertrophy. Consequently, BFR-RT could be used to reduce external mechanical loading while inducing a potent anabolic response proceeding to muscle hypertrophy.

Acknowledgement

This study is part of a collaboration project between the Norwegian School of Sports Sciences (NSSS) and the University of Queensland. I was fortunate enough to be given the chance to participate in the joint effort to bring new insight into strength training with blood flow restriction. I would like to thank the following people for the support that made this thesis possible:

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I would like to thank my parents, whose love and guidance are with me in whatever I pursue. And finally, last but by no means least, I wish to thank my dearest Kristin Haugen Granli for patience and encouragement, along with excellent proofreading of this thesis.

Lastly, I would like to end with a genuine quote from Richard Feynman that summarises one of my impressions of the completion of a masters of science degree: "Nobody ever figures out what life is all about, and it doesn't matter. Explore the world. Nearly everything is really interesting if you go into it deeply enough".

Tore Christian Næss
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Acronyms

1RM - 1 repetition maximum

4E-BP1 - Eukaryotic translation initiation factor 4 element-binding protein 1

AOP - Arterial occlusion pressure

APSS - Adult pre-exercise screening system

BFR - Blood flow restriction

BFR-RT - Blood flow restricted-resistance training

CMJ - Countermovement jump

CSA - Cross-sectional area

DEXA - Dual energy X-ray absorptiometry

ERK1/2 - Extracellular signal-regulated kinase 1/2

EDTA - Ethylene diamine triacetic acid

HL-RT - High load resistance training

mmHg - Millimeter of mercury

MRI - Magnetic resonance imaging

MVC - Maximal voluntary contraction

mRNA - Messenger ribonucleic acid

mTOR - Mammalian target of rapamycin

mTORC1 - Mammalian target of rapamycin complex 1

mTORC2 - Mammalian target of rapamycin complex 2

NIRS - Near infrared spectroscopy

p70S6K - protein 70 ribosomal S6 kinase

RFD - Rate of force development

RIR - Repetitions in reserve

rpS6 - Ribosomal protein S6

SJ - Squat jump

1. Introduction to Blood Flow Restriction Exercise

Since Dr. Sato pioneered the training modality of exercising with limited blood flow during the 70's in Japan, a multitude of experimental studies have proven its overall efficiency on several physiological and physical determinants (Araujo et al., 2015; Clarkson, Conway, & Warmington, 2017; de Oliveira, Caputo, Corvino, & Denadai, 2016; Kim et al., 2017; Sato, 2005; Yasuda, Fujita, Ogasawara, Sato, & Abe, 2010). It was originally thought that a high load of mechanical resistance (>70% of 1RM) was needed to induce hypertrophic adaptations in the muscle. However, emerging evidence support the notion that a lower mechanical resistance (10-40% of 1RM) accompanied by blood flow restriction (BFR) has the potential to promote similar hypertrophic adaptations (Kim et al., 2017; Laurentino et al., 2012; Lixandrao et al., 2018; Vechin et al., 2015). Thus, BFR resistance training (BFR-RT) has been scientifically established as a training modality to induce significant skeletal muscle hypertrophy and augment force production capacity (Slysz, Stultz, & Burr, 2016). From a sports performance aspect, combining BFR with different training modalities has been demonstrated to increase running economy and time to exhaustion with running exercise (Paton, Addis, & Taylor, 2017); increase 100 m running-speed (Behringer, Behlau, Montag, McCourt, & Mester, 2017); enhance aerobic power (Abe et al., 2010); and maximal power output and delayed onset of blood lactate accumulation with cycling intervals (de Oliveira et al., 2016). Considering the several athletic benefits associated with the use of BFR exercise, it remains an engaging and contemporary research field for further scientific investigations.

In addition to the direct sports performance aspects related to BFR exercise, a broader health perspective and the use of BFR-RT in rehabilitation training should be considered. Since the quality and the quantity of the skeletal muscle mass is important to maintain healthy function and to carry out daily activities, the elderly population should participate in regular resistance training to countermeasure some of the atrophy and loss of strength accompanied by aging (Goodpaster et al., 2006). However, some of the elderly population are frail and unable to perform traditional heavy resistance training using mechanical loads above 70% of maximum force capacity. Thus, BFR-RT seems to be an effective training method to induce similar strength and hypertrophy adaptations to the elderly population (Cook, LaRoche, Villa, Barile, & Manini, 2017; Vechin et al., 2015) while inducing a hypotensive effect to the same magnitude compared to traditional heavy resistance training (Neto et al., 2015). Considering the aspect of rehabilitation after different injuries, high mechanical loading are often bypassed, thus the resistance training for muscle hypertrophy and strength are compromised. However, BFR-RT has been demonstrated to be an efficient method for achilles tendon rupture rehabilitation (Yow, Tennent, Dowd, Loenneke, & Owens, 2018) and to

increase peak torque, power output and restore strength after extremity injuries (Hylden, Burns, Stinner, & Owens, 2015). Regarding the distinct advantages and useful scenarios of applied BFR-RT, a further profound investigation will potentially shed light on the anabolic signalling and the cellular responses underpinning the observed increases in muscle hypertrophy.

2. Project Description

This research project is a collaboration project between the Norwegian School of Sports Sciences and the University of Queensland, and serve as the preliminary study of the doctoral thesis to PhD student Charlie Davids: '*The effects of low load blood flow restricted resistance training on functional, morphological and molecular responses in trained individuals*'. Several investigators (Prof. Truls Raastad, master student Maria Moen and master student Tore Christian Næss) are included in the study with various research responsibilities. In this section (section 2), the author will briefly cover the entire project and give insight into the design of the study alongside a general overview of the BFR-study. In the following section (section 3), the author will review all the applied research methods and highlight his contribution and responsible methods from the BFR-study. Further, the author will delve into his unique segment: '*The underlying anabolic signalling of low load blood flow restriction induced skeletal muscle hypertrophy*', which will be the topic of his master thesis. In addition, a comprehensive theory chapter (section 4) is allocated to clarify the theoretical framework underpinning the author's master thesis. Subsequently (section 5) will discuss some ethical considerations regarding the BFR-study, while section 6 highlights the possible benefits of this research project. Lastly, a scientific article is crafted based on some of the results from the BFR-study.

2.1 Project Rationale

There is a need to understand what processes occur within the skeletal muscle in the acute phase following BFR-RT, and whether these differ from traditional heavy-load resistance training (HL-RT). Despite the shift from mechanical to a more metabolic stimulus that occurs with BFR-RT, there is some evidence to suggest a similar acute cellular signalling cascade is triggered following BFR-RT as is seen with HL-RT. Mammalian target of rapamycin (mTOR) is a key intracellular signalling protein that initiates the protein synthesis response that leads to the production of new contractile proteins, and ultimately induces muscle hypertrophy. Gundermann and colleagues (2014) have reported that the protein synthesis response is diminished when rapamycin (an mTOR inhibitor) is administered following BFR-RT, suggesting that, in a similar fashion to HL-RT, hypertrophy with BFR-RT is achieved via the mTOR pathway. Additional studies have also demonstrated that BFR-RT triggers similar processes for skeletal muscle remodelling (satellite cell proliferation) as traditional HL-RT (Fry et al., 2010; Fujita et al., 2007; Gundermann et al., 2014; Nielsen et al., 2012), as well as reducing proteolysis-related genes, and negative regulators of hypertrophy i.e. myostatin (Laurentino et al., 2012; Manini et al., 2011), despite the markedly

reduced levels of mechanical stress. However, none of these studies directly compared these responses to HL-RT. Therefore, while it appears that BFR-RT achieves muscle adaptations through similar cellular and molecular pathways as HL-RT, it is not clear if these pathways are activated to a similar extent, or if the magnitude of these responses are comparable to traditional HL-RT. A greater knowledge of these acute processes that occurs within the muscle would help shed light on the mechanistic underpinnings of BFR-RT. In addition to this, a deeper understanding of the acute processes would potentially explain and reinforce the findings of chronic studies comparing BFR-RT and HL-RT, and help determine whether BFR-RT is an equally effective strategy to maximise muscular adaptations in the absence of high levels of mechanical stress.

2.2 Aims and Hypotheses

The aim of this study was to examine (1) the effects of blood flow restriction on the morphological, molecular and functional responses to low load resistance training compared to high load resistance training, and (2) the acute anabolic signalling following a single blood flow restriction exercise bout that drives the chronic adaptations seen with training, and whether these differ to a traditional heavy load resistance exercise session. The following null hypothesis and alternative hypothesis has been constructed for this master thesis, with a mechanistic paradigm in mind, and will trial the process of falsification through the hypothetic-deductive structure proposed by Karl Popper and Carl Hempel (Popper, 1952).

Null hypothesis: Blood flow restriction exercise will result in an equivalent anabolic stimulus acutely following exercise when compared to a high load resistance exercise without blood flow restriction, which will translate to similar gains in muscle mass and muscle strength following a chronic period of training.

Alternative hypothesis: Blood flow restriction exercise will result in a different anabolic stimulus acutely following exercise when compared to a high load resistance exercise without blood flow restriction, which will translate into dissimilar increase in muscle mass and muscle strength following a chronic period of training.

2.3 Participants

A total of 22 strength trained males and females (age 18-35) were recruited to participate in the study (table 1). Strength trained individuals were defined as completing 1-2 strength training sessions for the legs per week, during the last two years. Further, an equal gender distribution were selected for the purpose of gender sub-analysis, thus a possible indication of different responses

based on gender. Previous BFR-RT studies that incorporated muscle biopsies and employed similar experimental designs with 20-24 sessions of lower body exercise have been able to detect significant differences between two conditions with sample sizes of 8-10 participants in each condition (Ellefsen et al., 2015; Nielsen et al., 2012). Allowing for a typical dropout rate of 10%, a target of 22 participants were recruited (1 early drop-out due to a foot injury outside the BFR-study) to ensure sufficient statistical power. Participants were recruited via word of mouth, e-mailing students at the Norwegian School of Sports Science, social media, and recruitment posters. The experimental procedures and the risks were explained to the participants before they provided their written informed consent to take part in the study. Participants were further screened using the Adult Pre-Exercise Screening System (APSS) tool (Norton, 2012) and were considered eligible to participate if free from musculoskeletal or cardiovascular conditions. Female participants were also screened for contraceptives and their menstrual cycle (see attachment 6, for detailed description).

Inclusion criteria BFR-study: (1) frequent resistance training the last two years (1-2 strength training sessions for legs per week), (2) free from musculoskeletal or cardiovascular conditions, (3) men and females age 18-35, (4) no other injuries that may prevent heavy load resistance training.

Exclusion criteria BFR-study: (1) performed any additional lower body resistance exercises outside the training program, (2) did not perform the pre and post measures, (3) unable to provide written informed consent, (4) performed more than two endurance sessions per week.

Table 1. Subject characteristics, Mean ± SD

Variable	BFR-RT (n = 11)	HL-RT (n = 10)
Sex (male/female)	5 males, 6 females	4 males, 6 females
Age (years)	24.3±3.2	24.2±3.1
Body Mass (kg)	75.8±10.2	76.6±12.7
DEXA Leg Lean Mass (g)	19.438±3.895	20.270±3.504
1RM Squat Strength (kg)	105±37	106±36
Isometric Peak Torque (Nm)	257±67	268±44
Training Status (years)	4.8±2.2	4.9±2.9

Abbreviations: Training status (years of frequent strength training)

2.4 Experimental Design

A number of morphological, molecular and performance measures were conducted over the space of 4 visits to gain baseline levels of muscle mass and muscle function. Subsequent to these baseline

measures, a total of 27 training sessions were performed over the course of nine weeks (three sessions per week). Following the completion of training, the same measures that were completed during baseline were repeated to evaluate increases in hypertrophy and muscle function from the training block. Subsequently to the training intervention and post-testing, another exercise bout was performed and served as the acute exercise session for the measurements of anabolic signalling responses and the cellular stress to a single bout of exercise with either BFR-RT or HL-RT. This final session was chosen in an effort to eliminate any exaggerated acute responses to unaccustomed exercise, as these often do not reflect the chronic changes seen after a period of training (Mitchell et al., 2014; Morton et al., 2016).

2.5 Training-Program

After completing the baseline visits (described detailed in section 3), participants were allocated to either (1): ~30% 1RM with blood flow restriction (12 cm cuff width) corresponding to a 60% arterial occlusion pressure (BFR-RT), or (2) 8-10RM loads with no blood flow restriction (HL-RT, table 2). Stratification was employed to ensure an equal group distribution based on gender, muscle size and muscle strength. Participants were supervised during the thrice-weekly lower body resistance training sessions. Sessions 1 and 3 consisted of barbell back squat, leg press, and leg extension while session 2 consisted of Bulgarian split squats and the leg extension exercise (Fig. 1). Participants allocated to the BFR-RT group performed 4 sets of each exercise comprising 30, 15, 15, and 15 repetitions, respectively. A rest period of 45 seconds was allocated between sets. In the HL-RT condition, 4 sets of 8 reps for each exercise was performed, with 120 seconds of rest between sets. For both conditions, loads were adjusted to ensure that participants exercised to muscular failure. If the specified number of repetitions were completed before muscular failure was achieved, the exercise load would be increased for the subsequent session, gauged from a repetitions in reserve (RIR) rating. On the contrary, if the desired number of repetitions could not be completed, the load would be decreased in the following session. Such load manipulation was chosen due to the inherent need for low-load resistance exercise to be performed until muscular failure in order to maximise the hypertrophic response to training (Fahs et al., 2015; Farup et al., 2015). Sets would also be performed until momentary muscular failure in the HL-RT condition i.e. 8-RM loads as opposed to ~80% of 1RM, to avoid confounding the criteria for set termination. For both conditions, 3 minutes rest was allocated between exercises. Prior to the exercise bout, a standardised warm-up containing 5 min of either bicycling, jogging or rowing, followed by 5 min of calisthenic exercises including: self selected dynamic stretches, dynamic warm-up drills and

bodyweight exercises, subjects performed 2 sets of the first exercise to gradually increase load. Training was performed on non-consecutive days to allow adequate recovery between sessions. A deload week in week 5 was incorporated for both conditions, for the purpose of promoting recovery. Training would still occur in this week, however, the number of sets performed for each exercise was reduced by 1. In addition, the third session of the deload week was changed with a mid-test for the 1RM squat and the MVC of the knee extensors.

Figure. 1. Illustrating the four different exercises performed during the training intervention. A: Barbell squat, B: Leg press, C: Bulgarian split squat and D: Leg extension.

Table 2. Resistance training program - exercises, sets, reps, and rest intervals for each weekly session for HL-RT and LL-BFR groups.

Condition	Session One and Three	Session Two
High Load resistance training (8-10RM, 120 sec inter-set rest)	Barbell Squat: 4 sets of 8 reps	Bulgarian Split Squats: 4 sets of 8 reps (each leg)
	Leg Press: 4 sets of 8 reps	Leg Extension: 4 sets of 8 reps
	Leg Extension: 4 sets of 8 reps	
Low Load BFR (~30% 1RM, 45 sec inter-set rest)	Barbell Squat: 1 set of 30 reps, 3 sets of 15 reps	Bulgarian Split Squats: 4 sets of 15 reps (each leg)
	Leg Press: 1 set of 30 reps, 3 sets of 15 reps	Leg Extension: 1 set of 30 reps, 3 sets of 15 reps
	Leg Extension: 1 set of 30 reps, 3 sets of 15 reps	

2.6 Project Timeline

The recruitment of participants started in mid-August, while the baseline visits were conducted during the following weeks. The training intervention lasted from September to mid-November

(Fig. 2). Further, the post-testing was conducted late-November followed by the postliminary acute study in early-December.

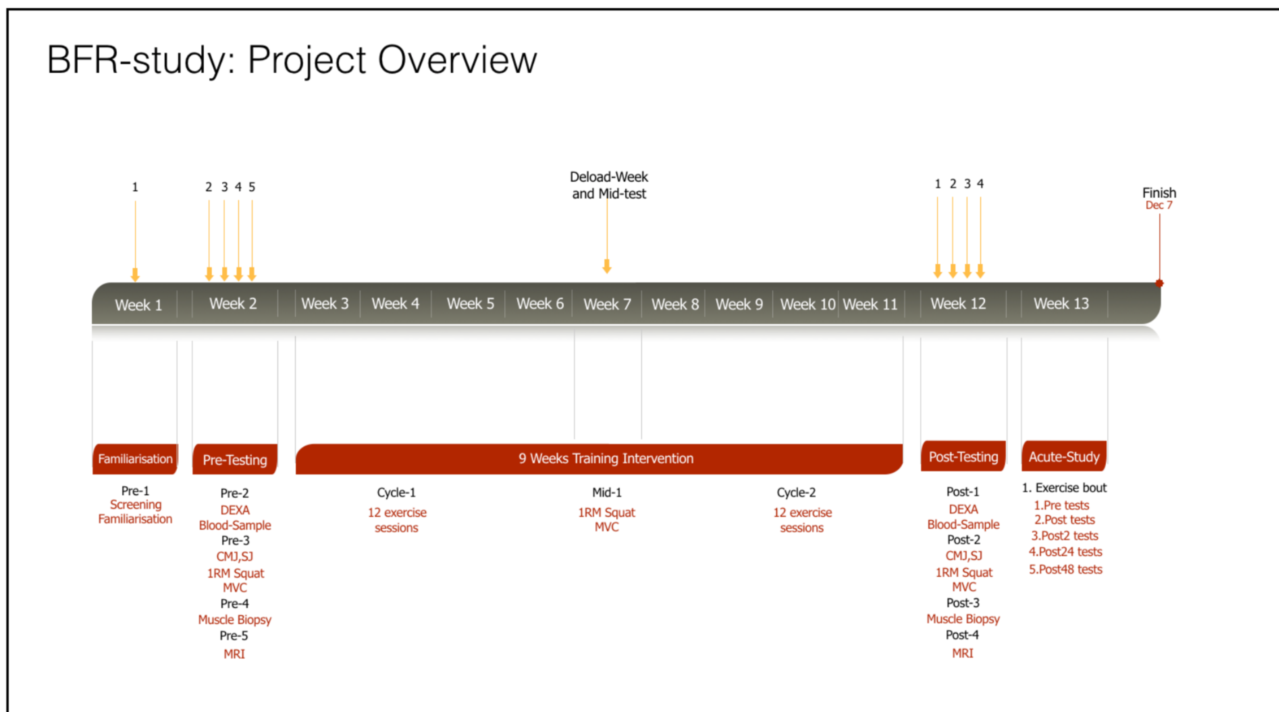

Figure. 2. Timeline for the training intervention followed by the acute experiment.

2.7 Acute Study Timeline

After all post-testing was conducted, 3 consecutive days were selected for the acute study (Fig. 3). First, a standardised meal containing oatmeal (1.4 g of oatmeal/kg bodyweight) and water were consumed by the participants. This was chosen due to its relative low protein content to minimise the influence of a protein intake regarding the acute anabolic signalling, and that the protein dose were standardised between subjects (0.16 g protein/kg bodyweight). Two hours following the meal, the first test session was performed comprising of: muscle biopsy sampling, blood sampling, thigh girth measurements, perceived muscle soreness, countermovement jumps and MVC knee extension (methods are described detailed in chapter 3). Subsequent to the test session, subjects performed an exercise bout in a similar fashion to the last training session of the training block. Subjects were accordingly accustomed to either blood flow restriction exercise or heavy resistance exercise. Loads corresponded to the maximum load that could be lifted within the ramification of each distinct exercise protocol. Immediately following the exercise session (lasting approximately 40 min), a protein shake (0.4 g whey/kg bodyweight, corresponding to 0.28 g protein/kg bodyweight) was ingested followed by a second test session identical to the one above, except for the muscle and

blood sampling. Two hours post exercise, a third test session was performed with all the tests. During the two consecutive days, another test session was performed at post 24-hours, and post 48-hours after exercise.

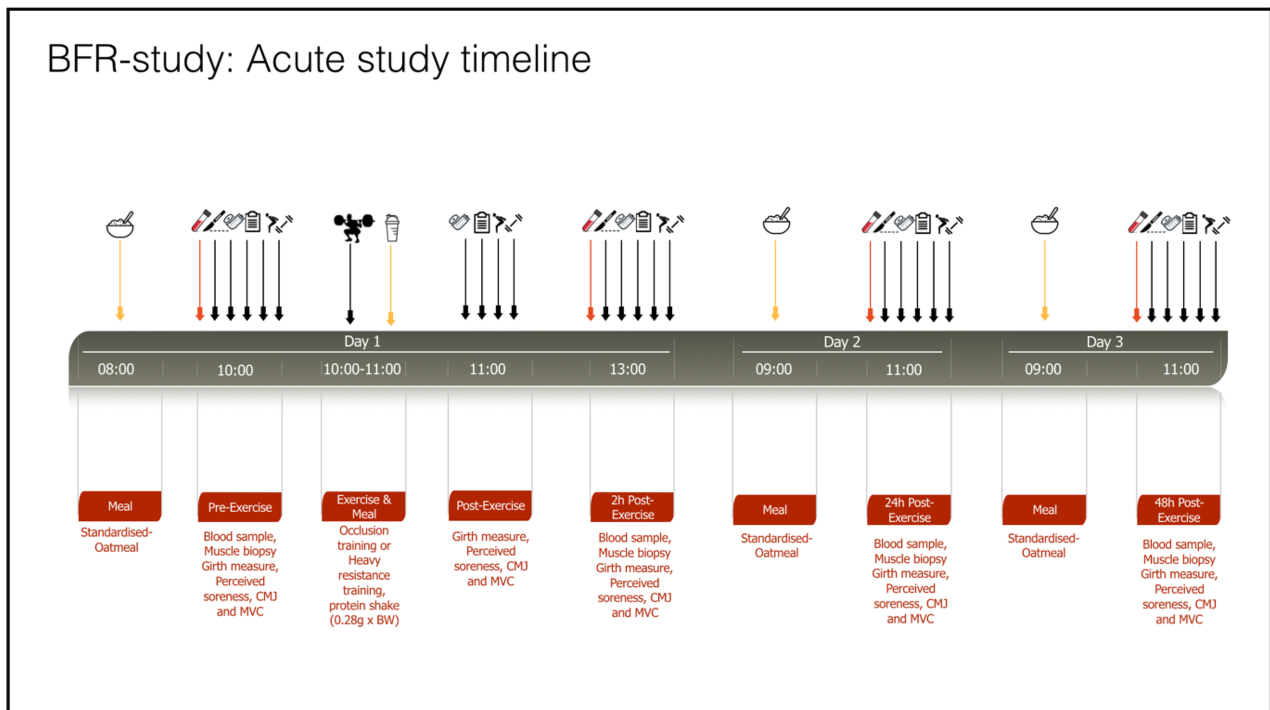

Figure. 3. Timeline for the acute experiment.

2.8 Stratification

Following the completion of all baseline measurements, subjects were allocated to either the BFR-RT group or the HL-RT group in a manner of stratification. Three main variables: 1RM squat strength, female/male gender, and the quantity of lean mass in the legs (DEXA), were chosen for the stratification. The mean and standard deviation value of the 1RM squat strength in the BFR-RT group (105±37kg) was similar to the high load condition (106±36kg). While the lean leg mass of the BFR-RT group (19.5±3.9kg) was comparable to the traditional strength training group (20.2±3.5kg). These three variables were selected in an effort to eliminate the possible confounder of training effects from uneven group distributions.

3. Material and Methods

The following section will review the different methodological considerations and the several research methods applied in this study. All of the measurements performed during the baseline visits, with the exception of the ultrasound to determine arterial occlusion pressure, were repeated to assess the changes in muscle mass and muscle function following the training block. The muscle mass assessments were repeated after a minimum of 5 days following the final session to avoid mistaking the acute swelling responses as increased contractile hypertrophy. Measures of muscle function were repeated after approximately 5 and 7 days following the final training session to allow full recovery of muscle strength and endurance capacity, and to assess any delayed adaptive responses to training (Nielsen et al., 2017).

3.1 Body Composition and Muscle Mass

Dual-Energy X-ray Absorptiometry (DEXA) and magnetic resonance imaging (MRI) were conducted before and after the nine week training intervention. The DEXA (Lunar iDXA, GE Healthcare, Buckinghamshire, United Kingdom) was used to assess the total body composition of the subjects. Participants were scanned in the morning in a fasted state, avoiding exercise in the 12-hours prior to the test, and were asked to void their bladder immediately prior to the scan to enhance the reproducibility of results. Further, the participants were asked to remove all clothing and jewellery containing metal (zippers, rings, bracelets etc.). Immediately before the scan, the subjects height and weight were measured on a stadiometer (Seca 217, Seca gmbh & co. kg., Hamburg, Germany) and enrolled in the DEXA software. Following, the participants were asked to sit in the middle of the scanning table while the investigator guided the subject to lay supine inside the borderline of the table, and instructed them to position the arms away from the body with the thumbs turning upward. Support bands were then placed around the subjects knees and ankles and the investigator told the participants to lay motionless for the duration of the DEXA scan (7 minutes and 16 seconds). Total body scan mode was selected and all scans were analysed with enCORE Sofware, Version 14.10.022 (GE Healthcare, Buckinghamshire, United Kingdom). During the data analysis, segmental borderlines were manually selected to separate the legs from the hips to quantify lean leg mass. Zona orbicularis was chosen as the cut-off borderline, and all pre and post images were matched to ensure equal segmental distribution before analysing the results. Researcher Mr. Tore Christian Næss was responsible for performing the DEXA scans and the data analysis of all subjects.

Transmission of X-rays through the body to measure bone mineral density has sustained a niche following the approval by the Food and Drug administration for clinical use in 1988 (Krugh & Langaker, 2018). The two-dimensional imaging technique relies on two different energy levels, thus the images can be divided into two compartments; Bone and soft tissue (Borga et al., 2018). In later years, the DEXA has been demonstrated as an effective method to measure total and regional distributions of lean body mass and fat mass, with statistical significant findings regarding the reliability and the validity of its use in several investigations (Chen et al., 2007; Hind, Oldroyd, & Truscott, 2011; Kohrt, 1998; Salamone et al., 2000; Smith-Ryan et al., 2017). Based on the existing literature of experimental studies investigating the efficiency and the accuracy of the DEXA scan, its application in research scenarios is widely accepted in the sport science discipline.

In addition to the DEXA scan, muscle cross-sectional area (CSA) and quadriceps volume were determined using magnetic resonance imaging (MRI) to provide a local measure of muscle mass. The MRI is currently viewed as the gold standard to quantify muscle mass and changes in local CSA (Delmonico, Kostek, Johns, Hurley, & Conway, 2008). The dominant leg was selected for the analysis. All participants underwent the MRI at a private radiology centre (Aleris Røntgen Oslo City, Norway). The coordination management of participants was allocated to researcher Ms. Maria Moen, while Aleris Røntgen helped out with a certified radiograph to conduct the MRI investigations. To provide a valid record of the CSA, the individual length of the femur bone was recorded for each participant. Transverse section images were captured of the dominant leg (GE Signa 1.5 Tesla Echospeed, GEMedical Systems, Madison, WI, USA) before and after the training period. The images [Digital Imaging and Communications in Medicine (DICOM)] were analysed using ITK-SNAP software (University of PA, Philadelphia, USA; www.itksnap.org). A total of 10 DICOM images were used to derive the CSA of the quadricep muscles along the dominant thigh. The CSA values from each of the 10 locations were combined to provide a total quadriceps CSA. The CV of these assessments was <2%. Charlie Davids was responsible for the data analysis of CSA from the MRI.

3.2 Blood Sampling

Subsequently to the DEXA scan in a fasted state, blood sampling was performed. Blood samples were collected (5 ml EDTA) by Mr. Charlie Davids at both pre- and post-conditions of the participants. In addition, blood samples were taken at four time points during the final acute study. Blood samples were further processed by Mr. Tore Christian Næss and Mr. Charlie Davids with the following procedure: centrifuged at 10 000G for the duration of 10

minutes in a temperature of 4ºC, pipetting two labelled epindorf tubes with 500 µg serum and then stored in a freezer at -70ºC. Blood samples would be analysed for acute anabolic hormones (testosterone and growth hormone) and myoglobin as a biomarker of muscle damage.

3.3 Functional Outcome Measures

Lower body strength and power performance were evaluated with the following tests, in the respective order, during a single session lasting approximately 90 min. All participants completed a familiarisation of the tests prior to the first test session. Subjects were instructed to not perform any vigorous exercise the day before the test session. Further, the investigators leading the test sessions (Ms. Maria Moen, Mr. Tore Christian Næss and Mr. Charlie Davids) noted the use of weightlifting-belts, shoes, music and other confounding variables that could affect the testing session. E.g. if the participant wanted to apply the use of weightlifting-shoes during the 1RM squat session, he/she could do so, however, the same equipment was used during the post test. Verbal encouragement was provided to all participants during the testing sessions to exert maximal effort in an attempt to reduce possible bias.

3.3.1 Countermovement Jump & Squat Jump

After a standardised warm-up containing 5 min of bicycling (Monark 839 Ergomedic, Vansbro, Sweden) and 5 min of calisthenic exercises including: self selected dynamic stretches, dynamic warm-up drills and bodyweight exercises, subjects performed 2 sets of 3 sub maximal countermovement jumps (hands held at the hips, performed with a fast eccentric motion before the concentric jump) at 60 and 80% effort, respectively. Using a force platform (HUR Labs, Tampere, Finland), participants performed 3 maximal countermovement jumps with a 30 second rest between each jump. Following the countermovement jumps, the squat jump technique (hands held at the hips, after the eccentric motion lowering the centre of mass, a two-second isometric position was held before the concentric ascend) was instructed to the participants, and 2 sets of 3 sub maximal squat jumps at 60 and 80%, respectively were performed. Subsequent to the warm-up jumps, 3 maximal squat jumps with a 30 second rest between each jump were performed. The best jump identified as the one producing the highest vertical displacement of the centre of mass was selected for each of the two jumping techniques. Additional characteristics such as rate of force development, peak power, acceleration, displacement and velocity were calculated and analysed by Charlie Davids, and will be used in his thesis.

3.3.2 One-Repetition Maximum

Barbell back squat one-repetition maximum (1RM) was performed to serve as a measure of baseline strength, but also for the determination of subsequent training loads during the study. Several warm up sets were performed, gradually increasing in load and decreasing in repetitions, i.e: 10-6-3-1 repetitions with adequate but self-selected rest in between. Following the warm-up, single repetitions were performed, with 3 minutes of rest in between, until an attempt was deemed unsuccessful. Failing to complete the concentric (raising) portion of the movement or not achieving the specified range of motion (femur approximately horizontal to the ground) were deemed as an unsuccessful attempt. The highest load that was successfully completed was recorded as the 1RM-score. Investigators recorded the individual rack heights and safety heights during the familiarisation, so the testing sessions were equivalent during the pre- and post-test.

3.3.3 Maximal Voluntary Isometric Contraction

Following the 1RM back squat, the maximal voluntary isometric contraction (MVC) strength of the knee extensors and the knee flexors of the dominant leg was assessed in an isokinetic dynamometer (CSMI Humac Norm Model 770, USA). Participants were seated upright with a chair angle of 85 degrees, the back of the knees positioned at the edge of the seat with their dominant leg strapped to the lever arm of the machine. The lever arm was fixed at an angle corresponding to 70 degrees of knee flexion (full knee extension defined as 0 degrees of flexion). The axis of rotation of the dynamometer lever arm was visually aligned to the lateral femoral condyle. Prior to the individual chair adjustments that was recorded, femur length and the position (distal and lateral) of the near infrared spectroscopy (NIRS) equipment was recorded. Identical pre- to post training positioning of the seat and dynamometer lever arm was employed for each subject. Following three warm-up sub-maximal voluntary contractions of the knee extensors, participants performed 3 x 3-second maximal voluntary contractions, each separated by 90 seconds of rest. The same procedure was repeated with the knee flexors. The participants were instructed to apply force as rapid and hard as possible for the entire 3 seconds. The maximum torque value generated out of the three isometric contractions was recorded as the MVC, and rate of torque development was calculated over the initial 250 ms of that contraction (Humac extremity software). Data analysis was performed by Charlie Davids.

In contrast to the 1RM test where the achievement relies upon a larger set of determinants, including a high demand of the technical execution of the movement, the Humac offers a more controlled and isolated form of strength testing. The method is demonstrated to provide a high intrarater reliability (Habets, Staal, Tijssen, & van Cingel, 2018) and inter-reliability score (de

Araujo Ribeiro Alvares et al., 2015), hence a commonly used method to assess changes directly in muscle strength while reducing possible confounders such as: Learning effects, coordination, stability, etc.

3.3.4 Isokinetic Knee Extensor Endurance Task

After sufficient rest (approximately 5-10 minutes), participants completed an isokinetic knee extensor endurance task (Cybex Humac Norm Model 770, USA). The task consisted of 50 x sequential isokinetic knee extension contractions at a rate of 90 degrees per second. Knee flexion speed was set at 300 degrees per second, to allow a natural passive flexion to occur. Participants were instructed to kick out against the lever arm as rapidly and as fast as possible during each of the contractions, and were asked not to attempt to pace themselves over the 50 repetitions. Subjects were provided visual feedback of the magnitude of force from the torque monitor in an attempt to encourage them to reach maximum force for each contraction, and to make the best effort in an attempt to maintain the force during each contraction. The decrement in torque following the 50 contractions was recorded as the fatigue index, and the accumulation of torque produced with each contraction was recorded as the total work capacity.

3.4 Tissue Oxygenation

During the isometric/isokinetic tasks, contraction-induced changes in the oxygenation of skeletal muscle was assessed via NIRS. This has previously been demonstrated to be both reliable and valid for the measurement of the skeletal muscle oxidative metabolism (Ferrari, Muthalib, & Quaresima, 2011). Prior to the measure, the NIRS device was placed on the distal portion of the m. vastus lateralis of the dominant leg and secured in place. The portable NIRS device emits light from three optodes between wavelengths of 760 to 850 nm. Oxyhaemoglobin (O2Hb) and deoxyhaemoglobin (HHb) absorb light at different wavelengths, therefore by emitting light at different wavelengths and calculating the absorption and refraction ratios of the light, this produces relative changes of haemoglobin concentrations within the capillaries of the skeletal muscle. To avoid interference of ambient light on the light emitted from the NIRS optodes, a black cloth was secured over the NIRS device. Based on the NIRS data acquired from the testing during familiarisation, the lighting in the room influenced the haemoglobin detection. Therefore, during the pre- and post-testing, light was turned off. Further, data from the NIRS probe were recorded continuously online at 10Hz for later analysis. Analysis of NIRS data was performed with the Oxysoft V.3.0.53 software (Artinis Medical Systems, Netherlands) which uses the modified Lambert Law to correct for the scattering of light within tissue. Using the relative concentrations of both oxy- and deoxy-haemoglobin, other

measures can be derived by the software, such as total haemoglobin (tHb) (tHb = O2Hb + HHb) and tissue saturation index (TSI) (TSI = [O2Hb / tHb] x 100), which provide an indication of the volume of blood within the skeletal muscle, and the skeletal muscle oxygen saturation, respectively. Researcher Mr. Charlie Davids was responsible for the acquisition and analysis of the NIRS data.

3.5 Determining the Occlusion Pressure

Subsequently to the baseline visits, participants that were allocated to the BFR-RT had their individual BFR cuff pressure determined using a Doppler ultrasound (table 3). Following 10 minutes of seated rest, the same BFR cuff that was used in the training intervention was positioned on the proximal thigh of the dominant leg. The pulse and blood flow at the posterior tibial artery was then detected using a hand-held Doppler probe. Both auditory and visual information from the ultrasound were used to determine if the pulse was present. Once the pulse was detected, the cuff was inflated to 100 mmHg for 30 seconds. Next, the cuff pressure was increased incrementally by 40 mmHg until the arterial flow was no longer detected. Pressure was then decreased in 10 mmHg increments until arterial flow returned, the least amount of pressure needed for total arterial occlusion was selected as the 100% occlusion pressure. Following the first trial, a second attempt was performed to check the reliability of the occlusion pressure measurement. Investigator Mr. Charlie Davids had the key responsibility of the individual occlusion pressure assessment, while investigator Mr. Tore Christian Næss assisted.

Table 3. Maximum and relative occlusion pressure.

Maximum AOP (mmHg): Mean ± SD (min-max)	60% AOP (mmHg): Mean ± SD (min-max)
180±23 (150-240)	108±14 (90-144)

Abbreviations: mmHg; millimeter of mercury

3.6 Muscle Biopsy

Muscle biopsies were collected prior and after the training block from the *m. vastus lateralis* to evaluate changes in muscle fiber cross-sectional area, fiber type distribution and anabolic signalling. Muscle tissue was sampled under local anesthetic from the participants' dominant leg before the first training session and after the last training session (approximately 7-10 days of recovery after the last exercise bout and approximately 3-5 days after the last test session). Further, 3 more muscle biopsies (0, 24 and 48-h) were collected after an acute resistance exercise session (28th), to study the phosphorylation of key intramuscular signalling proteins and important heat shock proteins, thus a total of 5 samples were collected from each participant. A certified and competent researcher

(professor Truls Raastad) with several years of experience in biopsy sampling was in charge of this procedure. The targeted area of the biopsy was first disinfected by chlorhexidine followed by local anesthesia (Xylocaine adrenaline, $10mg \cdot ml^{-1}$+$5\mu g \cdot ml^{-1}$, AstraZeneca, London, UK). Subsequently, a 1-2 cm wide cut and 1.5-2.2 cm deep section into the skin and muscle fascia was conducted with a scalpel. A 6 mm Bergström biopsy needle (Pelomi, Alberslund, Denmark) was inserted through the section, where 1-3 muscle samples were taken (approximately 150 mg in total). A vacuum tube needle was attached to the biopsy needle to pull the muscle tissue into the needle. Following the procedure, the section was closed with strips and the muscle sample was cleaned for connective tissue, adipose tissue and blood. Further, the cleansed samples were stored in eppendorf tubes and directly stored in a -70°C freezer. Participants were given wound care advice (see attachment 12) and were closely followed up during the subsequent training sessions.

3.7 Western Blotting

Western blotting was used to analyse muscle tissue for phosphorylation and total protein content of the following proteins associated with skeletal muscle hypertrophy: p-70S6kinase (protein 70 ribosomal S6 kinase), 4E-BP1 (Eukaryotic translation initiation factor 4 element-binding protein 1), ERK1/2 (Extracellular signal-regulated kinase 1/2) and rpS6 (Ribosomal protein S6). Investigator Mr. Tore Christian Næss was responsible for the analysis, which was conducted in the muscle laboratory at the Norwegian School of Sports Sciences. In western blotting, the proteins are separated based on their molecular weight through a process of gel electrophoresis. Further, the proteins are transferred to a membrane, producing a band for each distinct protein (Fig. 4), which are incubated with the specific antibodies to the protein of interest (Mahmood & Yang, 2012). The unbound antibody is then washed off, leaving the attached antibody to the specific protein of interest. At last, the chosen antibody binds to the specific protein. The thickness of the

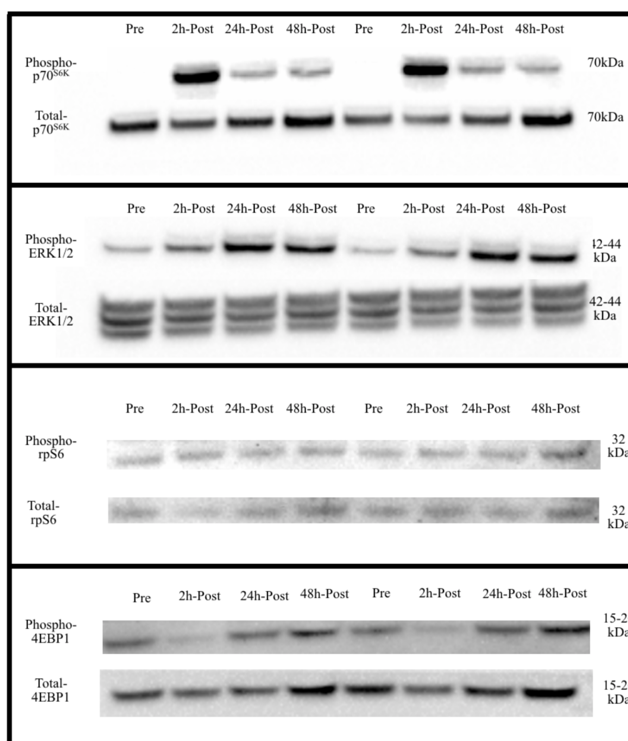

Figure. 4. Panel of representative protein immunblots for $p70^{S6K}$ (phosphorylated at Thr^{389} and total), ERK1/2 (phosphorylated at Thr^{202}/Tyr^{204} and total), rpS6 (phosphorylated at $Ser^{235/236}$ and total) and 4EBP1 (phosphorylated at $Thr^{37/46}$ and total). All samples from each participant were always quantified in the same blot, and all samples were run in duplicates.

bands resemble the quantity of protein present, thus a protein standard can indicate the quantity of proteins located to the band. Figure 4 is added to illustrate the end results by western blotting achieved from the BFR-study.

3.7.1 Homogenisation

The muscle biopsy samples were stored in a minus 80ºC freezer until they were homogenised. Approximately 50 mg of muscle tissue was homogenised in a solution of 1 ml T-PER (Tissue Protein Extraction Reagent, Thermo scientific, Rockford, IL, USA) and 20 µl EDTA (Ethylene Diamine Triacetic Acid, Thermo scientific, Rockford, IL, USA). For samples that were either above or below the threshold of 50 mg muscle tissue, the solution was adjusted thereafter. Each muscle sample was homogenised for the duration of 3-5 seconds, followed by another 3-5 seconds, or until no intact tissue was seen. Succeeding, the samples were shuddered in a refrigerator for 30 minutes and then centrifuged at 10 000G for the duration of 10 minutes at a temperature of 4ºC. The supernatant of each sample was transferred to 1.5 ml eppendorf tubes, before they were centrifuged one additional time with the same settings as above. After the second centrifugation, the supernatant was pipetted into a new eppendorf tube of 1.5 ml and further divided into 25 µl aliquots added to 10 x 0.2 ml eppendorf tubes. The final eppendorf tubes of 25 µl muscle sample solution were then stored in a minus 70ºC freezer.

3.7.2 Total Protein Content

Samples were first evaluated for the total concentration of protein by using the RC/DC Protein Assay kit from BioRad (Herkules, CA, USA). As a known standard protein concentration, the bovine gamma globulin was selected with a spectrum of 0.125; 0.25; 0.5; 1; 1.5 $\mu g \cdot ml^{-1}$. Samples were diluted in a 1:4 ratio with dH_2O, in such manner that the protein concentrations should be within the area defined by the standard protein concentration. In 96-well microplates (Greiner Bio-one International AG, Kremsmünster, Germany), the samples were pipetted in triplicates of 5 µl in each well. Following the pipetting of samples and the protein standard, a 25 µl reagent A*S (alkaline copper tartrate solution, Bio-Rad Laboratories Inc., USA) and a 200 µl reagent B (diluted folin reagent) were added into each well with a multichannel pipette. The 96-well microplates were then kept in a dark environment for the duration of 15 minutes, subsequently, the plate was analysed by ASYS Expert 96 from Biochrom (Cambridge, UK). The protein concentrations (CV<10%) were then calculated from the KIM Immunochemical Processing Software 32.

Investigator Mr. Tore Christian Næss had the key responsibility of the analysis of the total protein concentration.

3.7.3 Western Blotting Procedure

Muscle tissue samples were diluted in dH_2O and added a sample reducing agent (10x, NuPAGE, NP0009, Invitrogen) and a LDS sample buffer (4x, NuPAGE, NP0007, Invitrogen) in relation to the specific protein concentration of that sample (protein concentration of 15 µg) . Following, the samples were heated to reach 70ºC for the duration of 10 minutes before they were pipetted into a 10-well gel (NuPAGE 4-12% Bis-This Gel, 10 well, NP0321BOX, Invtrogen). A 5 µl weight marker (Protein Ladder PS 11, cat#310005, GeneOn., Germany) was pipetted into the first and last well of each specific gel. Further, a 30 µl solution of each sample were pipetted into the rest of the eight wells, with a duplicate of every participant on the same gel. Electrophoresis (SDS-PAGE electrophoresis) were conducted with chambers (Novex Mini-Cell, XCell SureLock, Invtrogen) at 200 Volt with a duration of 45 minutes using a running buffer: 100 ml MES/MOPS SDS running buffer (20x NuPAGE, NP0002, Invitrogen) and 1900 ml dH_2O. Subsequently, the samples were transferred to a polyvinylidene difluoride (PVDF) membrane (Immuno-blot, Cat.#1620177, Bio-Rad). Prior to the blotting stage, the membranes where activated with methanol (Merck KgaA, 1.06007.2500), dH_2O and a transfer buffer: 50 ml transfer buffer (NuPage, NP0005, Invitrogen), 100 ml methanol, 850 ml dH_2O and 1 ml antioxidant (NuPAGE, NP0005, Invitrogen). Membranes were further blocked for 2-hours at room temperature in 5% fat-free skimmed milk (Skimmed milk powder Merck KGaA, Germany), and a 0.05% TBS-t solution (Tris-buffered saline 10x TBS, cat#1706435, Bio-Rad and Tween 20 VWR International, Radnor, Pa, USA) and incubated over night at 4ºC with primary antibodies (table 4), followed by incubation with the specific secondary antibodies for 1-hour at room temperature. Membranes were washed in the 0.05% TBS-t solution between stages and protein stripping was performed by using Restore Western Blot Stripping Buffer (Pierce Biotechnology, Rockford, IL, USA) for 10 minutes. The membranes were finally incubated for the duration of 5 minutes of a 1:1 solution of a luminol enhancer solution (cat#1859024) and stable peroxide buffer (cat#1859025) immediately before the imaging was conducted with ChemiDoc MP Imaging system, and further analysed with Image Lab Software (Bio-Rad Laboratories, Hercules, CA, USA). All samples from each participant were always loaded on the same gel, and band intensity comparisons were only done within each blot.

Table 4. Primary- and secondary antibodies used in immunoblotting.

Antibody	Producer	Host	Ratio	Cat.nr
Total-p70^{S6} Kinase (49D7)	Cell Signaling	Rabbit	1:1000	#2708
Phospho-p70^{S6} Kinase (Thr^{389}) (108D2)	Cell Signaling	Rabbit	1:1000	#9234
Total-p44/42 MAPK (ERK1/2)	Cell Signaling	Rabbit	1:1000	#9102
Phospho-p44/42 MAPK (ERK1/2) (Thr^{202}/Tyr^{204})	Cell Signaling	Rabbit	1:1000	#9101
Total-S6 Ribosomal Protein (5G10)	Cell Signaling	Rabbit	1:1000	#2217
Phospho-S6 Ribosomal Protein (Ser^{235}/^{236})	Cell Signaling	Rabbit	1:1000	#2211
Total-4E-BP1 Antibody	Cell Signaling	Rabbit	1:1000	#9452
Phospho-4E-BP1 (Thr^{37}/^{46})(236B4)	Cell Signaling	Rabbit	1:1000	#2855
Anti-rabbit IgG, HRP-linked Antibody	Cell Signaling	Goat	1:3000	#7074

For the detailed procedure regarding the western blotting protocol used in this study, see section; List of attachments 10.

3.8 Immunohistochemistry

Immunohistochemistry was used to analyse the myonuclei concentration and satellite cells in the muscle samples. Further, the technique was applied to distinguish the type I and type II fibers a part, quantifying the corresponding numbers and size of fibers. Researcher Ms. Maria Moen was accountable for the immunohistochemistry analysis for the BFR-study and the results will be used in her master thesis.

3.9 Thigh Girth Measurement

During the postliminary acute study, segmental leg limb volume were quantified using the formula to calculate the volume of a truncated cone using girth and height: Vol = $h/12\pi \times (c_1^2 + c_2^2 + (c_1)(c_2))$ (Katch & Katch, 1974). A measurement band was used to assess the girth of the gluteal furrow (c_1), directly above the knee (c_2) and the distance between the two points (h). Thigh volume was evaluated at five time points during the acute study; pre-, post-, 2-h post-, 24-h post- and 48-h post-exercise, to evaluate the acute muscle swelling effects of BFR-RT and HL-RT. Subjects were asked to stand with their centre of mass above the dominant leg, while the measured leg was in a relaxed position, positioned at a 20 cm box. All segmental measurements were conducted by Mr. Tore Christian Næss to reduce possible measurement bias.

3.10 Perceived Muscle Soreness

Previous to the girth measure and functional tests conducted in the acute study, subjects were told to rate their perceived muscle soreness from their legs. They were first asked to rate the subjective muscle soreness in both of their thighs in a standing position from a 0-10 scale. An A4 paper sheet with representative icons (smileys representing different humanoid faces ranging from sad to smiling) corresponding to the numeric values of the test were placed on the wall in front of the subjects to aid the decision making. Participants were also told to try evaluate the muscle soreness without considering the influence of the muscle biopsy, which they had taken prior to this assessment. Following the perceived muscle soreness in a standing position, subjects were instructed to perform a few repetitions of the squat and further rate the perceived thigh muscle soreness. Thus, two numeric values were recorded for each subject regarding the perceived muscle soreness. Researcher Mr. Tore Christian Næss governed the test during the acute study and ensured that all participants were asked the same question and given the same information stated above.

3.11 Statistical Analysis

All data were tested for Gaussian distribution using the Shapiro-Wilk normality test. A two-way repeated measures ANOVA was used to assess the interaction of groups and time-point effects of phosphorylation status. Log transformation (base 10) was used to make phosphorylation data less skewed. Paired t-tests were used to assess pre and post changes of muscle function and muscle hypertrophy, while unpaired t-tests were used to evaluate the differences between groups. Data are presented as mean±standard deviation. The level of significance for all statistical analyses was set to $p < 0.05$ and data were analysed and graphically presented using Prism 8 (San Diego, CA, USA, https://www.graphpad.com).

4. Theory

The following chapter is allocated to the theoretical framework undermining this master thesis. A thorough review of BFR-RT, muscle hypertrophy and the cellular and molecular components underlying this physiological adaptation will be investigated. Further, a brief inquiry regarding the literature of the acute responses of a single bout of exercise driving the anabolic signalling responses involved with the two different training modalities (BFR-RT vs. HL-RT) will be explored.

4.1 Blood Flow Restriction Resistance Training

An abundant amount of manipulations and alterations can be rendered to create a BFR-RT protocol to induce hypertrophy. The main determinants that underpin these adaptations in regard to the BFR-RT protocol is: training load, exercise volume, applied limb pressure, continuously/intermittent occlusion, cuff size, inter-set recovery and whether taking the set to failure, or the use of a predefined sub-maximal amount of repetitions. Among the BFR-literature reviewed in the meta-analysis of Lixandrao and colleagues (2018), mounting evidence support the effectiveness of several BFR-RT protocols to induce muscular hypertrophy. For instance, Martin-Hernandez and colleagues (2013) used 30-15-15-15 reps with 20% of 1RM using continuously blood flow restriction with an absolute pressure at 110 mmHg with a 140 mm cuff, while Clark and colleagues (2011) used 3 sets to concentric failure with 30% of 1RM using continuously blood flow restriction inflated to 130% above the resting brachial systolic blood pressure with a 60 mm cuff. These studies demonstrated different alterations regarding important factors underpinning the stimulus to the muscle, hence the muscular adaptations of several training sessions performed consequently. Table 5 summaries a wide variety of different resistance training protocols frequently used in the BFR-literature reviewed by the author. In the subsequent section, two of the main determinants driving the hypertrophy from the BFR-RT protocol are highlighted.

4.1.1 Pressure

A distinctive feature of blood flow restriction is determining the degree of pressure applied to the extremity. Pressure could be measured with a sphygmomanometer consisting of an inflatable cuff and a measuring unit, i.e. a mercury manometer. An absolute pressure in X mmHg could then be applied to the limb, used in several investigations seen from table 5. The range of absolute pressure among these investigations vary greatly, 40-200 mmHg, in other terms, the largest value of pressure applied is five fold the lowest pressure. This can have direct implications of the magnitude and the variations of the stimulus induced by the resistance training of different subjects and increase the

risk for some subjects to be placed under complete arterial occlusion (Dankel, Buckner, et al., 2017). It is speculated that the quantity of pressure follows a dose responsive manner as the hormesis curve (Loenneke, Thiebaud, Abe, & Bemben, 2014). If so, an optimal pressure gradient will inhibit venous blood flow while reducing the arterial inflow of blood, thus a blood pooling will be evident in the muscle tissue (Iida et al., 2007).

Another way to assess limb pressure while minimising the potential errors associated with an absolute value of X mmHg, is to apply the pressure relative to the arterial occlusion pressure. AOP is often defined as the least amount of pressure required for a complete reduction in arterial

Table 5. A summary of BFR-resistance training studies showing the discrepancies in several important determinants underpinning the applied stimulus of a BFR-training session.

Study	Cuff width (cm)	Pressure	Training load (%1RM)	Volume (set x rep)
Barnett et al., 2016	unknown	40% AOP	30	30,15,15,15
Clark et al., 2011	6	130% resting SBP	30	3 x failure
Cook et al., 2017	6	150% resting SBP	30	3 x failure
Dankel et al., 2017	3	160 mmHg	unknown	4 x failure
Dankel et al., 2017	5	40% AOP	unknown	4 x failure
Drummond et al., 2008	unknown	200 mmHg	20	30,15,15,15
Ferguson et al., 2018	13	110 mmHg	20	30,15,15, failure
Freitas et al., 2017	5	160 mmHg	20	30,15,15,15
Fry et al., 2010	unknown	200 mmHg	20	30,15,15,15
Iversen et al., 2010	14	100 mmHg	unknown	30,15,15,15,15
Jessee et al., 2018	5	40% AOP	30 and 50	4 x failure
Laurentino et al., 2012	17.5	80% AOP	20	3 x 15
Laurentino et al., 2016	5 and 10	80% AOP	20	3 x 15
Lixandrao et al., 2015	unknown	40% and 80%	20 and 40	2-3 x 15
Loenneke et al., 2015	5	40%, 50% and 60%	20 and 30	30,15,15,15
Madarame et al., 2008	4	160 mmHg	30	30,15,15
Manini et al., 2012	11	150% resting SBP	20	4 x failure
Martin-Hernandez et al., 2013	14	110 mmHg	20	30,15,15,15
Neto et al., 2015	6 and 10	80% AOP	20	30,15,15,15
Nielsen et al., 2017	13.5	100 mmHg	20	4 x failure
Sousa et al., 2017	18	80% AOP	30	4 x failure
Souza et al., 2018	18	50% AOP	20	30,15,15,15
Suga et al., 2009	18.5	130% resting SBP	20	2 x 30
Vechin et al., 2015	18	50% AOP	20	30,15,15,15
Wernbom et al., 2013	13.5	90-100 mmHg	30	5 x failure
Yow et al., 2018	14	80% AOP	30	30,15,15,15

Abbreviations: mmHg; millimeter of mercury, AOP; arterial occlusion pressure, SBP; systolic blood pressure

blood flow, which could be measured with the use of ultrasound. Several blood flow restriction investigations have used this method to assess relative pressure for their test-subjects (table 5). The range of relative pressure across these investigations cover 40-90% of AOP.

A study conducted by Lixandrão and colleagues (2015) was carried out to explore muscle hypertrophy and strength comparing 40 and 80% of AOP in response to 12 weeks of resistance training with blood flow restriction using two different training intensities of 20 and 40% of 1RM. Regarding muscle mass, increasing pressure from 40 to 80% when training with 20% of 1RM, resulted in an increase in muscle mass by 2.44%. However, when exercising at 40% of 1RM, a larger pressure did not result in any meaningful improvements in either strength or hypertrophy (Lixandrao et al., 2015). These results are reproduced by Dankel and colleagues (2017) showing that occlusion protocols utilising a low training load (10-20% of 1RM), benefits from increased blood flow restriction pressures. Thus, an increase in AOP could elicit in a meaningful muscular adaptation only when the BFR-RT protocol utilise a training load at the lower range.

A recent study by Mouser and colleagues (2017) studied the hemodynamics of blood flow at different pressures (rest-100% of AOP). One interesting finding was that the arterial blood flow was relatively unchanged between 50 and 90% of the arterial occlusion pressure, thus the blood flow reduced in a non-linear fashion with the increasing pressure. It is also documented that a larger percentage of AOP is accompanied by increased perceptual responses such as discomfort and increased rating of perceived exertion leading to a decrease in exercise volume compared to lower restriction pressures (Mattocks et al., 2017). Another group of researchers studied the muscle adaptations in response to 8 weeks of BFR-RT with the use of 40% of AOP and 90% of AOP applied to the same cuff (unknown width) (Counts et al., 2016). Both training protocols induced muscle hypertrophy, enhanced peak isometric strength and muscular endurance with no significant differences between the two protocols. A secondary recent study indicate that arterial occlusion pressure as low as 50% can induce similar hypertrophy and strength increases compared to traditional heavy resistance training (Kim et al., 2017).

Despite the literature of pressure regarding blood flow restriction training, an optimal pressure is yet to be confirmed, and the existing methods are likely to be imperfect. However, based on the studies above, it could be more conservative to select a lower relative pressure to; (1) reduce the probability of prolonged exposure to ischemia (associated with skeletal muscle damage); (2) more likely able to achieve a sufficient training volume; (3) reduce unnecessary discomfort for the subjects, and (4) reduce dropout rate of participants. In addition, it is important to highlight that BFR-pressure should be considered in relation to the width of the cuff.

4.1.2 Cuff Width

Reviewing the literature reveals that a broad range of different cuff widths (30-185 mm) are commonly applied among the blood flow restriction investigations (table 5). An important aspect regarding cuff width is the amount of applied pressure required to achieve a venous blood pooling without a complete arterial occlusion, which is suggested as an important stimulus to induce muscle hypertrophy associated with muscle swelling (Loenneke, Fahs, Thiebaud, et al., 2012). Jessee and colleagues (2016) studied 249 subjects in relation to three commonly used cuff widths (50, 100 and 120 mm) in response to the pressure required for a complete AOP. An interesting finding was that the narrow cuff (50 mm) required the greatest pressure to occlude blood flow followed by the larger cuff (100 mm), while the largest cuff (120 mm) required the least pressure. Quantification data from another investigation supports that narrow cuffs (50 mm) compared to larger cuffs (135 mm), requires greater (61%) absolute pressure to induce complete arterial occlusion (235 mmHg vs. 144 mmHg, respectively) (Loenneke, Fahs, Rossow, Sherk, et al., 2012). This relationship is evident in several investigations (Crenshaw, Hargens, Gershuni, & Rydevik, 1988; Graham, Breault, McEwen, & McGraw, 1993; Loenneke, Fahs, Rossow, Sherk, et al., 2012). It is therefore proposed that the absolute pressure should be applied in relation to the width of the cuff. However, a recent study conducted by Mouser et al. (2018), examined the acute hemodynamic responses to the commonly applied 100 mm- and the 120 mm cuff. A noteworthy result from this investigation, revealed that when a relative equal pressure was applied to the cuffs, there were no significant differences among blood flow, mean blood velocity, peak blood velocity and artery diameter. This indicates that the 100 mm and the 120 mm cuff apply an equivalent blood flow stimulus when using equal relative AOP. Supporting this assertion is results from another study comparing 50, 100 and 120 mm cuffs applying 40 to 90% of AOP, showing that relative pressures appear to elicit similar blood flow responses, despite different cuff widths (Mouser et al., 2017).

The studies above are more concerned with pressure regulations and the acute responses to hemodynamic variables associated with the applied stimulus of a BFR-RT protocol. A further relevant aspect regards the physiological adaptations when frequent training sessions are performed with BFR-RT with a narrow cuff compared to a wider cuff. It is speculated that a more narrow cuff should theoretically compress less muscle tissue, thus it is plausible to theorise that a more narrow cuff is more efficient in increasing muscle growth. This question was addressed by Laurentino et al. (2016), studying the cross sectional area (CSA) and maximum strength of the elbow flexors in response to 6 weeks of either BFR-RT with narrow cuffs (50 mm) or BFR-RT with wider cuffs (100 mm). To equalise the BFR-RT stimulus of both groups, a relative pressure at 80% of AOP was

applied with an equivalent training volume. Both BFR-RT protocols resulted in increased CSA (≈10%) and strength (≈12%). Despite the differences of cuff widths, no significant changes were identified regarding changes in elbow flexors CSA (p=0.97) or muscle strength measured as 1RM (p=0.96) between the narrow and wide cuff protocol.

In defiance of the accumulated evidence supporting the efficiency of BFR-RT, little is currently known about the long term BFR-RT adaptations to different cuff widths. It is therefore too ambiguous for the author to give a direct answer in this short review on the paramount question: Does cuff width influence the muscular adaptations accompanied by BFR-RT? Nevertheless, it is important to consider the cuff width in relation to the quantity of the applied pressure to regulate and control the degree of added occlusion to the specific limb.

4.2 Muscle Hypertrophy

It is thoroughly established through the scientific endeavour of exercise physiology that the mammalian skeletal muscle can adapt to a progressive overload of resistance training, thus alter its morphological conditions i.e. increasing in muscle fiber hypertrophy (growth of existing cells) (Schoenfeld, 2010). It was previously believed that a training load in the higher range (>70% of 1RM) was needed to induce significant muscle hypertrophy (McDonagh & Davies, 1984). However, during the latest decade, experimental studies have demonstrated that training loads in the lower range (<40% of 1RM) can result in muscle hypertrophy if the sets are performed to exhaustion (Nóbrega, Ugrinowitsch, Pintanel, Barcelos, & Libardi, 2018; Ogasawara, P. Loenneke, Thiebaud, & Abe, 2013) or in combination with blood flow restriction (Lixandrao et al., 2018; Lixandrao et al., 2015). Through the physiological adaptations when several resistance training sessions are performed consecutively, the contractile elements enlarge and the extracellular matrix expands (Vierck et al., 2000). It is plausible to consider that the majority of the exercise-induced skeletal muscle hypertrophy results from an increase of sarcomeres and myofibrils added in parallel to the muscle fiber (Paul & Rosenthal, 2002). Further, the quantity of the myofibrillar contractile proteins; actin and myosin increases, thus amplify the individual diameter of the muscle fiber, leading to an increased overall muscle cross-sectional area (Toigo & Boutellier, 2006). The following sections will review the cellular and the molecular mechanisms underpinning the skeletal muscle hypertrophy seen with the resistance training modalities of traditional strength training and blood flow restricted strength training.

4.2.1 Muscle Protein Synthesis

Measuring the rate of muscle protein synthesis is adopted for its gauging efficiency in the assessment of the acute responses to different stimuli (i.e. training load, exercise volume, nutrition, etc.) (Atherton & Smith, 2012). Regarding the magnitude of the acute responses of the muscle protein synthesis to resistance training, a vast amount of experimental studies have been conducted over the last decades. For instance, when subjects exercised with low training load (≤ 40% of 1RM), no detectable increases of muscle protein synthesis was acquired, whereas increasing the training load to 60-90% of 1RM increased the muscle protein synthesis acutely up to 3 folded (Kumar et al., 2009). However, increases in the muscle protein synthesis accompanied by low-load training (≤ 40% of 1RM) is demonstrated to transpire if the sets are taken to failure (Burd et al., 2010) or performed with blood flow restriction (Fry et al., 2010; Fujita et al., 2007). Therefore, resistance training with low load accompanied by a reduction of the blood flow to the exercising muscle seems to be an effective strategy to increase the muscle protein synthesis. However, extrapolating the amplitude of an increased muscle protein synthesis cannot be directly compared to muscle hypertrophy; the muscle protein breakdown has to be considered.

4.2.2 Muscle Protein Breakdown

The amount of proteins within the muscle fibers are not only determined by the rate of the synthesis of newly formed proteins, but also the rates of breakdown of existing proteins. In mammalian skeletal muscle, three major pathways exists for the degradation of proteins: the ubiquitin-proteasome pathway, the lysosomal proteolysis and the calpain Ca^2 depended cysteine protease (Tipton, Hamilton, & Gallagher, 2018). Equally to the synthetic rate of proteins, the degradation of proteins can be measured with stable isotopic tracer methods (Kim, Suh, Lee, & Wolfe, 2016). From experimental studies investigating the muscle protein breakdown, the general view is that the muscle protein breakdown will acutely be upregulated the following hours after a resistance training session (Biolo, Maggi, Williams, Tipton, & Wolfe, 1995; Pasiakos & Carbone, 2014; Phillips, Tipton, Ferrando, & Wolfe, 1999). However, to the author's knowledge the relative increase of muscle protein breakdown comparing BFR-RT to traditional heavy resistance training is currently unknown. Nevertheless, it is tempting to speculate that the protein breakdown is slightly diminished with BFR-RT compared to heavy resistance training due to its subjective nature of low load training, resulting in a decreased degree of mechanical tension. This remains speculative and in either way, care should be selected if decreased/increased muscle degradation alone is extrapolated to muscle atrophy/hypertrophy, accordingly, the net protein balance should be acknowledged.

4.2.3 Net Protein Balance

The mammalian muscle protein are subjected to a perpetual modification through the adjustment of synthesising new proteins and the breakdown of existing proteins, briefly reviewed above. Since the magnitude of skeletal muscle hypertrophy is ultimately the product of the net protein balance (muscle protein synthesis minus muscle protein breakdown), schematic representation in figure 6, the total quantity of the skeletal muscle mass is therefore regulated by this ratio. Since the objective of this study is to investigate the acute anabolic effects of blood flow restriction training, a further delve into the mechanisms and pathways responsible for the up-regulation of the muscle protein synthesis (green box from figure 5) is necessary.

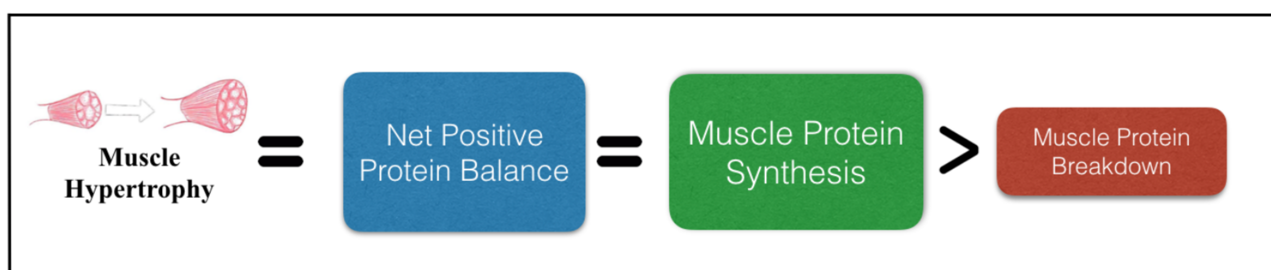

Figure. 5. Delineates the relationship of the muscle protein synthesis and the muscle protein breakdown obligatory to achieve muscle hypertrophy.

4.3 Mechanisms of BFR Strength Training Induced Muscle Hypertrophy

Despite the evident muscle hypertrophy achieved from BFR-RT studies reviewed in chapter 4.1, the attributed mechanisms underpinning the hypertrophy are poorly understood (Pearson & Hussain, 2015). It is theorised that the amplified levels of metabolic stress (i.e., accumulation of metabolites produced by an ischemic condition) seen with BFR-RT, is liable to further act on secondary mechanisms (figure 6) to increase muscle hypertrophy (Loenneke, Fahs, Wilson, & Bemben, 2011). Complementary to the metabolic stress, the mechanical tension (mechanically induced tension generated by force production and stretch) are regarded as fundamental to muscle growth (Schoenfeld, 2010). It is generally considered that the mechanical tension achieved from heavy resistance training agitate the integrity of skeletal muscle, resulting in mechanochemically transduced molecular and cellular reactions in the muscle fibers (Toigo & Boutellier, 2006). Nevertheless, the relative contribution of the metabolic stress and the mechanical tension achieved from BFR-RT is not well known, however, due to the subjective nature of low mechanical loads used in BFR-RT, it is plausible to speculate that the preeminent mechanism may be the metabolic stress. In either way, future studies should further highlight the proposed secondary mechanisms

deriving from the metabolic stress and the mechanical tension from BFR-RT, featured in figure 6 from Pearson & Hussain (2015). A brief review of two secondary mechanisms will be explored: Cell swelling and mechanotransduction.

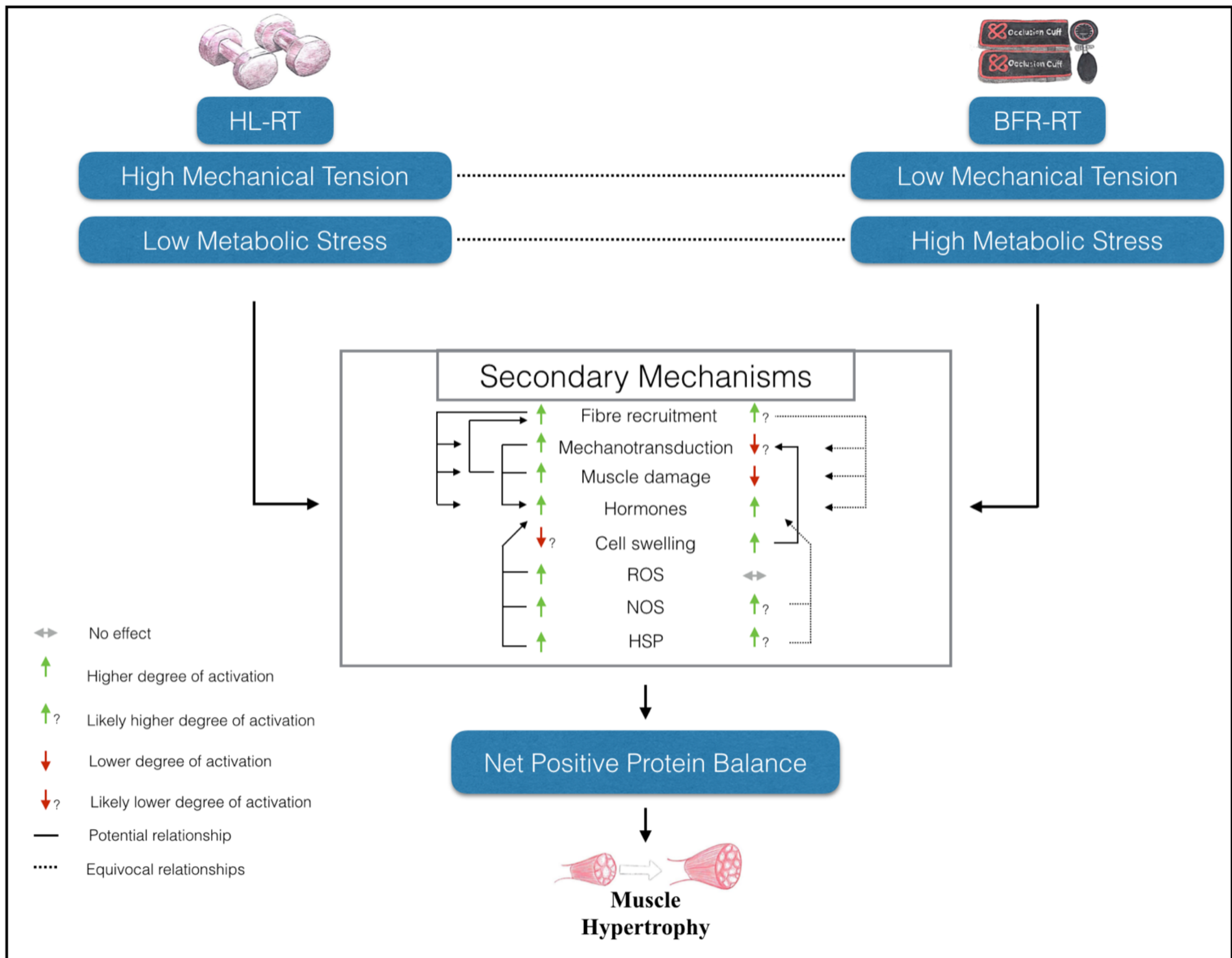

Figure. 6. Outlines the proposed undermining factors related to blood flow restriction exercise induced muscle hypertrophy. Note the unknown relative contributions of the secondary mechanisms and the ambivalent interrelationship of some of the mechanisms. Figure retrieved and modified from a review article by Pearson and Hussain (2015).

4.3.1 Cell Swelling

In light of recent evidence supporting the notion that the magnitude of the muscle swelling effect is equivalent comparing a bout of HL-RT (80% of 1RM) to a bout of BFR-RT (20% of 1RM, 160 mmHg) (Freitas et al., 2017), cell swelling augment its position as a potential secondary mechanism for BFR-RT induced hypertrophy. The results from a previous investigation of Loenneke and colleagues (2012) are consistent with the findings that BFR-RT induce significant muscle swelling post resistance exercise. Additionally, Jenkins and colleagues (2015) observed a greater increase in the acute muscle swelling with BFR-RT (30% of 1RM) than HL-RT (80% of 1RM). Thus, it is reasonable to consider that the mechanism of muscle swelling is one of the potential mechanisms of BFR-RT induced muscle hypertrophy. Further, the precise cellular mechanisms responsible for the

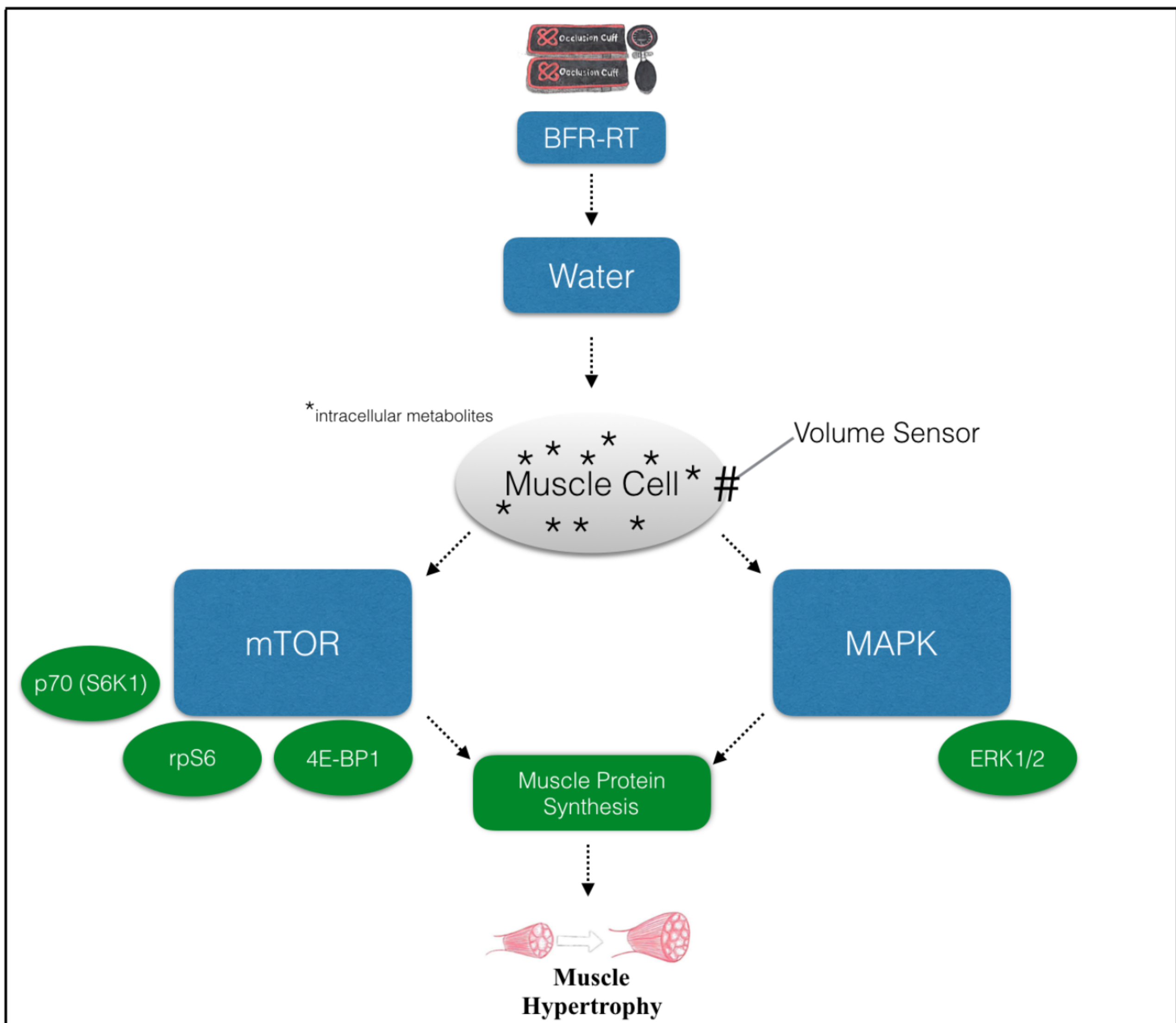

Figure. 7. A schematic framework of a possible mechanistic pathway crucial for blood flow restriction exercise induced muscle hypertrophy. Figure retrieved and slightly modified from Loenneke et al. (2012).

cell swelling with BFR-RT are not well understood. However, it is established that the applied pressure deriving from the cuff, creates a pressure gradient favouring arterial blood flow while inhibiting venous blood flow, hence an increased blood pooling (Pearson & Hussain, 2015). The subsequent intracellular swelling is thus considered to cause the cell to initiate a signalling cascade to augment its structural architecture (cell swelling signalling further reviewed in chapter 4.4.4). Figure 7 represent a hypothesised illustration of the mechanism adopted by Loenneke and colleagues (2012).

4.3.2 Mechanotransduction

Presented in chapter 4.3, external mechanical loading can ultimately induce skeletal muscle hypertrophy. Specifically, the mechanical tension activates sarcolemmal-bound mechanosensors (e.g., integrins and focal adhesions) that convert mechanical energy into chemical signals, thus the

mammalian/mechanistic target of rapamycin (mTOR) is activated (mTOR signalling reviewed in 4.4.1) and further promote an increase in the muscle protein synthesis (Fry et al., 2010). However, as previously discussed, the influence of mechanical tension in BFR-RT might not be significant due to low load resistance. While it could be disputed that the metabolic stress could mediate related mechanisms, thus the effects of mechanotransduction may be additive (Pearson & Hussain, 2015). Future studies should possibly emphasise the role of mechanotransduction as a mechanism of muscle hypertrophy regarding BFR-RT, since no direct evidence exists in the literature.

4.4 Molecular Signalling: the Underlying Mechanisms of Hypertrophy

The capacity of muscle fibers to hypertrophy in response to external loading and the molecular pathways underpinning this response have been extensively studied during the last decades (Marcotte, West, & Baar, 2015). This adaptation can derive from heavy load strength training or from low load BFR-RT, and several underlying mechanisms of the increased net protein synthesis and muscle hypertrophy have been investigated. To study the cellular and molecular mechanisms

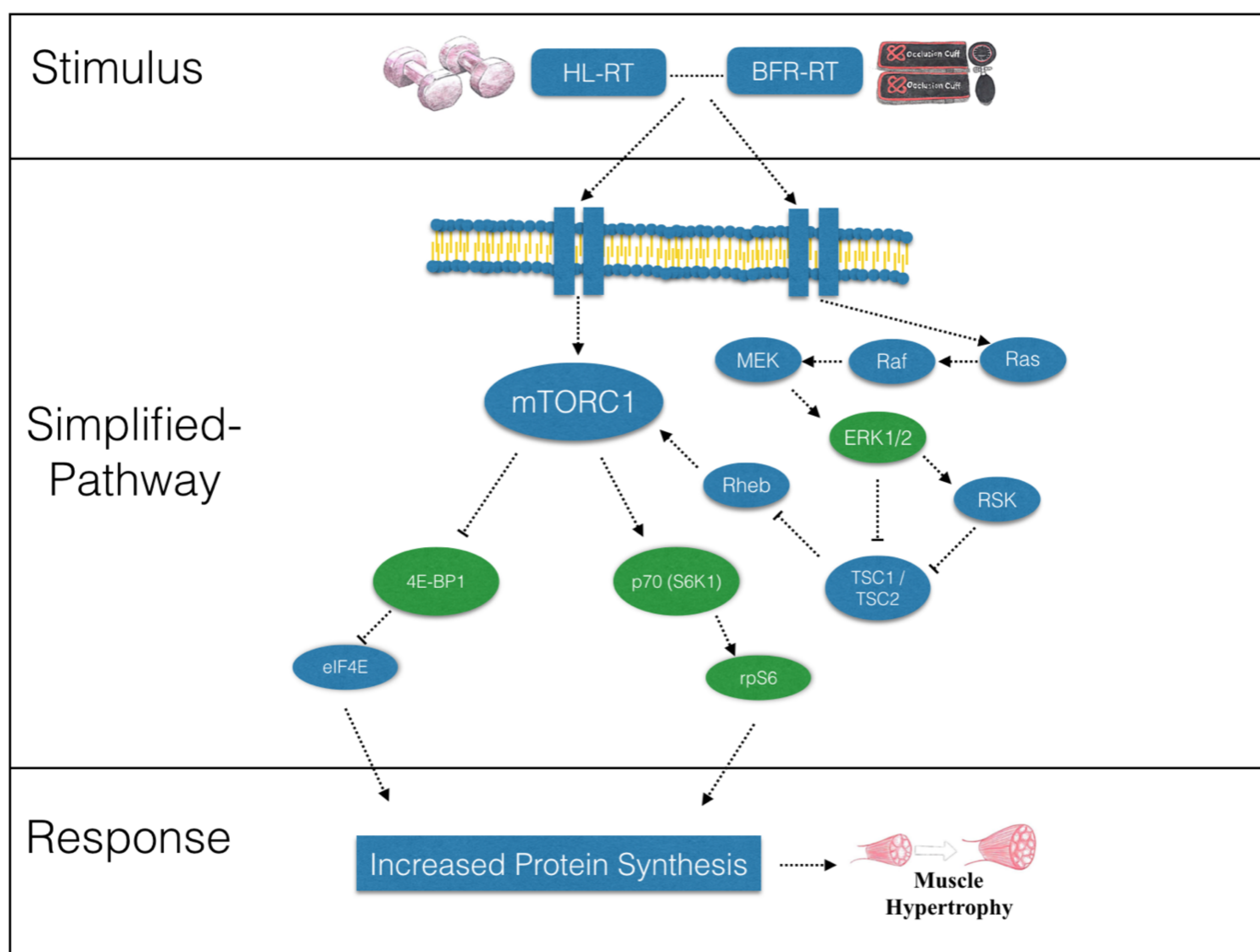

Figure. 8. A simplified delineation of the interrelationship of key anabolic signalling proteins working concurrently to induce muscle hypertrophy, analogous to a complex orchestra where each member has an essential role contributing to the overall goal. Green proteins are highlighted, thus the target for the BFR-study. Figure is based upon the complex anabolic signalling cascades reviewed by Laplante and Sabatini (2009).

underpinning the increased protein synthesis in response to strength training with BFR, muscle biopsies along with western blotting methodology were selected for this BFR-study. To the author's knowledge, an impartial amount of the studies investigating the specific hypertrophic responses to BFR-RT compared to HL-RT have implemented muscle biopsies. Consequently, the underlying molecular mechanisms responsible for the potent hypertrophic effects seen from BFR-RT are still being defined. A simplified schematic illustration of the key anabolic signalling proteins studied in the BFR-study are highlighted in green from figure 8.

4.4.1 Anabolic Signalling: mTor Signalling-Pathway

During the late 80's, the mTOR protein was discovered by a group of molecular exercise physiologists at the University of Basel (Wackerhage, 2006). The mTOR protein is a 289-kDa serine-threonine kinase that can increase protein synthesis through a process of translation of mRNA molecules into proteins inside the ribosome organelle of the muscle cell (Laplante & Sabatini, 2009). Further, the mTOR protein can activate the transcription of important genes related to the increased protein synthesis. Since the discovery of the mTOR, the protein has attracted a lot of scientific attention because of its capability to act as a master growth regulator of all cells (Laplante & Sabatini, 2009).

mTOR is divided into complex 1 (mTORC1) and complex 2 (mTORC2), where mTORC1 is at the centre of the mTOR pathway leading to the increased protein synthesis (Wackerhage, 2006). mTORC1 is activated by different stimuli, including resistance exercise, amino acids and hormones. An investigation sought out by Fry and colleagues (2010), found that mTORC1 activity (phosphorylated/total) increased significant 1 hour and 3 hours after a low-load BFR-RT workout compared to the same workout without any BFR. However, it is plausible to speculate that the differences of mTORC1 activity would be diminished if the low-load BFR workout was compared to heavy resistance training. Thus in our study, resistance training with blood flow restriction was matched with traditional heavy resistance training to get a better comparison and understanding of how the two training modalities equates. Accordingly, three important downstream proteins phosphorylated by mTORC1; p70S6kinase, RPS6 and 4E-BP1, will be studied in relation to BFR-RT vs. HL-RT.

4.4.2 Anabolic Signalling: p70S6kinase & rpS6

An activated mTORC1 can in turn phosphorylate the p70S6kinase protein, which further acts on several key proteins. The signalling cascade downstream from p70S6kinase includes activation through phosphorylation of eIF4B, rpS6 and SKAR, increasing protein synthesis through a cap-

depended translocation, ribosome biogenesis and mRNA biogenesis, respectively (Laplante & Sabatini, 2009). In addition, p70S6kinase inhibits eEF2k through phosphorylation, consequently increasing protein synthesis through an amplified process of translation and elongation.

Several investigations have been carried out to investigate the responses of p70S6kinase and rpS6 in relation to a traditional strength training stimuli; a significant phosphorylation of the proteins are prominent (Apro & Blomstrand, 2010; Farnfield, Breen, Carey, Garnham, & Cameron-Smith, 2012; Glover et al., 2008; J. J. Hulmi et al., 2009). Corresponding results are found when participants exercised with BFR-RT (Fry et al., 2010; Fujita et al., 2007; Gundermann et al., 2012; Wernbom et al., 2013). The phosphorylation of the p70S6kinase was increased by three-fold with low-load BFR-RT 1 hour post-workout, while the control group who performed the same exercise protocol with no occlusion did not reach statistical significance from baseline (Fujita et al., 2007). The increased phosphorylation of p70S6kinase following the BFR-RT resulted in a significant increase in rpS6 (phospho/total) along with an increased muscle protein synthesis (Fry et al., 2010), supporting the notion that BFR-RT does indeed enhance mTORC1-signalling and muscle protein synthesis through phosphorylations of both p70S6kinase and rpS6. However, the low-load BFR studies above used control groups who exercised with the same load, ergo, the training modality (BFR-RT vs. HL-RT) that is the most effective in inducing the acute muscle anabolism could still be questioned. To address this, we compared a frequent used BFR-RT protocol to a traditional resistance training program on the relative contribution of phosphorylation of p70S6kinase and rpS6.

A rather recent project from Nakajima and colleagues (2018) studied the effects of low microvascular O_2 partial pressures (P mvO_2) during contractions on muscle hypertrophy signalling. A noteworthy demonstration that muscle hypoxia is not obligatory for the hypertrophic responses to low-force electrical induced muscle contractions, however the reduced P mvO_2 did enhance the ribosomal protein S6 phosphorylation and potentiated the hypertrophic response. Although, the results are based on rodent subjects, future studies should investigate if these responses are comparable to human test subjects.

4.4.3 Anabolic Signalling: 4E-BP1

mTORC1 acts downstream on 4E-BP1, thus the 4E-BP1 gets inhibited through phosphorylation, which in turn detaches from the eIF4E protein (Holz, Ballif, Gygi, & Blenis, 2005). This specific detachment allows the ribosome to bind to the mRNA to initiate translation, thus increase protein synthesis (Wackerhage, 2006). In addition, the phosphorylation of 4E-BP1 regulates the availability

of elF4E which regulates the cap-depended translation in response to cell stress (Ayuso, Hernandez-Jimenez, Martin, Salinas, & Alcazar, 2010). Resistance exercise is indeed showed to inhibit the activity of 4E-BP1 immediately following exercise when mTORC1 is phosphorylated, gradually returning to baseline over the next 2-hours (Dreyer et al., 2006; Farnfield et al., 2012). However, during BFR-RT, the decreased phosphorylation of 4E-BP1 did not reach statistical significance, despite the tendency to a reduction in activation 1-hour and 3-hour post-workout (Fry et al., 2010). The literature is rather sparse to draw any certain conclusions on the relative contribution of the phosphorylation of 4E-BP1 regarding the hypertrophic adaptations attained through frequent low-load BFR-RT (Pearson & Hussain, 2015). This study will potentially provide further insight into the causal relationship of the molecular mechanisms associated with the hypertrophy observed with the BFR-RT exercise modality.

4.4.4 Anabolic Signalling: ERK1/2

As previously reviewed, cell swelling has been postulated to be one of the plausible cellular mechanisms responsible for the augmented protein synthesis, thus hypertrophy accompanied by BFR-RT (reviewed in chapter: 4.3.1). It is speculated that during BFR-RT, muscle swelling is detected by intrinsic volume sensors which leads to an activation of the mitogen-activated protein-kinase (MAPK) pathway. The extracellular signal-regulated kinase 1/2 (ERK1/2) which are proteins linked to osmosensing (Miyamoto, Teramoto, Gutkind, & Yamada, 1996), display an important MAPK signalling module, responsible for the adaptation of skeletal muscle in response to exercise stimuli (Loenneke, Fahs, Rossow, Abe, et al., 2012). In brief notion, the MAPK pathway includes a small G protein (RAS) and three protein kinases (RAF, MEK, ERK), and starts when a ligand bind to a transmembrane protein (receptor tyrosine kinase), and initiate the signalling cascade with translocation of ERK to the nucleus, resulting in increased transcription factors, thus increased gene expression (McCain, 2013). Research have demonstrated that the MAPK signalling pathway is increased with BFR-RT, including the phosphorylation of the ERK1/2 protein acutely up to 3-hours post-exercise from a BFR-RT exercise session (Gundermann et al., 2012). Lending support to this finding is work conducted by Fry and colleagues (2010). Hence, it could be reasonable to hypothesise that to achieve the maximum anabolic stimuli after a resistance training session, both of the mTORC1 and MAPK signalling pathway is needed to be activated. Anyhow, a further potential mechanisms of the BFR-RT induced hypertrophy may derive from its capability to induce hypoxia, which affects the activity of the reactive oxygen species (ROS). Experimental studies have shown that ROS generated during resistance training can activate MAPK signalling in skeletal myoblasts

(Kefaloyianni, Gaitanaki, & Beis, 2006). However, the literature is insufficient to draw any certain conclusion on the molecular mechanisms underpinning the increased protein synthesis seen with BFR-RT in strength trained subjects. More data should be warranted to seek a better understanding of the anabolic responses of BFR-RT compared to HL-RT.

4.4.5 Anabolic Signalling: Summary

Figure 9 summarises the selected anabolic proteins for the BFR-study associated with an increased muscle protein synthesis following a bout of resistance training with and without the conjunction of blood flow restriction. Despite the prominent relationship of the proteins at either side of the mechanical loading pattern, non of these studies have directly compared the magnitude of influence regarding the comparison of HL-RT and BFR-RT. Thus, the studies investigating the signalling cascades concerning HL-RT have frequently compared it to a control condition without exercise, while several studies from the BFR-literature compared low load resistance training without BFR to BFR-RT. Accordingly, insufficient evidence exists to evaluate the relationship regarding the key anabolic proteins matching the two different training modalities.

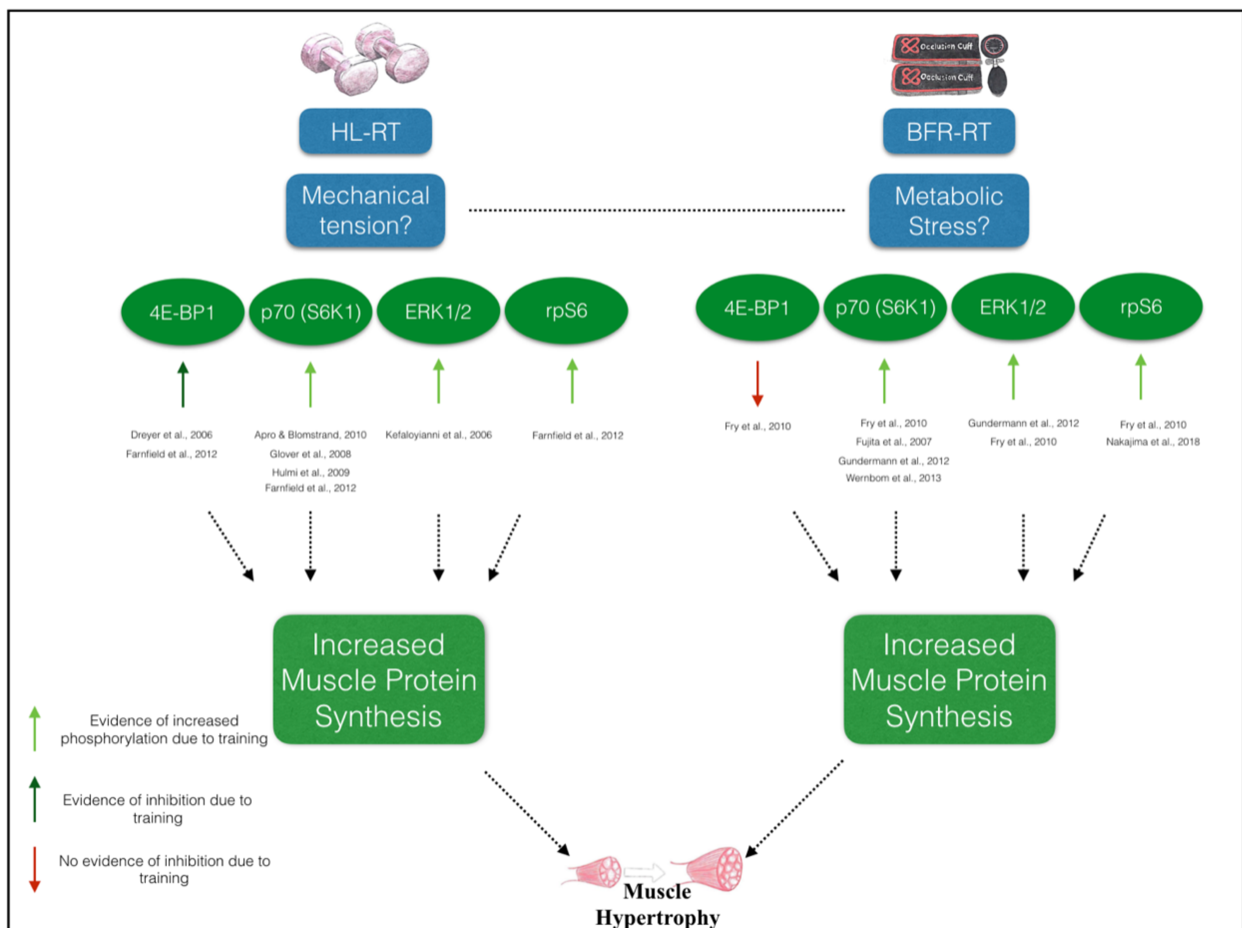

Figure. 9. Overview of studies investigating the acute anabolic signalling responses to a bout of either heavy resistance training or blood flow restriction resistance training. Dotted arrows indicate a possible outcome (not measured directly in most of the investigations). Figure is based upon several articles: Dreyer et al., (2006), Fairfield et al., (2012), Apro & Blomstrand, (2010), Glover et al., (2008), Hulmi et al., (2009), Farnfield et al., (2012), Kefalovianni et al., (2006), Fry et al., (2010), Fujita et al., (2007), Gundermann et al., (2012), Wernbom et al., (2013) and Nakajima et al., (2018).

5. Ethical Considerations

During 1964, the Declaration of Helsinki was formed by the World Medical Association, describing a set of ethical principles regarding human experiments ("World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects," 2014). This landmark document focused crucial attention to the fundamental rights of research participants and to the responsibilities of the scientists, especially that the voluntary consent of the human subject is absolutely indispensable. This includes that: (1) test subjects could leave the experiment at any time without being asked for reason, (2) the risk included in participation should be minimised and (3) that participation is voluntary. All participants included in the BFR-study went through a pre-exercise screening before completing any component of the study (see list of attachment. 3), and was only eligible to participate if they were free from any musculoskeletal, metabolic or neurological disorders that would hinder their capacity to exercise. Further, participants were fully informed of all aspects of the study by reading through a study information sheet (see list of attachment. 4). After reading the sheet, participants signed a participant consent form, acknowledging that they were fully aware of all requirements of the study, have had all questions pertaining to the study answered to their satisfaction, and agree to participate in the study. They were also presented with the first fundamental right of the Helsinki Declaration, that they were free to withdraw from the study at any time, without prejudice.

Regarding the second aspect related to risk minimisation, some key considerations were made. First, there is a minor risk of bruising and infection from muscle biopsy and blood collection procedures. To minimise this risk, muscle samples and blood collections were only performed by qualified professionals who adhere strict procedural guidelines. Secondly, during the DEXA-scan, participants were exposed to a very small amount of radiation. However, the dose from a single scan is estimated to be approximately 4 microsieverts (µSv) (Sheu & Diamond, 2016). This is a very small dose, and is considered to have a very low risk. As a reference, a visit at the dentist conducting one set of dental radiographs corresponds to a radiation of approximately 5-10 µSv, while a mammogram procedure frequently used in breast imaging to assess cancer risks is roughly 400-600 µSv (Hart & Wall, 2004; Hendrick, 2010). Lastly, all training performed during the 9-weeks training block were supervised by qualified personnel to minimise the risk of potential injuries associated with unwarranted technical execution of the exercises, and to assist if any acute injuries would have happened.

Ethical approval was attained by the ethical comité of the Norwegian School of Sport Science (see list of attachments. 1). Nevertheless, two further important ethical considerations are highlighted below: sex and safety concerns.

5.1 Sex

Historically, women have in general been used less frequent as test subjects (Beery & Zucker, 2011; Kim, Carrigan, & Menon, 2008; Yoon et al., 2014). This feministic discrimination cultivate a major issue in the advancement of modern scientific knowledge. The scientific BFR literature is off no exception regarding selection of males over females as study participants. A recently published review article summarised the demographics of those included (4335 participants) in the BFR research literature (Counts et al., 2018). Looking into the experimental studies with a training intervention of BFR, only 17% of the participants constitutes from the female sex. The underrepresentation of females in the BFR literature represent a prejudice and possess an adverse effect when generalisation from scientific studies are performed. Therefore, this study is conducted with an equal sex distribution to add substance regarding possible sex differences on the responses of a chronic training block with BFR-RT.

5.2 Safety Concerns

A recently published study by Patterson and colleagues (2018) retrieved a questionnaire from 99 participants implementing regular BFR-RT in their weekly training routine. The incidences of the reported side effects were delayed onset muscle soreness (39.2%), numbness (18.5%) and dizziness (14.6%). However, some have reported larger detrimental effects and incidences associated with the use of BFR in the literature. Two cases of extensive muscle damage, rhabdomyolysis are known (Iversen & Rostad, 2010; Tabata, Suzuki, Azuma, & Matsumoto, 2016) and one case of vision loss by central retinal vein occlusion (Ozawa, Koto, Shinoda, & Tsubota, 2015). One could argue that the loss of vision reported by Ozawa and colleagues, could not be directly linked to the BFR-RT, and that the subject was prone to such complications based on the patient's history of hypertension and diabetes, thus increased risk of central retinal vein occlusion. Further, Bunevicius and colleagues (2016) studied the responses of the cardiovascular system to a single bout of blood flow restriction training, to determine the risk factors for cardiac patients and physically inactive. Asides the small tendency to an increased systolic blood pressure, low intensity exercise carried out with BFR-RT did not result in significant overload of the cardiac function (Bunevicius et al., 2016). However, one may dispute that the adverse events seen from these studies could be accompanied by any training modalities, and little published evidence suggest that BFR-RT offer any greater health risk than

traditional resistance training (Clark et al., 2011; Loenneke, Wilson, Wilson, Pujol, & Bemben, 2011). Despite the above, the focal point of existing literature has merely focused on the safety aspects related to the long-term training with restricted blood flow, thus the relative safety aspects should be further elucidated with more research on BFR exercise to draw a more certain conclusion.

6. Impact of Research

The expected results of this project could have important ramifications for the large variety of populations that seek the muscular benefits of BFR-RT. Despite the recent popularity of BFR-RT, it is currently limited to research-minded practitioners and coaches as the efficacy and safety of this novel mode of exercise is still being questioned by some. The proposed study would help add weight to the body of evidence supporting low load BFR-RT as a viable alternative to traditional heavy load resistance exercise, and encourage its application for clinical, healthy and athletic populations. BFR-RT could also be utilised to promote health and minimise muscle wasting in people who are temporarily immobilised due to injury, demonstrated by Kubota and colleagues (2008). Finally, athletic populations could utilise this method of exercise to reduce weekly training loads, or temporarily minimise the stress on tendons and joints while maintaining sufficient stress to skeletal muscle to maintain adaptation. Accordingly, this research project will benefit athletes, sport scientists and coaches involved with prescribing resistance exercise. This benefit will be achieved from providing information that will assist in designing efficient and safe resistance training programs involving BFR-RT for the development and/or maintenance of muscle mass and strength, by achieving a sufficient stimulus for adaptation, with reduced mechanical loading. In addition, the new knowledge derived from this project may ultimately assist in (1) reducing total training stress during busy competitive periods, thereby potentially reducing risk of injury and promoting athlete health and wellbeing, (2) maintaining muscular strength during period of reduced training e.g. de-loading or tapering, (3) expediting recovery and return to play/competition following musculoskeletal injury and (4) shed light on the molecular mechanisms underpinning the increased skeletal muscle hypertrophy.

References

Abe, T., Fujita, S., Nakajima, T., Sakamaki, M., Ozaki, H., Ogasawara, R., . . . Ishii, N. (2010). Effects of Low-Intensity Cycle Training with Restricted Leg Blood Flow on Thigh Muscle Volume and VO2MAX in Young Men. *J Sports Sci Med, 9*(3), 452-458.

Apro, W., & Blomstrand, E. (2010). Influence of supplementation with branched-chain amino acids in combination with resistance exercise on p70S6 kinase phosphorylation in resting and exercising human skeletal muscle. *Acta Physiol (Oxf), 200*(3), 237-248. doi:10.1111/j.1748-1708.2010.02151.x

Araujo, J. P., Neto, G. R., Loenneke, J. P., Bemben, M. G., Laurentino, G. C., Batista, G., . . . Sousa, M. S. (2015). The effects of water-based exercise in combination with blood flow restriction on strength and functional capacity in post-menopausal women. *Age (Dordr), 37*(6), 110. doi:10.1007/s11357-015-9851-4

Atherton, P. J., & Smith, K. (2012). Muscle protein synthesis in response to nutrition and exercise. *J Physiol, 590*(Pt 5), 1049-1057. doi:10.1113/jphysiol.2011.225003

Ayuso, M. I., Hernandez-Jimenez, M., Martin, M. E., Salinas, M., & Alcazar, A. (2010). New hierarchical phosphorylation pathway of the translational repressor eIF4E-binding protein 1 (4E-BP1) in ischemia-reperfusion stress. *J Biol Chem, 285*(45), 34355-34363. doi:10.1074/jbc.M110.135103

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews, 35*(3), 565-572. doi:http://dx.doi.org/10.1016/j.neubiorev.2010.07.002

Behringer, M., Behlau, D., Montag, J. C. K., McCourt, M. L., & Mester, J. (2017). Low-Intensity Sprint Training With Blood Flow Restriction Improves 100-m Dash. *J Strength Cond Res, 31*(9), 2462-2472. doi:10.1519/jsc.0000000000001746

Biolo, G., Maggi, S. P., Williams, B. D., Tipton, K. D., & Wolfe, R. R. (1995). Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol, 268*(3 Pt 1), E514-520. doi:10.1152/ajpendo.1995.268.3.E514

Bjornsen, T., Wernbom, M., Kirketeig, A., Paulsen, G., Samnoy, L., Baekken, L., . . . Raastad, T. (2019). Type 1 Muscle Fiber Hypertrophy after Blood Flow-restricted Training in Powerlifters. *Med Sci Sports Exerc, 51*(2), 288-298. doi:10.1249/mss.0000000000001775

Borga, M., West, J., Bell, J. D., Harvey, N. C., Romu, T., Heymsfield, S. B., & Dahlqvist Leinhard, O. (2018). Advanced body composition assessment: from body mass index to body composition profiling. *Journal of Investigative Medicine, 66*(5), 1-9. doi:10.1136/jim-2018-000722

Bunevicius, K., Sujeta, A., Poderiene, K., Zachariene, B., Silinskas, V., Minkevicius, R., & Poderys, J. (2016). Cardiovascular response to bouts of exercise with blood flow restriction. *Journal of Physical Therapy Science, 28*(12), 3288-3292. doi:10.1589/jpts.28.3288

Burd, West, D. W., Staples, A. W., Atherton, P. J., Baker, J. M., Moore, D. R., . . . Phillips, S. M. (2010). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS ONE, 5*(8), e12033. doi:10.1371/journal.pone.0012033

Chen, Z., Wang, Z., Lohman, T., Heymsfield, S. B., Outwater, E., Nicholas, J. S., . . . Going, S. (2007). Dual-Energy X-Ray Absorptiometry Is a Valid Tool for Assessing Skeletal Muscle Mass in Older Women. *The Journal of Nutrition, 137*(12), 2775-2780. doi:10.1093/jn/137.12.2775

Clark, B. C., Manini, T. M., Hoffman, R. L., Williams, P. S., Guiler, M. K., Knutson, M. J., . . . Kushnick, M. R. (2011). Relative safety of 4 weeks of blood flow-restricted resistance

exercise in young, healthy adults. *Scand J Med Sci Sports, 21*(5), 653-662. doi:10.1111/j.1600-0838.2010.01100.x

Clarkson, M. J., Conway, L., & Warmington, S. A. (2017). Blood flow restriction walking and physical function in older adults: A randomized control trial. *J Sci Med Sport, 20*(12), 1041-1046. doi:10.1016/j.jsams.2017.04.012

Cook, C. J., Kilduff, L. P., & Beaven, C. M. (2014). Improving strength and power in trained athletes with 3 weeks of occlusion training. *Int J Sports Physiol Perform, 9*(1), 166-172. doi:10.1123/ijspp.2013-0018

Cook, S. B., LaRoche, D. P., Villa, M. R., Barile, H., & Manini, T. M. (2017). Blood flow restricted resistance training in older adults at risk of mobility limitations. *Exp Gerontol, 99*, 138-145. doi:10.1016/j.exger.2017.10.004

Counts, B. R., Dankel, S. J., Barnett, B. E., Kim, D., Mouser, J. G., Allen, K. M., . . . Loenneke, J. P. (2016). Influence of relative blood flow restriction pressure on muscle activation and muscle adaptation. *Muscle Nerve, 53*(3), 438-445. doi:10.1002/mus.24756

Counts, B. R., Rossow, L. M., Mattocks, K. T., Mouser, J. G., Jessee, M. B., Buckner, S. L., . . . Loenneke, J. P. (2018). Let's talk about sex: where are the young females in blood flow restriction research? *Clin Physiol Funct Imaging, 38*(1), 1-3. doi:10.1111/cpf.12394

Crenshaw, A. G., Hargens, A. R., Gershuni, D. H., & Rydevik, B. (1988). Wide tourniquet cuffs more effective at lower inflation pressures. *Acta Orthop Scand, 59*(4), 447-451.

Cumming, K. T., Paulsen, G., Wernbom, M., Ugelstad, I., & Raastad, T. (2014). Acute response and subcellular movement of HSP27, alphaB-crystallin and HSP70 in human skeletal muscle after blood-flow-restricted low-load resistance exercise. *Acta Physiol (Oxf), 211*(4), 634-646. doi:10.1111/apha.12305

Dankel, S. J., Buckner, S. L., Counts, B. R., Jessee, M. B., Mouser, J. G., Mattocks, K. T., . . . Loenneke, J. P. (2017). The acute muscular response to two distinct blood flow restriction protocols. *Physiol Int, 104*(1), 64-76. doi:10.1556/2060.104.2017.1.1

Dankel, S. J., Jessee, M. B., Buckner, S. L., Mouser, J. G., Mattocks, K. T., & Loenneke, J. P. (2017). Are higher blood flow restriction pressures more beneficial when lower loads are used? *Physiol Int, 104*(3), 247-257. doi:10.1556/2060.104.2017.3.2

de Araujo Ribeiro Alvares, J. B., Rodrigues, R., de Azevedo Franke, R., da Silva, B. G., Pinto, R. S., Vaz, M. A., & Baroni, B. M. (2015). Inter-machine reliability of the Biodex and Cybex isokinetic dynamometers for knee flexor/extensor isometric, concentric and eccentric tests. *Phys Ther Sport, 16*(1), 59-65. doi:10.1016/j.ptsp.2014.04.004

de Oliveira, M. F., Caputo, F., Corvino, R. B., & Denadai, B. S. (2016). Short-term low-intensity blood flow restricted interval training improves both aerobic fitness and muscle strength. *Scand J Med Sci Sports, 26*(9), 1017-1025. doi:10.1111/sms.12540

Delmonico, M. J., Kostek, M. C., Johns, J., Hurley, B. F., & Conway, J. M. (2008). Can dual energy X-ray absorptiometry provide a valid assessment of changes in thigh muscle mass with strength training in older adults? *Eur J Clin Nutr, 62*(12), 1372-1378. doi:10.1038/sj.ejcn.1602880

Dreyer, H. C., Fujita, S., Cadenas, J. G., Chinkes, D. L., Volpi, E., & Rasmussen, B. B. (2006). Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J Physiol, 576*(Pt 2), 613-624. doi:10.1113/jphysiol.2006.113175

Ellefsen, S., Hammarstrom, D., Strand, T. A., Zacharoff, E., Whist, J. E., Rauk, I., . . . Ronnestad, B. R. (2015). Blood flow-restricted strength training displays high functional and biological efficacy in women: a within-subject comparison with high-load strength training. *Am J Physiol Regul Integr Comp Physiol, 309*(7), R767-779. doi:10.1152/ajpregu.00497.2014

Fahs, C. A., Loenneke, J. P., Thiebaud, R. S., Rossow, L. M., Kim, D., Abe, T., . . . Bemben, M. G. (2015). Muscular adaptations to fatiguing exercise with and without blood flow restriction. *Clin Physiol Funct Imaging, 35*(3), 167-176. doi:10.1111/cpf.12141

Farnfield, M. M., Breen, L., Carey, K. A., Garnham, A., & Cameron-Smith, D. (2012). Activation of mTOR signalling in young and old human skeletal muscle in response to combined resistance exercise and whey protein ingestion. *Appl Physiol Nutr Metab, 37*(1), 21-30. doi:10.1139/h11-132

Farup, J., de Paoli, F., Bjerg, K., Riis, S., Ringgard, S., & Vissing, K. (2015). Blood flow restricted and traditional resistance training performed to fatigue produce equal muscle hypertrophy. *Scand J Med Sci Sports, 25*(6), 754-763. doi:10.1111/sms.12396

Ferrari, M., Muthalib, M., & Quaresima, V. (2011). The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments. *Philos Trans A Math Phys Eng Sci, 369*(1955), 4577-4590. doi:10.1098/rsta.2011.0230

Freitas, E., Poole, C., Miller, R., Heishman, A., Kaur, J., Bemben, D., & Bemben, M. (2017). *Time Course Change in Muscle Swelling: High-Intensity vs. Blood Flow Restriction Exercise* (Vol. 38).

Fry, C. S., Glynn, E. L., Drummond, M. J., Timmerman, K. L., Fujita, S., Abe, T., . . . Rasmussen, B. B. (2010). Blood flow restriction exercise stimulates mTORC1 signaling and muscle protein synthesis in older men. *J Appl Physiol (1985), 108*(5), 1199-1209. doi:10.1152/japplphysiol.01266.2009

Fujita, S., Abe, T., Drummond, M. J., Cadenas, J. G., Dreyer, H. C., Sato, Y., . . . Rasmussen, B. B. (2007). Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *Journal of Applied Physiology, 103*(3), 903-910. doi:10.1152/japplphysiol.00195.2007

Glover, E. I., Oates, B. R., Tang, J. E., Moore, D. R., Tarnopolsky, M. A., & Phillips, S. M. (2008). Resistance exercise decreases eIF2Bepsilon phosphorylation and potentiates the feeding-induced stimulation of p70S6K1 and rpS6 in young men. *Am J Physiol Regul Integr Comp Physiol, 295*(2), R604-610. doi:10.1152/ajpregu.00097.2008

Goodpaster, B. H., Park, S. W., Harris, T. B., Kritchevsky, S. B., Nevitt, M., Schwartz, A. V., . . . Newman, A. B. (2006). The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci, 61*(10), 1059-1064.

Graham, B., Breault, M. J., McEwen, J. A., & McGraw, R. W. (1993). Occlusion of arterial flow in the extremities at subsystolic pressures through the use of wide tourniquet cuffs. *Clin Orthop Relat Res*(286), 257-261.

Gundermann, D. M., Fry, C. S., Dickinson, J. M., Walker, D. K., Timmerman, K. L., Drummond, M. J., . . . Rasmussen, B. B. (2012). Reactive hyperemia is not responsible for stimulating muscle protein synthesis following blood flow restriction exercise. *J Appl Physiol (1985), 112*(9), 1520-1528. doi:10.1152/japplphysiol.01267.2011

Gundermann, D. M., Walker, D. K., Reidy, P. T., Borack, M. S., Dickinson, J. M., Volpi, E., & Rasmussen, B. B. (2014). Activation of mTORC1 signaling and protein synthesis in human muscle following blood flow restriction exercise is inhibited by rapamycin. *Am J Physiol Endocrinol Metab, 306*(10), E1198-1204. doi:10.1152/ajpendo.00600.2013

Habets, B., Staal, J. B., Tijssen, M., & van Cingel, R. (2018). Intrarater reliability of the Humac NORM isokinetic dynamometer for strength measurements of the knee and shoulder muscles. *BMC Res Notes, 11*(1), 15. doi:10.1186/s13104-018-3128-9

Hart, D., & Wall, B. F. (2004). UK population dose from medical X-ray examinations. *Eur J Radiol, 50*(3), 285-291. doi:10.1016/s0720-048x(03)00178-5

Hendrick, R. E. (2010). Radiation Doses and Cancer Risks from Breast Imaging Studies. *Radiology, 257*(1), 246-253. doi:10.1148/radiol.10100570

Hind, K., Oldroyd, B., & Truscott, J. G. (2011). In vivo precision of the GE Lunar iDXA densitometer for the measurement of total body composition and fat distribution in adults. *Eur J Clin Nutr, 65*(1), 140-142. doi:10.1038/ejcn.2010.190

Holz, M. K., Ballif, B. A., Gygi, S. P., & Blenis, J. (2005). mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell, 123*(4), 569-580. doi:10.1016/j.cell.2005.10.024

Hulmi, Tannerstedt, J., Selänne, H., Kainulainen, H., Kovanen, V., & Mero, A. A. (2009). Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *Journal of Applied Physiology, 106*(5), 1720-1729. doi:10.1152/japplphysiol.00087.2009

Hulmi, J. J., Tannerstedt, J., Selanne, H., Kainulainen, H., Kovanen, V., & Mero, A. A. (2009). Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *J Appl Physiol (1985), 106*(5), 1720-1729. doi:10.1152/japplphysiol.00087.2009

Hylden, C., Burns, T., Stinner, D., & Owens, J. (2015). Blood flow restriction rehabilitation for extremity weakness: a case series. *J Spec Oper Med, 15*(1), 50-56.

Iida, H., Kurano, M., Takano, H., Kubota, N., Morita, T., Meguro, K., . . . Nakajima, T. (2007). Hemodynamic and neurohumoral responses to the restriction of femoral blood flow by KAATSU in healthy subjects. *Eur J Appl Physiol, 100*(3), 275-285. doi:10.1007/s00421-007-0430-y

Iversen, E., & Rostad, V. (2010). Low-load ischemic exercise-induced rhabdomyolysis. *Clin J Sport Med, 20*(3), 218-219. doi:10.1097/JSM.0b013e3181df8d10

Jenkins, N. D., Housh, T. J., Bergstrom, H. C., Cochrane, K. C., Hill, E. C., Smith, C. M., . . . Cramer, J. T. (2015). Muscle activation during three sets to failure at 80 vs. 30% 1RM resistance exercise. *Eur J Appl Physiol, 115*(11), 2335-2347. doi:10.1007/s00421-015-3214-9

Jessee, M. B., Buckner, S. L., Dankel, S. J., Counts, B. R., Abe, T., & Loenneke, J. P. (2016). The Influence of Cuff Width, Sex, and Race on Arterial Occlusion: Implications for Blood Flow Restriction Research. *Sports Med, 46*(6), 913-921. doi:10.1007/s40279-016-0473-5

Katch, V. L., & Katch, F. I. (1974). A Simple Anthropometric Method for Calculating Segmental Leg Limb Volume. *Research Quarterly. American Alliance for Health, Physical Education and Recreation, 45*(2), 211-214. doi:10.1080/10671188.1974.10615262

Kefaloyianni, E., Gaitanaki, C., & Beis, I. (2006). ERK1/2 and p38-MAPK signalling pathways, through MSK1, are involved in NF-kappaB transactivation during oxidative stress in skeletal myoblasts. *Cell Signal, 18*(12), 2238-2251. doi:10.1016/j.cellsig.2006.05.004

Kim, D., Loenneke, J. P., Ye, X., Bemben, D. A., Beck, T. W., Larson, R. D., & Bemben, M. G. (2017). Low-load resistance training with low relative pressure produces muscular changes similar to high-load resistance training. *Muscle Nerve, 56*(6), E126-e133. doi:10.1002/mus.25626

Kim, E. S. H., Carrigan, T. P., & Menon, V. (2008). Enrollment of Women in National Heart, Lung, and Blood Institute-Funded Cardiovascular Randomized Controlled Trials Fails to Meet Current Federal Mandates for Inclusion. *Journal of the American College of Cardiology, 52*(8), 672-673. doi:10.1016/j.jacc.2008.05.025

Kim, I.-Y., Suh, S.-H., Lee, I.-K., & Wolfe, R. R. (2016). Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research. *Experimental & Molecular Medicine, 48*(1), e203. doi:10.1038/emm.2015.97

Kohrt, W. M. (1998). Preliminary evidence that DEXA provides an accurate assessment of body composition. *Journal of Applied Physiology, 84*(1), 372-377. doi:10.1152/jappl.1998.84.1.372

Krugh, M., & Langaker, M. D. (2018). Dual Energy Xray Absorptiometry (DEXA) *StatPearls*. Treasure Island (FL): StatPearls Publishing StatPearls Publishing LLC.

Kubo, K., Komuro, T., Ishiguro, N., Tsunoda, N., Sato, Y., Ishii, N., . . . Fukunaga, T. (2006). Effects of low-load resistance training with vascular occlusion on the mechanical properties of muscle and tendon. *J Appl Biomech, 22*(2), 112-119.

Kubota, A., Sakuraba, K., Sawaki, K., Sumide, T., & Tamura, Y. (2008). Prevention of disuse muscular weakness by restriction of blood flow. *Med Sci Sports Exerc, 40*(3), 529-534. doi: 10.1249/MSS.0b013e31815ddac6

Kumar, V., Selby, A., Rankin, D., Patel, R., Atherton, P., Hildebrandt, W., . . . Rennie, M. J. (2009). Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol, 587*(1), 211-217. doi:10.1113/jphysiol. 2008.164483

Laplante, M., & Sabatini, D. M. (2009). mTOR signaling at a glance. *Journal of Cell Science, 122*(20), 3589-3594. doi:10.1242/jcs.051011

Laurentino, G. C., Loenneke, J. P., Teixeira, E. L., Nakajima, E., Iared, W., & Tricoli, V. (2016). The Effect of Cuff Width on Muscle Adaptations after Blood Flow Restriction Training. *Med Sci Sports Exerc, 48*(5), 920-925. doi:10.1249/mss.0000000000000833

Laurentino, G. C., Ugrinowitsch, C., Roschel, H., Aoki, M. S., Soares, A. G., Neves, M., Jr., . . . Tricoli, V. (2012). Strength training with blood flow restriction diminishes myostatin gene expression. *Med Sci Sports Exerc, 44*(3), 406-412. doi:10.1249/MSS.0b013e318233b4bc

Libardi, C. A., Chacon-Mikahil, M. P., Cavaglieri, C. R., Tricoli, V., Roschel, H., Vechin, F. C., . . . Ugrinowitsch, C. (2015). Effect of concurrent training with blood flow restriction in the elderly. *Int J Sports Med, 36*(5), 395-399. doi:10.1055/s-0034-1390496

Lixandrao, M. E., Ugrinowitsch, C., Berton, R., Vechin, F. C., Conceicao, M. S., Damas, F., . . . Roschel, H. (2018). Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med, 48*(2), 361-378. doi: 10.1007/s40279-017-0795-y

Lixandrao, M. E., Ugrinowitsch, C., Laurentino, G., Libardi, C. A., Aihara, A. Y., Cardoso, F. N., . . . Roschel, H. (2015). Effects of exercise intensity and occlusion pressure after 12 weeks of resistance training with blood-flow restriction. *Eur J Appl Physiol, 115*(12), 2471-2480. doi:10.1007/s00421-015-3253-2

Loenneke, J. P., Fahs, C. A., Rossow, L. M., Abe, T., & Bemben, M. G. (2012). The anabolic benefits of venous blood flow restriction training may be induced by muscle cell swelling. *Med Hypotheses, 78*(1), 151-154. doi:10.1016/j.mehy.2011.10.014

Loenneke, J. P., Fahs, C. A., Rossow, L. M., Sherk, V. D., Thiebaud, R. S., Abe, T., . . . Bemben, M. G. (2012). Effects of cuff width on arterial occlusion: implications for blood flow restricted exercise. *Eur J Appl Physiol, 112*(8), 2903-2912. doi:10.1007/s00421-011-2266-8

Loenneke, J. P., Fahs, C. A., Thiebaud, R. S., Rossow, L. M., Abe, T., Ye, X., . . . Bemben, M. G. (2012). The acute muscle swelling effects of blood flow restriction. *Acta Physiol Hung, 99*(4), 400-410. doi:10.1556/APhysiol.99.2012.4.4

Loenneke, J. P., Fahs, C. A., Wilson, J. M., & Bemben, M. G. (2011). Blood flow restriction: the metabolite/volume threshold theory. *Med Hypotheses, 77*(5), 748-752. doi:10.1016/j.mehy. 2011.07.029

Loenneke, J. P., Thiebaud, R. S., Abe, T., & Bemben, M. G. (2014). Blood flow restriction pressure recommendations: the hormesis hypothesis. *Med Hypotheses, 82*(5), 623-626. doi:10.1016/j.mehy.2014.02.023

Loenneke, J. P., Wilson, J. M., Wilson, G. J., Pujol, T. J., & Bemben, M. G. (2011). Potential safety issues with blood flow restriction training. *Scand J Med Sci Sports, 21*(4), 510-518. doi: 10.1111/j.1600-0838.2010.01290.x

Mahmood, T., & Yang, P.-C. (2012). Western Blot: Technique, Theory, and Trouble Shooting. *North American Journal of Medical Sciences, 4*(9), 429-434. doi:10.4103/1947-2714.100998

Manini, T. M., Vincent, K. R., Leeuwenburgh, C. L., Lees, H. A., Kavazis, A. N., Borst, S. E., & Clark, B. C. (2011). Myogenic and proteolytic mRNA expression following blood flow restricted exercise. *Acta Physiol (Oxf), 201*(2), 255-263. doi:10.1111/j.1748-1716.2010.02172.x

Marcotte, G. R., West, D. W., & Baar, K. (2015). The molecular basis for load-induced skeletal muscle hypertrophy. *Calcif Tissue Int, 96*(3), 196-210. doi:10.1007/s00223-014-9925-9

Martin-Hernandez, J., Marin, P. J., Menendez, H., Ferrero, C., Loenneke, J. P., & Herrero, A. J. (2013). Muscular adaptations after two different volumes of blood flow-restricted training. *Scand J Med Sci Sports, 23*(2), e114-120. doi:10.1111/sms.12036

Martin-Hernandez, J., Marin, P. J., Menendez, H., Loenneke, J. P., Coelho-e-Silva, M. J., Garcia-Lopez, D., & Herrero, A. J. (2013). Changes in muscle architecture induced by low load blood flow restricted training. *Acta Physiol Hung, 100*(4), 411-418. doi:10.1556/APhysiol.100.2013.011

Mattocks, K. T., Jessee, M. B., Counts, B. R., Buckner, S. L., Grant Mouser, J., Dankel, S. J., . . . Loenneke, J. P. (2017). The effects of upper body exercise across different levels of blood flow restriction on arterial occlusion pressure and perceptual responses. *Physiol Behav, 171*, 181-186. doi:10.1016/j.physbeh.2017.01.015

McCain, J. (2013). The MAPK (ERK) Pathway: Investigational Combinations for the Treatment Of BRAF-Mutated Metastatic Melanoma. *P & T : a peer-reviewed journal for formulary management, 38*(2), 96-108.

McDonagh, M. J., & Davies, C. T. (1984). Adaptive response of mammalian skeletal muscle to exercise with high loads. *Eur J Appl Physiol Occup Physiol, 52*(2), 139-155.

Mitchell, C. J., Churchward-Venne, T. A., Parise, G., Bellamy, L., Baker, S. K., Smith, K., . . . Phillips, S. M. (2014). Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men. *PLoS ONE, 9*(2), e89431. doi:10.1371/journal.pone.0089431

Miyamoto, S., Teramoto, H., Gutkind, J. S., & Yamada, K. M. (1996). Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors. *J Cell Biol, 135*(6 Pt 1), 1633-1642.

Moore, D. R., Burgomaster, K. A., Schofield, L. M., Gibala, M. J., Sale, D. G., & Phillips, S. M. (2004). Neuromuscular adaptations in human muscle following low intensity resistance training with vascular occlusion. *Eur J Appl Physiol, 92*(4-5), 399-406. doi:10.1007/s00421-004-1072-y

Morton, R. W., Oikawa, S. Y., Wavell, C. G., Mazara, N., McGlory, C., Quadrilatero, J., . . . Phillips, S. M. (2016). Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men. *J Appl Physiol (1985), 121*(1), 129-138. doi:10.1152/japplphysiol.00154.2016

Mouser, J. G., Dankel, S. J., Jessee, M. B., Mattocks, K. T., Buckner, S. L., Counts, B. R., & Loenneke, J. P. (2017). A tale of three cuffs: the hemodynamics of blood flow restriction. *Eur J Appl Physiol, 117*(7), 1493-1499. doi:10.1007/s00421-017-3644-7

Mouser, J. G., Dankel, S. J., Mattocks, K. T., Jessee, M. B., Buckner, S. L., Abe, T., & Loenneke, J. P. (2018). Blood flow restriction and cuff width: effect on blood flow in the legs. *Clin Physiol Funct Imaging*. doi:10.1111/cpf.12504

Nakajima, T., Koide, S., Yasuda, T., Hasegawa, T., Yamasoba, T., Obi, S., . . . Kano, Y. (2018). Muscle hypertrophy following blood flow-restricted, low-force isometric electrical stimulation in rat tibialis anterior: role for muscle hypoxia. *J Appl Physiol (1985), 125*(1), 134-145. doi:10.1152/japplphysiol.00972.2017

Neto, G. R., Sousa, M. S., Costa, P. B., Salles, B. F., Novaes, G. S., & Novaes, J. S. (2015). Hypotensive effects of resistance exercises with blood flow restriction. *J Strength Cond Res, 29*(4), 1064-1070. doi:10.1519/jsc.0000000000000734

Nielsen, J. L., Aagaard, P., Bech, R. D., Nygaard, T., Hvid, L. G., Wernbom, M., . . . Frandsen, U. (2012). Proliferation of myogenic stem cells in human skeletal muscle in response to low-load resistance training with blood flow restriction. *J Physiol, 590*(17), 4351-4361. doi:10.1113/jphysiol.2012.237008

Nielsen, J. L., Frandsen, U., Prokhorova, T., Bech, R. D., Nygaard, T., Suetta, C., & Aagaard, P. (2017). Delayed Effect of Blood Flow-restricted Resistance Training on Rapid Force Capacity. *Med Sci Sports Exerc, 49*(6), 1157-1167. doi:10.1249/mss.0000000000001208

Nóbrega, S. R., Ugrinowitsch, C., Pintanel, L., Barcelos, C., & Libardi, C. A. (2018). Effect of Resistance Training to Muscle Failure vs. Volitional Interruption at High- and Low-Intensities on Muscle Mass and Strength. *The Journal of Strength & Conditioning Research, 32*(1), 162-169. doi:10.1519/jsc.0000000000001787

Norton, K. (2012). New Australian standard for adult pre-exercise screening. *Sport Health, 30*(2).

Ogasawara, R., P. Loenneke, J., Thiebaud, R., & Abe, T. (2013). Low-Load Bench Press Training to Fatigue Results in Muscle Hypertrophy Similar to High-Load Bench Press Training (Vol. 4).

Ozaki, H., Yasuda, T., Ogasawara, R., Sakamaki-Sunaga, M., Naito, H., & Abe, T. (2013). Effects of high-intensity and blood flow-restricted low-intensity resistance training on carotid arterial compliance: role of blood pressure during training sessions. *Eur J Appl Physiol, 113*(1), 167-174. doi:10.1007/s00421-012-2422-9

Ozawa, Y., Koto, T., Shinoda, H., & Tsubota, K. (2015). Vision Loss by Central Retinal Vein Occlusion After Kaatsu Training: A Case Report. *Medicine (Baltimore), 94*(36), e1515. doi:10.1097/md.0000000000001515

Pasiakos, S. M., & Carbone, J. W. (2014). Assessment of skeletal muscle proteolysis and the regulatory response to nutrition and exercise. *IUBMB Life, 66*(7), 478-484. doi:10.1002/iub.1291

Paton, C. D., Addis, S. M., & Taylor, L. A. (2017). The effects of muscle blood flow restriction during running training on measures of aerobic capacity and run time to exhaustion. *Eur J Appl Physiol, 117*(12), 2579-2585. doi:10.1007/s00421-017-3745-3

Patterson, S. D., & Brandner, C. R. (2018). The role of blood flow restriction training for applied practitioners: A questionnaire-based survey. *J Sports Sci, 36*(2), 123-130. doi:10.1080/02640414.2017.1284341

Paul, A. C., & Rosenthal, N. (2002). Different modes of hypertrophy in skeletal muscle fibers. *J Cell Biol, 156*(4), 751-760. doi:10.1083/jcb.200105147

Pearson, S. J., & Hussain, S. R. (2015). A Review on the Mechanisms of Blood-Flow Restriction Resistance Training-Induced Muscle Hypertrophy. *Sports Medicine, 45*(2), 187-200. doi:10.1007/s40279-014-0264-9

Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E., & Wolfe, R. R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *American Journal of*

Physiology-Endocrinology and Metabolism, 273(1), E99-E107. doi:10.1152/ajpendo.1997.273.1.E99

Phillips, S. M., Tipton, K. D., Ferrando, A. A., & Wolfe, R. R. (1999). Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *American Journal of Physiology-Endocrinology and Metabolism, 276*(1), E118-E124. doi:10.1152/ajpendo.1999.276.1.E118

Popper, K. R. (1952). The Nature of Philosophical Problems and Their Roots in Science. *The British Journal for the Philosophy of Science, 3*(10), 124-156.

Salamone, L. M., Fuerst, T., Visser, M., Kern, M., Lang, T., Dockrell, M., . . . Lohman, T. G. (2000). Measurement of fat mass using DEXA: a validation study in elderly adults. *Journal of Applied Physiology, 89*(1), 345-352. doi:10.1152/jappl.2000.89.1.345

Sato, Y. (2005). The history and future of KAATSU training (Vol. 1).

Schoenfeld, B. J. (2010). The mechanisms of muscle hypertrophy and their application to resistance training. *J Strength Cond Res, 24*(10), 2857-2872. doi:10.1519/JSC.0b013e3181e840f3

Sheu, A., & Diamond, T. (2016). Bone mineral density: testing for osteoporosis. *Australian Prescriber, 39*(2), 35-39. doi:10.18773/austprescr.2016.020

Slysz, J., Stultz, J., & Burr, J. F. (2016). The efficacy of blood flow restricted exercise: A systematic review & meta-analysis. *J Sci Med Sport, 19*(8), 669-675. doi:10.1016/j.jsams.2015.09.005

Smith-Ryan, A. E., Mock, M. G., Ryan, E. D., Gerstner, G. R., Trexler, E. T., & Hirsch, K. R. (2017). Validity and reliability of a 4-compartment body composition model using dual energy x-ray absorptiometry-derived body volume. *Clin Nutr, 36*(3), 825-830. doi:10.1016/j.clnu.2016.05.006

Tabata, S., Suzuki, Y., Azuma, K., & Matsumoto, H. (2016). Rhabdomyolysis After Performing Blood Flow Restriction Training: A Case Report. *J Strength Cond Res, 30*(7), 2064-2068. doi:10.1519/jsc.0000000000001295

Tarnopolsky, M. A., Atkinson, S. A., MacDougall, J. D., Chesley, A., Phillips, S., & Schwarcz, H. P. (1992). Evaluation of protein requirements for trained strength athletes. *J Appl Physiol (1985), 73*(5), 1986-1995. doi:10.1152/jappl.1992.73.5.1986

Taylor, L. W., Wilborn, C. D., Kreider, R. B., & Willoughby, D. S. (2012). Effects of resistance exercise intensity on extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase activation in men. *J Strength Cond Res, 26*(3), 599-607. doi:10.1519/JSC.0b013e318242f92d

Thiebaud, R. S., Loenneke, J. P., Fahs, C. A., Rossow, L. M., Kim, D., Abe, T., . . . Bemben, M. G. (2013). The effects of elastic band resistance training combined with blood flow restriction on strength, total bone-free lean body mass and muscle thickness in postmenopausal women. *Clin Physiol Funct Imaging, 33*(5), 344-352. doi:10.1111/cpf.12033

Tipton, K. D., Hamilton, D. L., & Gallagher, I. J. (2018). Assessing the Role of Muscle Protein Breakdown in Response to Nutrition and Exercise in Humans. *Sports Medicine (Auckland, N.z.), 48*(Suppl 1), 53-64. doi:10.1007/s40279-017-0845-5

Tipton, K. D., & Witard, O. C. (2007). Protein requirements and recommendations for athletes: relevance of ivory tower arguments for practical recommendations. *Clin Sports Med, 26*(1), 17-36. doi:10.1016/j.csm.2006.11.003

Toigo, M., & Boutellier, U. (2006). New fundamental resistance exercise determinants of molecular and cellular muscle adaptations. *Eur J Appl Physiol, 97*(6), 643-663. doi:10.1007/s00421-006-0238-1

Vanwye, W. R., Weatherholt, A. M., & Mikesky, A. E. (2017). Blood Flow Restriction Training: Implementation into Clinical Practice. *International Journal of Exercise Science, 10*(5), 649-654.

Vechin, F. C., Libardi, C. A., Conceicao, M. S., Damas, F. R., Lixandrao, M. E., Berton, R. P., . . . Ugrinowitsch, C. (2015). Comparisons between low-intensity resistance training with blood flow restriction and high-intensity resistance training on quadriceps muscle mass and strength in elderly. *J Strength Cond Res, 29*(4), 1071-1076. doi:10.1519/jsc.0000000000000703

Vierck, J., O'Reilly, B., Hossner, K., Antonio, J., Byrne, K., Bucci, L., & Dodson, M. (2000). Satellite cell regulation following myotrauma caused by resistance exercise. *Cell Biol Int, 24*(5), 263-272. doi:10.1006/cbir.2000.0499

Wackerhage, H. (2006). Introduction to molecular exercise physiology.

Wernbom, Apro, W., Paulsen, G., Nilsen, T. S., Blomstrand, E., & Raastad, T. (2013). Acute low-load resistance exercise with and without blood flow restriction increased protein signalling and number of satellite cells in human skeletal muscle. *Eur J Appl Physiol, 113*(12), 2953-2965. doi:10.1007/s00421-013-2733-5

Wernbom, M., & Aagaard, P. (2019). Muscle fibre activation and fatigue with low-load blood flow restricted resistance exercise – An integrative physiology review. *Acta Physiologica, 0*(ja), e13302. doi:10.1111/apha.13302

World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. (2014). *J Am Coll Dent, 81*(3), 14-18.

Yasuda, T., Fujita, S., Ogasawara, R., Sato, Y., & Abe, T. (2010). Effects of low-intensity bench press training with restricted arm muscle blood flow on chest muscle hypertrophy: a pilot study. *Clin Physiol Funct Imaging, 30*(5), 338-343. doi:10.1111/j.1475-097X.2010.00949.x

Yasuda, T., Ogasawara, R., Sakamaki, M., Ozaki, H., Sato, Y., & Abe, T. (2011). Combined effects of low-intensity blood flow restriction training and high-intensity resistance training on muscle strength and size. *Eur J Appl Physiol, 111*(10), 2525-2533. doi:10.1007/s00421-011-1873-8

Yoon, D. Y., Mansukhani, N. A., Stubbs, V. C., Helenowski, I. B., Woodruff, T. K., & Kibbe, M. R. (2014). Sex bias exists in basic science and translational surgical research. *Surgery, 156*(3), 508-516. doi:http://dx.doi.org/10.1016/j.surg.2014.07.001

Yow, B. G., Tennent, D. J., Dowd, T. C., Loenneke, J. P., & Owens, J. G. (2018). Blood Flow Restriction Training After Achilles Tendon Rupture. *J Foot Ankle Surg*. doi:10.1053/j.jfas.2017.11.008

Low Load Blood Flow Restricted Exercise Augment Anabolic Signalling Comparable to High Load Resistance and Translates Into Similar Muscle Growth During 9 Weeks of Training

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Purpose: It is well established that high-load resistance training (HL-RT, >70%/1RM) activates mTORC1 and elevates muscle protein synthesis. In addition, evidence from the last decade point to the direction that lower mechanical loads (20-40% 1RM) combined with blood flow restriction (BFR-RT) can augment protein synthesis to a similar extent (Fry et al., 2010). Corresponding signalling pathways have been identified, however, no studies have directly compared the anabolic signalling succeeding a bout of BFR-RT vs. HL-RT. Thus, we aim to elucidate some of the anabolic signalling regulating muscle hypertrophy induced by BFR-RT compared to HL-RT.
Methods: Twenty-one strength trained males and females (24±3y) performed 9 weeks of lower body strength training (3/week) with either BFR-RT or HL-RT. Before and after the intervention, muscle mass was quantified with DEXA and MRI, and muscle function was assessed with MVIC of knee extensors. Biopsies were obtained from m. vastus lateralis before and 2, 24 and 48-h after the last exercise session.
Results: Quadriceps CSA increased after BFR-RT (7.4±4.3%, p<.001) and HL-RT (4.6±2.9%, p=.007) with no differences between groups (p=.152). HL-RT increased MVIC (9.7±12.2%, p=.030) whereas no significant changes were observed after BFR-RT (5.2±12.9%, p=.28); no group interaction (p=.416). Phosphorylation of p70S6K^{Thr389} was elevated from baseline at 2-h (31-fold increase, p=.008) and 24-h (14.6-fold increase, p=.018) in HL-RT and elevated at 2-h (9.8-fold increase, p=.001) in BFR-RT, with no differences between groups (p=.826). Phosphorylation of rpS6$^{Ser235/23}$ (p=.564) and 4E-BP1$^{Thr37/46}$ (p=.474) was not different between HL-RT and BFR-RT at any time point. Phosphorylation of ERK1/2$^{Thr202/Tyr204}$ increased from baseline at 24-h (65±53%, p=.009) in HL-RT and increased from baseline at 2-h (77±56%, p=.002) and 24-h (140±144%, p=.011) in BFR-RT, with no differences between groups (p=.563).
Conclusion: Overall, these data support the application of BFR-RT to induce hypertrophic adaptations comparable to HL-RT. In addition, our data suggest that the key anabolic signalling proteins driving the hypertrophic responses from BFR-RT and HL-RT are activated following an exercise bout of either training modalities. This suggests that the activation of the mTORC1 pathway along with ERK1/2 to be important as a potential underlying signalling pathway of BFR-RT induced muscle hypertrophy. Consequently, BFR-RT could be used to reduce external mechanical loading while inducing a potent anabolic response proceeding to muscle hypertrophy.

Keywords: Hypertrophy, Occlusion Training, p70S6K, ERK1/2, rpS6, 4E-BP1

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Introduction

Since Dr. Sato pioneered the training modality of exercising with limited blood flow during the 70's in Japan (Sato, 2005), a multitude of experimental studies has proven its overall efficiency (Wernbom & Aagaard, 2019). For instance, combining blood flow restriction (BFR) with different training modalities has demonstrated to increase several physical determinants: improved running economy and time to exhaustion with running exercise (Paton et al., 2017); increased 100m running-speed (Behringer et al., 2017); enhanced aerobic power (Abe et al., 2010); maximal power output and delayed onset of blood lactate accumulation with cycling intervals (de Oliveira et al., 2016). In addition, BFR resistance training (BFR-RT) has been demonstrated to be an efficient method for achilles tendon rupture rehabilitation (Yow et al., 2018), increase peak torque, power output and restore strength after extremity injury (Hylden et al., 2015). Further, BFR-RT is demonstrated to induce a hypotensive effect to the same magnitude compared to high load resistance training (HL-RT) (Neto et al., 2015), and achieve similar hypertrophic adaptations in the elderly population compared to HL-RT (Cook et al., 2017; Vechin et al., 2015).

Furthermore, it is well established that HL-RT (>70% 1RM) activates the mammalian target of rapamycin complex 1 (mTORC1) and elevates the muscle protein synthesis (Hulmi et al., 2009; Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997). In addition, evidence from the last decade point in the direction that lower mechanical loads (20-40%/ 1RM) combined with BFR can activate mTORC1 and augment protein synthesis to a similar extent (Fry et al., 2010). Preliminary studies indicates that BFR-RT appears to increase the muscle protein synthesis through the mTOR-p70S6K-pathway and the MAPK-pathway (Fry et al., 2010; Fujita et al., 2007; Wernbom et al., 2013). The aforementioned are reflected in evidence supporting that BFR-RT has shown comparable effects on muscle hypertrophy equal to HL-RT (Lixandrao et al., 2018). However, even though the alleged efficiency of BFR-RT to induce significant skeletal muscle hypertrophy is evident (Kim et al., 2017; Laurentino et al., 2012; Lixandrao et al., 2018; Vechin et al., 2015), the underlying molecular mechanisms of BFR-RT induced muscle hypertrophy are less known. Currently, no studies to the authors' knowledge have directly compared the two training modalities in relation to the acute signalling responses following an exercise bout of BFR-RT vs. HL-RT. Therefore, the primary aim of the current study was to investigate the key anabolic signalling candidates regulating muscle hypertrophy to better elucidate the molecular mechanisms underlying muscle growth with BFR-RT compared to HL-RT. While the secondary aim of the study was to investigate the increased muscle hypertrophy and strength responses following an intervention of either training modalities.

Material and Methods

Subjects

Twenty-one (males n=9, females n=12) healthy and strength trained participants (age 24.3±3.1 years, body mass 76.2±11.5 kg, 1RM squat 105±36 kg, leg lean mass 19.9±3.7 kg) were recruited to participate in the study. Subjects were familiar with frequent resistance training (4.9±2.6 years).

The experimental procedures and the risks were explained to the participants before they provided their written informed consent to take part in the study. Participants were further screened using the Australian adult pre-exercise screening system tool (Norton, 2012) and they were considered eligible to participate if free from musculoskeletal or cardiovascular conditions, and had performed weekly resistance training on legs for the previous 2 years. The study was approved by the ethical committee of the Norwegian School of Sports Sciences and the Norwegian Centre for Research Data. All researchers complied with the standards set by the Declaration of Helsinki regarding the use of human subjects.

Study Design

Baseline muscle mass were assessed with magnetic resonance imaging (MRI) and dual energy X-ray absorptiometry (DEXA), while muscle function was quantified with maximal voluntary isometric contraction (MVIC) force of the knee extensors on an isokinetic dynamometer. Subsequent to the baseline measures, subjects were stratified based on sex, lean leg mass and muscle strength, and further randomised to either BFR-RT or HL-RT (Table 1). Training intervention lasted 9 weeks (3 sessions/ week). Following the completion of the training, the same measures that were completed during baseline were repeated after 5 days of recovery (Fig. 1). The subsequent week after the post-testing, another exercise bout similar to the one performed during the intervention was performed, and served as the acute exercise session for the measurements of anabolic signalling responses.

Training Program

Participants were supervised during the three-weekly lower body resistance training sessions. Session 1 and 3 consisted of barbell back squat, leg press and leg extensions, while session 2 consisted of Bulgarian split squats and leg extension exercises. Participants allocated to BFR-RT performed 4 sets of each exercise comprising of 30, 15, 15 and 15 repetitions respectively, with a rest period of 45 seconds between sets. Prior to the exercise, 12 mm nylon occlusion cuffs were inflated to 60% of the individuals total arterial occlusion pressure (AOP), and monitored between sets. Cuff pressure was deflated immediately following the final set of each exercise. In the HL-RT condition, 4 sets of 8 repetitions for each exercise were performed, with 120 seconds of rest between sets. For both conditions, loads were adjusted to ensure that participants exercised close to muscular failure (1-2 reps in reserve). If the specified number of repetitions was completed

Table 1. Subject characteristics, Mean ± SD

Variable	BFR-RT (n = 11)	HL-RT (n = 10)
Sex (male/female)	5 m, 6 f	4 m, 6 f
Age (years)	24.3±3.2	24.2±3.1
Body Mass (kg)	75.8±10.2	76.6±12.7
Leg Lean Mass (kg)	19.4±3.8	20.2±3.5
1RM Squat Strength (kg)	105±37	106±36
Isometric Peak Torque (Nm)	257±67	268±44
Training Status (years)	4.8±2.2	4.9±2.9

Abbreviations: Training status (years of strength training), 1RM; One repetition maximum

before muscular failure was achieved, the exercise load would be increased for the subsequent session, gauged from a rating of repetitions in reserve (RIR). On the contrary, if the desired number of repetitions could not be completed, the load would be decreased in the following session. Three minutes of rest was allocated between exercises. Training were performed on non-consecutive days, and a deload week (one fewer set per exercise) in week 5 was incorporated for both groups. Prior to the exercise session, all subjects performed a general warm-up routine of 5 minutes of either jogging, cycling or rowing followed by 5 minutes of calisthenic exercises including: self selected dynamic stretches, dynamic warm-up drills and bodyweight exercises. Following the warm-up routine, 2 sets of the first exercise were performed to gradually increase the external load.

Subjects were allowed a maximum of 2 endurance sessions per week and would be excluded if they performed any additional lower body resistance exercise outside the study. A training log was used at the end of every week to monitor and control participants' additional exercises. In addition, subjects were advised to incorporate a sufficient protein intake (1.5-1.8 gram protein/kg bodyweight) to maximise hypertrophic adaptations during the training intervention (Tarnopolsky et al., 1992; Tipton & Witard, 2007). A weekly 24-h diet recall was employed on different days (e.g. training vs. non-training, and week day vs. weekend) to gain insight into participants' eating habits, and to ensure adequate energy and macronutrient intake. Researchers would encourage a higher protein intake for participants below the recommended protein intake range. After each training session, a protein shake (20-30 gram protein) was provided to all participants.

Blood Flow Restriction

Participants allocated to the BFR-RT group had their individual BFR cuff pressure determined using a Doppler ultrasound. Following 10 minutes of seated rest, the same BFR cuff that was used during the training intervention, was positioned on the inguinal fold region of the dominant leg. Both auditory and visual information were used to determine if the pulse was present. The cuff was inflated to 100 mmHg for 30 seconds, followed by incremental increases of 40 mmHg until arterial flow no longer was detected. Pressure was then decreased in 10 mmHg increments until arterial flow returned, and the least amount of pressure needed for total arterial occlusion was selected as the 100% AOP. The mean pressure applied to the lower limbs required for 100% AOP and the 60% AOP used during the experiment was 180.5±23.7 and 108±14.2 mmHg, respectively.

Acute Exercise Session

Following the 9 weeks training program and post-testing in week 10, subjects performed one final bout with either HL-RT or BFR-RT accordingly to their intervention group. The exercise session 1 (squat, leg press and leg extension) was selected. The workout was closely monitored, and researchers applied verbal encouragement to all participants. Exercise session lasted approximately 40 minutes and loads corresponded to the maximum load that could be lifted within the ramification of each distinct exercise protocol. Total external load (reps x sets x external load) of BFR-RT (11426±4471 kg) and HL-RT (10124±3143 kg) was approximately similar.

Maximal Voluntary Contraction

Isometric strength of the knee extensors was assessed on an isokinetic dynamometer (CSMI Humac Norm Model 770, USA). Participants were seated upright with a chair angle of 85 degrees with their dominant leg strapped to the lever fixed at an angle corresponding to 70 degrees of knee flexion (full knee extension defined as 0 degrees of flexion). Following 5 min of cycling (Monark, Ergomedic 828E, Sweden) at a self selected pace, and three warm-up sub-maximal voluntary contractions, participants performed 3 x 3 second maximal voluntary contractions, each separated by 90 seconds of rest. Participants were instructed to apply force as rapid and hard as possible for the entire 3 seconds. A familiarisation session was performed prior to the first test session.

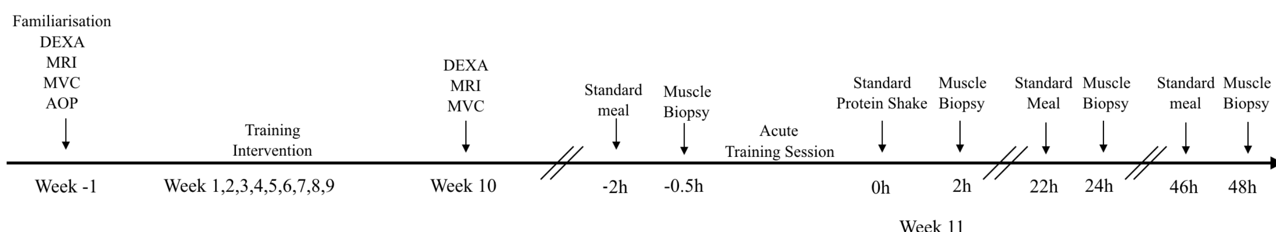

Figure 1. Timeline for the training intervention followed by the acute experiment
MVC, maximal voluntary contraction.
MRI, magnetic resonance imaging.
DEXA, dual energy X-ray absorptiometry.
AOP, arterial occlusion pressure.

Body Composition and Muscle CSA

DEXA (Lunar iDXA, GE Healthcare, Buckinghamshire, United Kingdom) was used before and after the intervention period to assess the total body composition. In a fasted state, participants were scanned in the morning, avoiding exercise in the 12-h prior to the test and were then asked to void their bladder immediately before the scan. All subjects were scanned head to toe in a supine position, quantifying lean tissue, fat mass and bone mineral content. Total lean leg mass was provided with further assessments.

Muscle cross-sectional area (CSA) and quadriceps volume were determined using MRI to provide a local measure of muscle mass. Transverse section images were captured of the dominant leg (GE Signa 1.5 Tesla Echospeed, GEMedical Systems, Madison, WI, USA) before and after the training period. The images [Digital Imaging and Communications in Medicine (DICOM)] were analysed using ITK-SNAP software (University of PA, Philadelphia, USA; www.itksnap.org). A total of 10 DICOM images were used to derive the CSA of the quadricep muscles along the dominant thigh. The CSA values from each of the 10 locations were combined to provide a total quadriceps CSA. The CV of these assessments was <2%.

Biopsy Collection

Biopsies were obtained from vastus lateralis of the dominant leg before, and 2, 24 and 48-h after the acute exercise session. The targeted area of the biopsy was first disinfected by chlorhexidine followed by local anesthesia (Xylocaine adrenaline, 10 $mg \cdot ml^{-1}$ + 5$\mu g \cdot ml^{-1}$, AstraZeneca, London, UK). Subsequently, a 1-2 cm wide cut and 1.5-2.2 cm deep section into the skin and muscle fascia was conducted with a scalpel. A 6 mm Bergström biopsy needle (Pelomi, Alberslund, Denmark) was inserted through the section, where 1-3 muscle samples were taken (approximately 150 mg in total). A vacuum tube needle was attached to the biopsy needle to pull the muscle tissue into the needle. Following the procedure, the section was closed with strips and the muscle sample was cleaned for connective tissue, adipose tissue and blood. The muscle tissue were weighted and quickly frozen in isopentane cooled on dry ice. Further, the cleansed samples were stored in eppendorf tubes and directly stored in a minus 80°C freezer. Participants were given wound care advice and were closely followed up after the procedure.

Two hours prior to the first, third and fourth biopsy, a standardised meal of oatmeal was consumed (0.16 g protein/kg bodyweight), while two hours prior to the second biopsy, a protein shake (0.28 g protein/kg bodyweight) was ingested.

Tissue Processing: Homogenisation

The muscle biopsy samples were stored in a minus 80ºC freezer until they were homogenised. Approximately 50 mg of muscle tissue was homogenised in a solution of 1 ml T-PER (Tissue Protein Extraction Reagent, Thermo scientific, Rockford, IL, USA) and 20 µl EDTA (Ethylene Diamine Triacetic Acid, Thermo scientific, Rockford, IL, USA). For samples that were either above or below the threshold of 50 mg muscle tissue, the solution was adjusted thereafter. Each muscle sample was homogenised for the duration of 3-5 seconds, followed by another 3-5 seconds, or until no intact tissue was seen. Succeeding, the samples were shuddered in a refrigerator for 30 minutes and then centrifuged at 10 000G for the duration of 10 minutes at a temperature of 4ºC. The supernatant of each sample was transferred to 1.5 ml eppendorf tubes, before they were centrifuged one additional time with the same settings as above. After the second centrifugation, the supernatant was pipetted into a new eppendorf tube of 1.5 ml and further divided into 25 µl aliquots added to 10 x 0.2 ml eppendorf tubes. The final eppendorf tubes of 25 µl muscle sample solution were then stored in a minus 70ºC freezer.

Tissue Processing: Total Protein Content

Samples were first evaluated for the total concentration of protein by using the RC/DC Protein Assay kit from BioRad (Herkules, CA, USA). As a known standard protein concentration, the bovine gamma globulin was selected with a spectrum of 0.125; 0.25; 0.5; 1; 1.5 $\mu g \cdot ml^{-1}$. Samples were diluted in a 1:4 ratio with dH_2O, in such manner that the protein concentrations should be within the area defined by the standard protein concentration. In 96-well microplates (Greiner Bio-one International AG, Kremsmünster, Germany), the samples were pipetted in triplicates of 5 µl in each well. Following the pipetting of samples and the protein standard, a 25 µl reagent A*S (alkaline copper tartrate solution, Bio-Rad Laboratories Inc., USA) and a 200 µl reagent B (diluted folin reagent) were added into each well with a multichannel pipette. The 96-well microplates were then kept in a dark environment for the duration of 15 minutes, subsequently, the plate was analysed by ASYS Expert 96 from Biochrom (Cambridge, UK). The protein concentrations (CV<10%) were then calculated from the KIM Immunochemical Processing Software 32.

Western Blotting

Muscle tissue samples were diluted in dH_2O and added a sample reducing agent (10x, NuPAGE, NP0009, Invitrogen) and a LDS sample buffer (4x, NuPAGE, NP0007, Invitrogen) in relation to the

specific protein concentration of that sample. Further, the samples were heated to 70ºC for the duration of 10 minutes before they were pipetted and separated by a 10-well gel (NuPAGE 4-12% Bis-This Gel, 10-well, NP0321BOX, Invitrogen). A weight marker (Protein Ladder PS 11, GeneOn., Germany) was added to the first and last well of each specific gel. Further, a 30 µl solution of each sample were pipetted into the rest of the eight wells, with a duplicate of each participant on the same gel. Electrophoresis (SDS-PAGE electrophoresis) were conducted with chambers (Novex Mini-Cell, XCell SureLock, Invitrogen) at 200 Voltage with a duration of 45 minutes using a running buffer: MES/MOPS SDS running buffer (20x NuPAGE, NP0002, Invitrogen) and dH_2O. Subsequently, the samples were transferred to a polyvinylidene difluoride (PVDF) membrane (Immuno-blot, Bio-Rad). Prior to the blotting stage, the membranes where activated with methanol (Merck KgaA, 1.06007.2500), dH_2O and a transfer buffer: transfer buffer (NuPage, NP0005, Invitrogen), methanol, dH_2O and antioxidant (NuPAGE, NP0005, Invitrogen). Membranes were further blocked for 2-h at room temperature in 5% fat-free skimmed milk (Skimmed milk powder Merck KgaA, Germany), and a 0.05% TBS-t solution (Tris-buffered saline 10x TBS, Bio-Rad and Tween 20 VWR International, Radnor, Pa, USA) and incubated over night at 4ºC with primary antibodies, followed by incubation with the specific secondary antibodies for 1-h at room temperature. Membranes were washed in the 0.05% TBS-t solution between stages and protein stripping was performed by using Restore Western Blot Stripping Buffer (Pierce Biotechnology, Rockford, IL, USA) for 10 minutes at room temperature. The membranes were finally incubated for the duration of 5 minutes of a 1:1 solution of a luminol enhancer solution and stable peroxide buffer immediately before the imaging was conducted with ChemiDoc MP Imaging system, and further analysed with Image Lab Software (Bio-Rad Laboratories, Hercules, CA, USA). All samples from each participant were always loaded on the same gel, and band intensity comparisons were only done within each blot.

Antibodies

The following primary antibodies were used and purchased from Cell Signalling Technology: Phospho-p70S6 Kinase (Thr^{389}), Phospho-p44/42 MAPK (ERK1/2) (Thr^{202}/Tyr^{204}), Phospho-S6 Ribosomal Protein ($Ser^{235/236}$), Phospho-4E-BP1 ($Thr^{37/46}$), Total p70S6 Kinase, Total-p44/42 MAPK (ERK1/2), Total-S6 Ribosomal Protein and Total-4E-BP1 Antibody. All primary antibodies were diluted in 1:1000. The secondary antibody, Anti-rabbit IgG, HRP-linked Antibody, was diluted in 1:3000.

Statistical Analysis

All data were tested for Gaussian distribution using the Shapiro-Wilk normality test. A two-way repeated measures ANOVA was used to assess the interaction of groups and time-point effects of phosphorylation status. Log transformation (base 10) was used to make phosphorylation data less skewed. Paired t-tests were used to assess pre and post changes of muscle function and muscle hypertrophy, while unpaired t-tests were used to evaluate the differences between groups. Data are presented as mean±standard deviation. The level of significance for all statistical analyses was set to $p < 0.05$ and data were analysed and graphically presented using Prism 8 (San Diego, CA, USA, https://www.graphpad.com).

Results

Participant Characteristics

The participants' characteristics at baseline are shown in Table 1. There were no significant baseline differences between HL-RT and BFR-RT ($p > 0.05$).

The participants in the HL-RT and the BFR-RT group completed the same number of strength training sessions 27±0 and 27±0 sessions, respectively. Total session load (repetitions x sets x external load) increased from 7179±3463 kg to 12317±4480 kg (72%, p=.001) with the BFR-RT group, while HL-RT increased from 7796±3027 kg to 10279±2969 kg (32%, p<0.001); no significant difference between volumes at baseline (p=.680) or post (p=.258).

Muscle Strength

Following the 9 week training intervention, the HL-RT group increased peak torque in isometric knee extension from pre to post (9.7±12.2%, p=0.030), whereas no significant increase was observed with BFR-RT (5.2±12.9%, p=0.28); no significant group interaction between the two groups (p=0.276).

Muscle Hypertrophy

Following the training period, total lean leg mass was increased irrespective of training modality: BFR-RT (1.95±1.78%, p=0.005) and HL-RT (1.05±1.34%, p=0.041), with no significant group interaction (p=0.213).

The cross-sectional area of the vastus lateralis increased following the training intervention with BFR-RT (7.4±4.3%, p<.0001) and with HL-RT (4.6±2.9%, p=.007), with no significant difference between training modality (p=.152).

Protein Signalling

Phosphorylation of $p70S6K^{Thr389}$ was elevated from baseline at 2-h (31-fold increase, p=0.009), 24-h (14.6-fold increase, p=0.022) and 48-h (6.6-fold increase, p=0.031) in HL-RT and elevated at 2-h

(9.8-fold increase, p=0.002) in BFR-RT, with no differences between groups (p=0.826).

There was no difference in rpS6$^{Ser235/2}$ phosphorylation at any time point after exercise in the BFR-RT group. HL-RT obtained a time-point interaction only at 24-h (3-fold increase, p=0.049). However, phosphorylation of rpS6$^{Ser235/23}$ was not different between HL-RT and BFR-RT (p=0.564).

There was no statistical significant group interaction of the phosphorylation of 4E-BP1$^{Thr37/46}$ (p=0.474), despite the time-point interaction of HL-RT at 2-h (-31±26%, p=0.049).

Phosphorylation of ERK1/2$^{Thr202/Tyr204}$ increased from baseline at 24-h (65±53%, p=0.009) and 48-h (63±50%, p=0.006) in HL-RT and increased from baseline at 2-h (77±56%, p=0.002) 24-h (140±144%, p=0.011) and 48-h (121±107%, p=0.001) in BFR-RT, with no differences between groups (p=0.563).

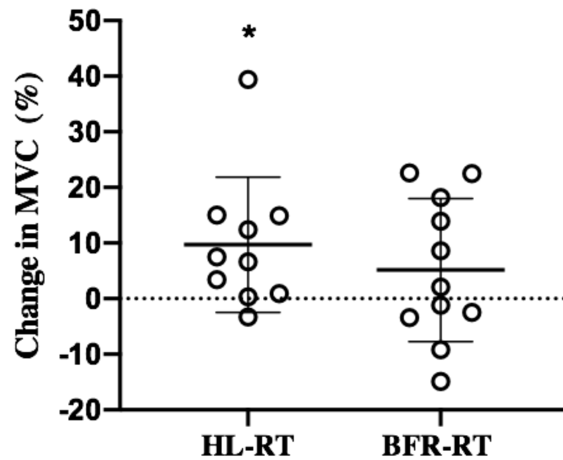

Fig. 2 Changes in MVC in HL-RT and BFR-RT following the training intervention. *p<0.05 versus baseline.

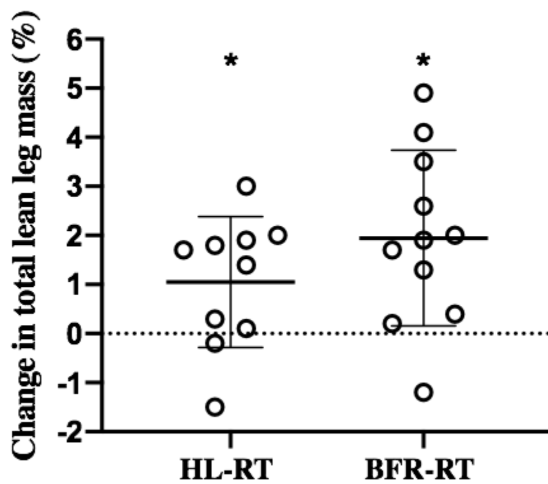

Fig. 3 Changes in total lean leg mass in HL-RT and BFR-RT following the training intervention. *p<0.05 versus baseline.

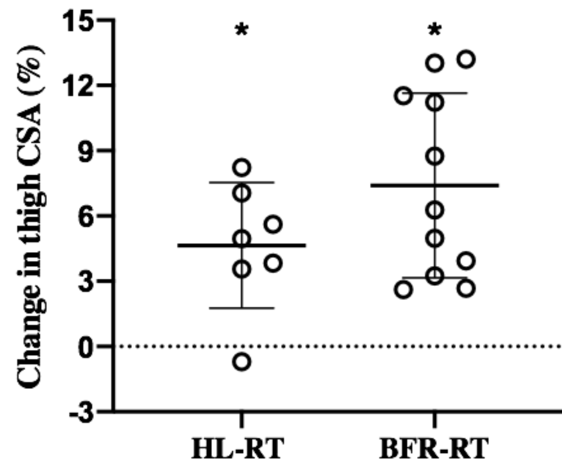

Fig. 4 Changes in thigh CSA in HL-RT and BFR-RT following the training intervention. *p<0.05 versus baseline.

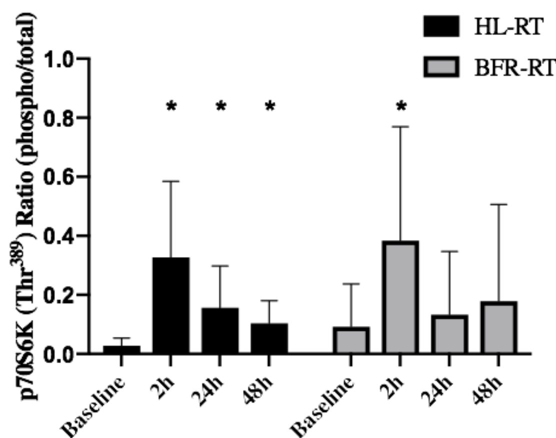

Fig. 5 Phosphorylation of p70S6K at Thr^{389} in HL-RT and BFR-RT at baseline and 2, 24 and 48-h following exercise. *p<0.05 versus baseline.

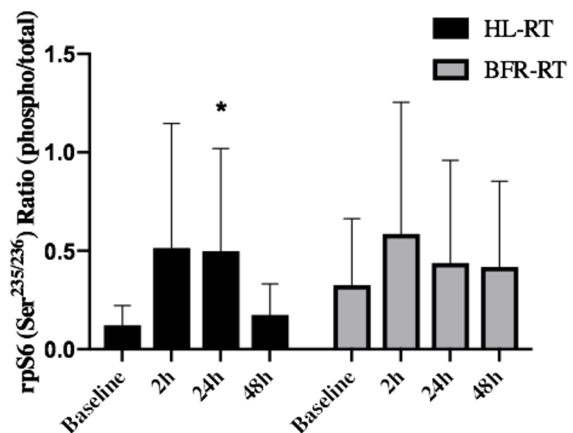

Fig. 6 Phosphorylation of rpS6 at Ser$^{235/236}$ in HL-RT and BFR-RT at baseline and 2, 24 and 48-h following exercise. *p<0.05 versus baseline.

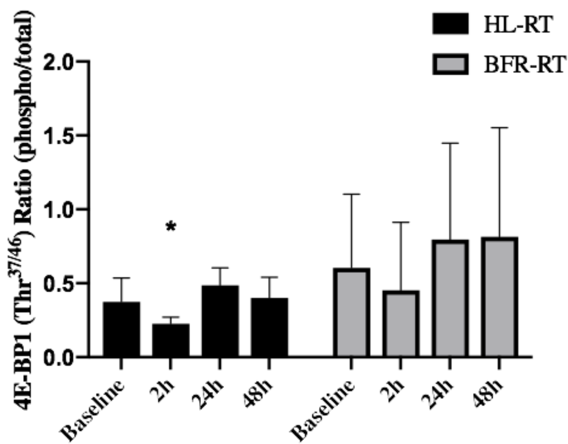

Fig. 7 Phosphorylation of 4E-BP1 at $Thr^{37/46}$ in HL-RT and BFR-RT at baseline and 2, 24 and 48-h following exercise. *p<0.05 versus baseline.

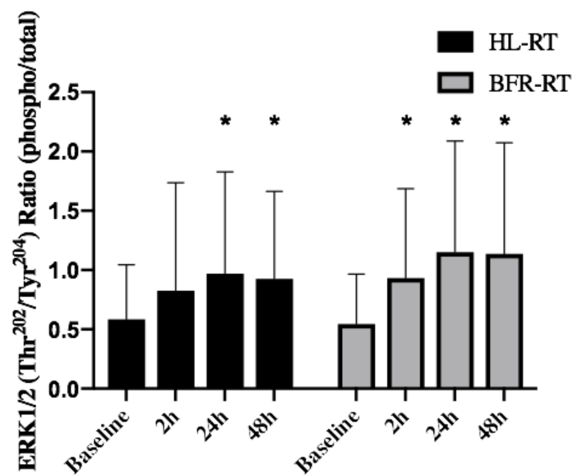

Fig. 8 Phosphorylation of ERK1/2 at Thr^{202}/Tyr^{204} in HL-RT and BFR-RT at baseline and 2, 24 and 48-h following exercise. *p<0.05 versus baseline.

Discussion

To the best of our knowledge, this is the first study to directly compare the anabolic signalling responses following a bout of BFR-RT matched with HL-RT. Thus, the primary and novel finding from our study was that BFR-RT augmented anabolic signalling to a similar magnitude as HL-RT following an exercise session. Further, there were two secondary findings: (1) resistance trained subjects who exercised with either BFR-RT or HL-RT for 9 weeks achieved similar muscle hypertrophy, and (2) only HL-RT transferred to improvements of strength in isometric knee extensions.

Protein Signalling

It was initially hypothesised that due to the subjective nature of lower mechanical stress accompanied by BFR-RT, along with the notion that the secondary mechanisms (i.e mechanotransduction, cell swelling, reactive oxygen species) may differ between HL-RT and BFR-RT (Pearson & Hussain, 2015), a potential difference in anabolic signalling underlying the increased protein synthesis could be apparent. Notably, the signalling responses were almost identical between conditions following the recovery phase of an acute exercise bout. A possible explanation could be that the differences of external mechanical loads used in the current study between BFR-RT and HL-RT were inadequate to induce a significant different response. For instance, BFR-RT and HL-RT exercised with an external load during the squats corresponding to 50.5±9.8%/1RM and 72.8±6.2%/1RM, respectively. Hence, it is possible that a more distinct difference in relative external load between exercise conditions (e.g., 30%/1RM vs. 80%/1RM) could have influenced the signalling responses, although this remains speculative and unknown .

We investigated several steps in the signalling pathways involved in the up-regulation of protein synthesis, and observed that p-p70S6K (Thr^{389}) was significantly elevated at 2-h post-exercise in both exercise conditions (Fig. 5). Interestingly, Gundermann et al. (2012) did not find any significant increase of p-p70S6K (Thr^{389}) at 1-h post BFR-RT. The subjects in Gundermann et al. (2012) exercised with the same protocol (30-15-15-15 repetitions), despite using a lower relative load (20%/1RM compared to our 50%/1RM). In addition, the leg extension was the only exercise performed, thus the protocol used by Gundermann et al. (2012) may have been sub maximal. Nonetheless, several investigations have reported elevated p-p70S6K (Thr^{389}) at 3-h post-BFR-RT (Fry et al., 2010; Fujita et al., 2007; Gundermann et al., 2012).

A recent study from Nakajima and colleagues (2018) studied the effects of low microvascular O_2 partial pressures (P mvO_2) during low-force electrical induced muscle contractions on muscle hypertrophy signalling in rodents. The reduction of P mvO_2 enhanced rpS6 phosphorylation and potentiated the hypertrophic response. Lending support to the rodent study is Fry et al. (2010) demonstrating an increased rpS6 phosprylation in the early phase (1-h) following BFR-RT in human subjects. In the present study, phosphorylation of $rpS6^{Ser235/23}$ was not different between HL-RT and BFR-RT at any time points compared to baseline (Fig. 6). Only HL-RT obtained a significant time point interaction at 24-h, which is in line with other studies demonstrating that HL-RT potentiates the phosphorylation of rpS6 (Glover et al., 2008; Mitchell et al., 2014). The

same pattern was observed with BFR-RT, although not significant (Fig. 6).

The multi-factor regulated protein 4E-BP1 becomes inhibited through phosphorylation, which in turn detaches from the eIF4E protein (Holz et al., 2005). This detachment allows the ribosome to bind to the mRNA to initiate translation, and thus increase the protein synthesis (Wackerhage, 2006). HL-RT is indeed showed to inhibit the activity of 4E-BP1 immediately following exercise when mTORC1 is phosphorylated (Dreyer et al., 2006; Farnfield et al., 2012), which was also demonstrated in our study (Fig. 7). Interestingly, the BFR-RT did not reach a significant interaction, despite a tendency (p=0.11) that was observed 2-h post-exercise. This tendency corroborates the results of the investigation of Fry and colleagues (2010), that demonstrated a tendency to a reduction in the activation of 4E-BP1 following (1 and 3-h) a BFR-RT session.

Intriguingly, our observations are in opposition to the study of Wernbom and colleagues (2013), that observed no changes of p-ERK1/2 ($^{Thr202/Tyr204}$) compared to baseline at any time points (1, 24 and 48-h) following BFR-RT exercise. Although the subjects in Wernbom et al. (2013) performed 5 sets to concentric failure (30%/1RM), the exercise volume are lower than in our study (5 vs. 12 sets). Moreover, others have reported that p-ERK1/2 ($^{Thr202/Tyr204}$) was elevated at 4-h post-exercise with 4 sets of 30%/1RM to failure (Burd et al., 2010) and elevated at 2 and 6-h post-exercise with HL-RT (65%/1RM and 80%/1RM) (Taylor, Wilborn, Kreider, & Willoughby, 2012). Moreover, Fry et al. (2010) demonstrated an early (1 and 3-h) increase of ERK1/2 and Mnk1 in response to a BFR-RT session (30-15-15-15, 20%/1RM). However, in our study, ERK1/2 showed a delayed phosphorylated response in both exercise conditions, which peaked at 24 and 48-h following the exercise bout (Fig. 8).

Additionally, it is important to consider the possible influence of different BFR-RT exercise protocols regarding the signalling responses in type I vs. type II fibers. Our lab has previously observed a more pronounced cellular stress response in type I than in type II fibers (Cumming, Paulsen, Wernbom, Ugelstad, & Raastad, 2014).

Collectively, ERK1/2 phosphorylation and the downstream regulators of mTORC1 attained an equalised post-exercise response with both exercising modalities in the present study. This indicates that the activation of both the mTORC1 and the MAPK signalling pathway to be important as an anabolic signalling response underlying the observed muscle hypertrophy in either training modality. This may benefit our understanding of the molecular mechanisms underpinning muscle hypertrophy induced by BFR-RT and lend support to the BFR-RT strategies aiming to increase muscle mass.

Strength Adaptations

The HL-RT group achieved a significant improvement of strength, whereas the BFR-RT group did not improve strength (Fig. 2). The improvement of strength for HL-RT may be attributed to several mechanisms (i.e neural adaptations, training specificity, fascicle changes). To the authors' knowledge, only one study has compared voluntary activation level through twitch interpolation technique following a training intervention of BFR-RT vs. HL-RT (Kubo et al., 2006). Kubo and colleagues (2006) demonstrated an increased voluntary activation level by approximately 3% following 12 weeks of training with HL-RT, with no significant change in the BFR-RT group. Further, Moore and colleagues (2004) assessed voluntary activation before and after a long-term BFR-RT intervention and observed no significant increase in muscle activation. Moreover, we did not measure any fascicle lengths of the muscles in m. quadriceps, which could theoretically be influenced differently between the training modalities (HL-RT vs. BFR-RT). This could potentially affect the peak force during the MVIC at the measured knee angle (70°) due to an improved myofilament overlap at that fascicle length. Furthermore, it is also plausible to speculate that the HL-RT group exercised with a greater intensity which mimics the subjective nature of a maximal voluntary contraction test, thus the training was more specific to the test setting. Despite the test specificity, theoretically favouring HL-RT, all participants were resistance trained and familiar with maximum force testing. In either way, most studies find HL-RT to be slightly superior regarding strength adaptations when compared to BFR-RT (Kubo et al., 2006; Libardi et al., 2015; Lixandrao et al., 2015; Martin-Hernandez, Marin, Menendez, Loenneke, et al., 2013; Ozaki et al., 2013; Yasuda et al., 2011). Thus, in conjunction with the findings from our study, BFR-RT may not be as efficient as HL-RT to increase peak force output, which is in conformity with the existing literature (Lixandrao et al., 2018).

It is worth commenting that our participants were strength trained individuals, and although no significant strength increase was obtained, no significant strength decrease was achieved either. This imply the possibility to use BFR-RT to maintain force production capacity e.g. during periods of mechanical de-loading. Finally, despite BFR-RT might not be considered optimal to induce strength adaptations, the literature demonstrates that individuals engaged in BFR-RT can increase muscle strength capacity (Ellefsen et al., 2015; Kubo et al., 2006; Vechin et al., 2015).

Muscle Hypertrophy

Most of the previous studies (Ellefsen et al., 2015; Laurentino et al., 2012; Ozaki et al., 2013; Thiebaud et al., 2013; Vechin et al., 2015; Yasuda et al., 2011) as well as the current study (Fig. 3) indicate that BFR-RT may be an equally effective strategy to maintain and augment muscle hypertrophic adaptations compared to HL-RT. This can be employed by the athletic populations to temporarily minimise the stress on tendons and joints while maintaining sufficient stress to skeletal muscle to maintain adaptation (Cook, Kilduff, & Beaven, 2014). In addition, BFR-RT could be utilised to promote health and minimise muscle wasting in people who are briefly immobilised due to injury (Kubota et al., 2008) or used during rehabilitation when high external loading is often restricted (Hylden et al., 2015; Vanwye, Weatherholt, & Mikesky, 2017; Yow et al., 2018).

Despite being non-significant, there was a weak tendency (p=0.15) to favour BFR-RT vs. HL-RT regarding hypertrophic adaptations measured with MRI (Fig. 4. 7.4±4.3% vs. 4.6±2.9%). In the recent meta-analysis of Lixandrao and colleagues (2018), only one BFR-protocol within one study of a total of 10 studies, demonstrated to favour BFR-RT compared to HL-RT regarding muscle hypertrophic adaptations. Collectively, our results corroborate the assertion and key findings from the meta-analysis of Lixandrao et al., (2018), that BFR-RT demonstrates similar efficacy regarding hypertrophic adaptations compared to HL-RT.

Finally, a more pronounced muscle hypertrophy may occur in type I fibers with BFR-RT than type II fibers, recently demonstrated in our lab (Bjornsen et al., 2019). Whether BFR-RT induce muscle hypertrophy in type I fibers to a greater extent than type II fibers and whether the training modality BFR-RT vs. HL-RT targets different fiber types should be further examined.

Safety Concerns

During our 9 weeks exercise intervention, comprising of a total of 297 sessions of BFR-RT, only one adverse event transpired. One participant perceived strong pain near the occipital process during the leg press exercise. Participant was not breathing continuously during leg press, which may have elevated cerebral pressure. The headache lasted the remainder of the day (12-h) and the same pain was reported during the two consecutive exercise sessions, accordingly two weeks of rest was allocated to heal with a slow introduction to sessions after break. Besides the incidence, some minor bruises from the cuffs were reported by female participants and one participant noted some trivial dizziness at the onset of the exercise intervention. Hence, the findings from the literature (Bunevicius et al., 2016; Clark et al., 2011; Loenneke, Fahs, et al., 2011; Patterson & Brandner, 2018) and our study, indicate that BFR-RT is a relative safe training method, however it should be noted that the possibilities of adverse effects cannot be ruled out unconditionally as with any exercise modality.

Training Program

The larger increase in session loads in BFR-RT (72%) compared to HL-RT (32%) during the intervention deserves a short comment. The difference is reasonably an outcome based on three conditions: (1) since our subjects were familiarised with the HL-RT method, they were able to start with a more correct load within the predefined sets and repetitions for the exercises than the BFR-RT group, (2) our subjects had not previously done any BFR-RT, thus the BFR-RT group required an introduction with slightly lower loads, (3) BFR-RT was initially (1-3 weeks) a painful method (based on the ratings of perceived pain from our subjects), however, following a couple of weeks subjects were getting accustomed to the BFR-RT protocol and managed to exercise with incremental loads. These factors might explain how the overall increase of external load were favouring the BFR-RT vs. HL-RT.

The initial training loads for BFR-RT were ~30%/1RM, however an unforeseen consequence of the study was that participants in the BFR condition appeared to tolerate higher loads (50±9%/1RM) than typically prescribed, despite still maintaining the 30-15-15-15 protocol. By utilising the repetitions in reserve method to standardise effort with training, we used a more ecologically valid way on increasing loads, as opposed to those typically used in tightly controlled research studies.

Limitations

Although an increased activation of the measured anabolic signalling proteins in the current study is linked with an increased muscle protein synthesis (Burd et al., 2010; Farnfield et al., 2012; Kumar et al., 2009; Nakajima et al., 2018), we did not measure the muscle protein synthesis directly. Since the acute study was performed succeeding the training intervention that demonstrated significant muscle hypertrophy, it is probable that the post-exercise signalling proteins were responsible for an up-regulation of the muscle protein synthesis accumulating into a net positive protein balance.

Another limitation regards the possibility of a type II error due to the relative low sample size (BFR-RT n=11, HL-RT n=10). The current study could conceivably benefit with additional subjects.

Finally, we cannot separate the anabolic signalling responses between type I and type II fibers. Thus we can't rule out the possibility of a distinct signalling pattern between fiber types, or

the possibility that the two training modalities (HL-RT vs. BFR-RT) triggers signalling cascades differently in relation to fiber types. Although, this is only apparent if one of the groups would have a higher distribution of either fiber types.

Conclusion

Overall, this study supports the application of BFR-RT to induce hypertrophic adaptations comparable to HL-RT. Notably, BFR-RT and HL-RT demonstrated an equivalent activation of the investigated anabolic signalling proteins following an exercise bout of either training modalities. This indicates that performing low load resistance training accompanied by blood flow restriction close to concentric failure can trigger multiple hypertrophic pathways to a similar extent as seen with heavy load resistance training. This suggests that the activation of the mTORC1 pathway along with ERK1/2 to be important as a potential underlying anabolic signalling response of BFR-RT induced muscle hypertrophy. Consequently, BFR-RT appears to be a well-tolerated and novel intervention that may be used to reduce external mechanical loading while inducing a potent anabolic response proceeding to muscle hypertrophy.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

References

Abe, T., Fujita, S., Nakajima, T., Sakamaki, M., Ozaki, H., Ogasawara, R., . . . Ishii, N. (2010). Effects of Low-Intensity Cycle Training with Restricted Leg Blood Flow on Thigh Muscle Volume and VO2MAX in Young Men. *J Sports Sci Med, 9*(3), 452-458.

Behringer, M., Behlau, D., Montag, J. C. K., McCourt, M. L., & Mester, J. (2017). Low-Intensity Sprint Training With Blood Flow Restriction Improves 100-m Dash. *J Strength Cond Res, 31*(9), 2462-2472. doi:10.1519/jsc.0000000000001746

Bjornsen, T., Wernbom, M., Kirketeig, A., Paulsen, G., Samnoy, L., Baekken, L., . . . Raastad, T. (2019). Type 1 Muscle Fiber Hypertrophy after Blood Flow-restricted Training in Powerlifters. *Med Sci Sports Exerc, 51*(2), 288-298. doi: 10.1249/mss.0000000000001775

Bunevicius, K., Sujeta, A., Poderiene, K., Zachariene, B., Silinskas, V., Minkevicius, R., & Poderys, J. (2016). Cardiovascular response to bouts of exercise with blood flow restriction. *Journal of Physical Therapy Science, 28*(12), 3288-3292. doi:10.1589/jpts.28.3288

Burd, West, D. W., Staples, A. W., Atherton, P. J., Baker, J. M., Moore, D. R., . . . Phillips, S. M. (2010). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS ONE, 5*(8), e12033. doi:10.1371/journal.pone.0012033

Clark, B. C., Manini, T. M., Hoffman, R. L., Williams, P. S., Guiler, M. K., Knutson, M. J., . . . Kushnick, M. R. (2011). Relative safety of 4 weeks of blood flow-restricted resistance exercise in young, healthy adults. *Scand J Med Sci Sports, 21*(5), 653-662. doi:10.1111/j.1600-0838.2010.01100.x

Cook, C. J., Kilduff, L. P., & Beaven, C. M. (2014). Improving strength and power in trained athletes with 3 weeks of occlusion training. *Int J Sports Physiol Perform, 9*(1), 166-172. doi:10.1123/ijspp.2013-0018

Cook, S. B., LaRoche, D. P., Villa, M. R., Barile, H., & Manini, T. M. (2017). Blood flow restricted resistance training in older adults at risk of mobility limitations. *Exp Gerontol, 99*, 138-145. doi:10.1016/j.exger.2017.10.004

Cumming, K. T., Paulsen, G., Wernbom, M., Ugelstad, I., & Raastad, T. (2014). Acute response and subcellular movement of HSP27, alphaB-crystallin and HSP70 in human skeletal muscle after blood-flow-restricted low-load resistance exercise. *Acta Physiol (Oxf), 211*(4), 634-646. doi:10.1111/apha.12305

de Oliveira, M. F., Caputo, F., Corvino, R. B., & Denadai, B. S. (2016). Short-term low-intensity blood flow restricted interval training improves both aerobic fitness and muscle strength. *Scand J Med Sci Sports, 26*(9), 1017-1025. doi: 10.1111/sms.12540

Dreyer, H. C., Fujita, S., Cadenas, J. G., Chinkes, D. L., Volpi, E., & Rasmussen, B. B. (2006). Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J Physiol, 576*(Pt 2), 613-624. doi:10.1113/jphysiol.2006.113175

Ellefsen, S., Hammarstrom, D., Strand, T. A., Zacharoff, E., Whist, J. E., Rauk, I., . . . Ronnestad, B. R. (2015). Blood flow-restricted strength training displays high functional and biological efficacy in women: a within-subject comparison with high-load strength training. *Am J Physiol Regul Integr Comp Physiol, 309*(7), R767-779. doi:10.1152/ajpregu.00497.2014

Farnfield, M. M., Breen, L., Carey, K. A., Garnham, A., & Cameron-Smith, D. (2012). Activation of mTOR signalling in young and old human skeletal muscle in response to combined resistance exercise and whey protein ingestion.

Appl Physiol Nutr Metab, 37(1), 21-30. doi: 10.1139/h11-132

Fry, C. S., Glynn, E. L., Drummond, M. J., Timmerman, K. L., Fujita, S., Abe, T., . . . Rasmussen, B. B. (2010). Blood flow restriction exercise stimulates mTORC1 signaling and muscle protein synthesis in older men. *J Appl Physiol (1985), 108*(5), 1199-1209. doi:10.1152/japplphysiol.01266.2009

Fujita, S., Abe, T., Drummond, M. J., Cadenas, J. G., Dreyer, H. C., Sato, Y., . . . Rasmussen, B. B. (2007). Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *Journal of Applied Physiology, 103*(3), 903-910. doi:10.1152/japplphysiol.00195.2007

Glover, E. I., Oates, B. R., Tang, J. E., Moore, D. R., Tarnopolsky, M. A., & Phillips, S. M. (2008). Resistance exercise decreases eIF2Bepsilon phosphorylation and potentiates the feeding-induced stimulation of p70S6K1 and rpS6 in young men. *Am J Physiol Regul Integr Comp Physiol, 295*(2), R604-610. doi: 10.1152/ajpregu.00097.2008

Gundermann, D. M., Fry, C. S., Dickinson, J. M., Walker, D. K., Timmerman, K. L., Drummond, M. J., . . . Rasmussen, B. B. (2012). Reactive hyperemia is not responsible for stimulating muscle protein synthesis following blood flow restriction exercise. *J Appl Physiol (1985), 112*(9), 1520-1528. doi:10.1152/japplphysiol.01267.2011

Holz, M. K., Ballif, B. A., Gygi, S. P., & Blenis, J. (2005). mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell, 123*(4), 569-580. doi:10.1016/j.cell.2005.10.024

Hulmi, J. J., Tannerstedt, J., Selänne, H., Kainulainen, H., Kovanen, V., & Mero, A. A. (2009). Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *Journal of Applied Physiology, 106*(5), 1720-1729. doi:10.1152/japplphysiol.00087.2009

Hylden, C., Burns, T., Stinner, D., & Owens, J. (2015). Blood flow restriction rehabilitation for extremity weakness: a case series. *J Spec Oper Med, 15*(1), 50-56.

Kim, D., Loenneke, J. P., Ye, X., Bemben, D. A., Beck, T. W., Larson, R. D., & Bemben, M. G. (2017). Low-load resistance training with low relative pressure produces muscular changes similar to high-load resistance training. *Muscle Nerve, 56*(6), E126-e133. doi:10.1002/mus.25626

Kubo, K., Komuro, T., Ishiguro, N., Tsunoda, N., Sato, Y., Ishii, N., . . . Fukunaga, T. (2006). Effects of low-load resistance training with vascular occlusion on the mechanical properties of muscle and tendon. *J Appl Biomech, 22*(2), 112-119.

Kubota, A., Sakuraba, K., Sawaki, K., Sumide, T., & Tamura, Y. (2008). Prevention of disuse muscular weakness by restriction of blood flow. *Med Sci Sports Exerc, 40*(3), 529-534. doi: 10.1249/MSS.0b013e31815ddac6

Kumar, V., Selby, A., Rankin, D., Patel, R., Atherton, P., Hildebrandt, W., . . . Rennie, M. J. (2009). Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol, 587*(1), 211-217. doi:10.1113/jphysiol.2008.164483

Laurentino, G. C., Ugrinowitsch, C., Roschel, H., Aoki, M. S., Soares, A. G., Neves, M., Jr., . . . Tricoli, V. (2012). Strength training with blood flow restriction diminishes myostatin gene expression. *Med Sci Sports Exerc, 44*(3), 406-412. doi:10.1249/MSS.0b013e318233b4bc

Libardi, C. A., Chacon-Mikahil, M. P., Cavaglieri, C. R., Tricoli, V., Roschel, H., Vechin, F. C., . . . Ugrinowitsch, C. (2015). Effect of concurrent training with blood flow restriction in the elderly. *Int J Sports Med, 36*(5), 395-399. doi: 10.1055/s-0034-1390496

Lixandrao, M. E., Ugrinowitsch, C., Berton, R., Vechin, F. C., Conceicao, M. S., Damas, F., . . . Roschel, H. (2018). Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med, 48*(2), 361-378. doi:10.1007/s40279-017-0795-y

Lixandrao, M. E., Ugrinowitsch, C., Laurentino, G., Libardi, C. A., Aihara, A. Y., Cardoso, F. N., . . . Roschel, H. (2015). Effects of exercise intensity and occlusion pressure after 12 weeks of resistance training with blood-flow restriction. *Eur J Appl Physiol, 115*(12), 2471-2480. doi:10.1007/s00421-015-3253-2

Loenneke, J. P., Fahs, C. A., Wilson, J. M., & Bemben, M. G. (2011). Blood flow restriction: the metabolite/volume threshold theory. *Med Hypotheses, 77*(5), 748-752. doi:10.1016/j.mehy.2011.07.029

Martin-Hernandez, J., Marin, P. J., Menendez, H., Loenneke, J. P., Coelho-e-Silva, M. J., Garcia-Lopez, D., & Herrero, A. J. (2013). Changes in muscle architecture induced by low load blood flow restricted training. *Acta Physiol Hung, 100*(4), 411-418. doi:10.1556/APhysiol.100.2013.011

Mitchell, C. J., Churchward-Venne, T. A., Parise, G., Bellamy, L., Baker, S. K., Smith, K., . . . Phillips, S. M. (2014). Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle

hypertrophy in young men. *PLoS ONE, 9*(2), e89431. doi:10.1371/journal.pone.0089431

Moore, D. R., Burgomaster, K. A., Schofield, L. M., Gibala, M. J., Sale, D. G., & Phillips, S. M. (2004). Neuromuscular adaptations in human muscle following low intensity resistance training with vascular occlusion. *Eur J Appl Physiol, 92*(4-5), 399-406. doi:10.1007/s00421-004-1072-y

Nakajima, T., Koide, S., Yasuda, T., Hasegawa, T., Yamasoba, T., Obi, S., . . . Kano, Y. (2018). Muscle hypertrophy following blood flow-restricted, low-force isometric electrical stimulation in rat tibialis anterior: role for muscle hypoxia. *J Appl Physiol (1985), 125*(1), 134-145. doi:10.1152/japplphysiol.00972.2017

Neto, G. R., Sousa, M. S., Costa, P. B., Salles, B. F., Novaes, G. S., & Novaes, J. S. (2015). Hypotensive effects of resistance exercises with blood flow restriction. *J Strength Cond Res, 29*(4), 1064-1070. doi:10.1519/jsc.0000000000000734

Norton, K. (2012). New Australian standard for adult pre-exercise screening. *Sport Health, 30*(2).

Ozaki, H., Yasuda, T., Ogasawara, R., Sakamaki-Sunaga, M., Naito, H., & Abe, T. (2013). Effects of high-intensity and blood flow-restricted low-intensity resistance training on carotid arterial compliance: role of blood pressure during training sessions. *Eur J Appl Physiol, 113*(1), 167-174. doi:10.1007/s00421-012-2422-9

Paton, C. D., Addis, S. M., & Taylor, L. A. (2017). The effects of muscle blood flow restriction during running training on measures of aerobic capacity and run time to exhaustion. *Eur J Appl Physiol, 117*(12), 2579-2585. doi:10.1007/s00421-017-3745-3

Patterson, S. D., & Brandner, C. R. (2018). The role of blood flow restriction training for applied practitioners: A questionnaire-based survey. *J Sports Sci, 36*(2), 123-130. doi:10.1080/02640414.2017.1284341

Pearson, S. J., & Hussain, S. R. (2015). A Review on the Mechanisms of Blood-Flow Restriction Resistance Training-Induced Muscle Hypertrophy. *Sports Medicine, 45*(2), 187-200. doi:10.1007/s40279-014-0264-9

Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E., & Wolfe, R. R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *American Journal of Physiology-Endocrinology and Metabolism, 273*(1), E99-E107. doi:10.1152/ajpendo.1997.273.1.E99

Sato, Y. (2005). The history and future of KAATSU training (Vol. 1).

Tarnopolsky, M. A., Atkinson, S. A., MacDougall, J. D., Chesley, A., Phillips, S., & Schwarcz, H. P. (1992). Evaluation of protein requirements for trained strength athletes. *J Appl Physiol (1985), 73*(5), 1986-1995. doi:10.1152/jappl.1992.73.5.1986

Taylor, L. W., Wilborn, C. D., Kreider, R. B., & Willoughby, D. S. (2012). Effects of resistance exercise intensity on extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase activation in men. *J Strength Cond Res, 26*(3), 599-607. doi:10.1519/JSC.0b013e318242f92d

Thiebaud, R. S., Loenneke, J. P., Fahs, C. A., Rossow, L. M., Kim, D., Abe, T., … Bemben, M. G. (2013). The effects of elastic band resistance training combined with blood flow restriction on strength, total bone-free lean body mass and muscle thickness in postmenopausal women. *Clin Physiol Funct Imaging,* 33(5), 344-352. dog:10.1111/cpf.12033

Tipton, K. D., & Witard, O. C. (2007). Protein requirements and recommendations for athletes: relevance of ivory tower arguments for practical recommendations. *Clin Sports Med, 26*(1), 17-36. doi:10.1016/j.csm.2006.11.003

Vanwye, W. R., Weatherholt, A. M., & Mikesky, A. E. (2017). Blood Flow Restriction Training: Implementation into Clinical Practice. *International Journal of Exercise Science, 10*(5), 649-654.

Vechin, F. C., Libardi, C. A., Conceicao, M. S., Damas, F. R., Lixandrao, M. E., Berton, R. P., . . . Ugrinowitsch, C. (2015). Comparisons between low-intensity resistance training with blood flow restriction and high-intensity resistance training on quadriceps muscle mass and strength in elderly. *J Strength Cond Res, 29*(4), 1071-1076. doi:10.1519/jsc.0000000000000703

Wackerhage, H. (2006). Introduction to molecular exercise physiology.

Wernbom, Apro, W., Paulsen, G., Nilsen, T. S., Blomstrand, E., & Raastad, T. (2013). Acute low-load resistance exercise with and without blood flow restriction increased protein signalling and number of satellite cells in human skeletal muscle. *Eur J Appl Physiol, 113*(12), 2953-2965. doi:10.1007/s00421-013-2733-5

Wernbom, M., & Aagaard, P. (2019). Muscle fibre activation and fatigue with low-load blood flow restricted exercise - An integrative physiology review. *Acta Physiologica*, 0(ja), e13302. doi:10.1111/apha.13302

Yasuda, T., Ogasawara, R., Sakamaki, M., Ozaki, H., Sato, Y., & Abe, T. (2011). Combined effects of low-intensity blood flow restriction training and high-intensity resistance training on muscle strength and size. *Eur J Appl Physiol, 111*(10), 2525-2533. doi:10.1007/s00421-011-1873-8

Yow, B. G., Tennent, D. J., Dowd, T. C., Loenneke, J. P., & Owens, J. G. (2018). Blood Flow Restriction Training After Achilles Tendon

Rupture. *J Foot Ankle Surg*. doi:10.1053/j.jfas.
2017.11.008

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List of Attachments

1) Approval from the Local Ethics Committee at the Norwegian School of Sports Sciences

2) Approval from the Norwegian Centre for Research Data

3) Health declaration form

4) Informed consent form

5) Questionnaire regarding screening of training history

6) Questionnaire for female participants regarding menstrual cycle and contraception

7) Diet recording log during the training intervention

8) Exercise recording log during the training intervention

9) Project cost/funding

10) Western blotting procedure, NuPAGE Invitrogen

11) Total protein procedure, DC Protein Assay, Bio-Rad

12) Wound care of biopsy

Att. 1: Approval from the Local Ethics Committee at the Norwegian School of Sports Sciences

Truls Raastad
Seksjon for fysisk prestasjon

OSLO 13. august 2018

Søknad 63 -190618 – Effekten av okklusjonstrening med lav motstand på muskelmasse, styrke og funksjon hos godt trente friske unge menn

Vi viser til søknad, prosjektbeskrivelse, informasjonsskriv og innsendt søknad til NSD, vedtak datert 22. juni 2018 fra etisk komite og svar på vedtak datert 9. august fra prosjektleder.

I henhold til retningslinjer for behandling av søknad til etisk komite for idrettsvitenskapelig forskning på mennesker, har leder av komiteen på fullmakt konkludert med følgende:

Vurdering:

I redegjørelsen fra prosjektleder fremgår følgende:

"Årsaken til at vi i utgangspunktet bare ønsket å inkludere bare menn i denne studien var på grunn av at potensielle kjønnsforskjeller og påvirkning av menstruasjonssyklus kunne gi mer støy i enkelte av målingene. Etter en nøyere vurdering har vi nå kommet frem til at potensielle kjønnsforskjeller og påvirkning fra menstruasjonssyklus vil tillegge så liten ekstra variasjon at det er tilregnelig å inkludere både menn og kvinner i denne studien uten at vi behøver å øke det total antallet deltagere. Vi vil rekrutter halvparten av hvert kjønn slik at vi også kan gjøre en sub-analyse på mulige kjønnsforskjeller."

Prosjektet godkjennes.

Vedtak:

På bakgrunn av forelagte dokumentasjon og innhentet informasjon finner komiteen at prosjektet er forsvarlig. Til vedtaket har komiteen lagt følgende forutsetning til grunn:

- *At vilkår fra NSD følges*

NIH NORGES IDRETTSHØGSKOLE

Besøksadresse: Sognsveien 220, Oslo
Postadresse: Pb 4014 Ullevål Stadion, 0806 Oslo
Telefon: +47 23 26 20 00, postmottak@nih.no
www.nih.no

Personvernombudet for forskning

Prosjektvurdering - Kommentar

Prosjektnr: 61071

GODKJENNING FRA ANDRE INSTANSER - REK

Prosjektet omhandler helse og personvernombudet rettet spørsmål til daglig ansvarlig om prosjektet har vært fremlagt for REK i e-post datert 02.07.18. Daglig ansvarlig oppgir i e-postkorrespondanse 03.07.18 at han tidligere har meldt inn liknende prosjekter til REK uten at disse har vært søknadspliktige etter helseforskningsloven. På bakgrunn av opplysningene fra daglig ansvarlig, legger personvernombudet til grunn at prosjektet ikke skal behandles av REK.

FORMÅL

Formålet med prosjektet er å undersøkt om okklusjonstrening med lav motstand fører til like god muskelvekst og styrkeøkning som vanlig tung styrketrening.

SENSITIVE OPPLYSNINGER

Studien innebærer behandling av sensitive opplysninger om helse. Personvernombudet vurderer data til å være relevante og nødvendige for å gjennomføre prosjektet, og har ingen innvendinger til datainnsamlingen.

UTVALG OG REKRUTTERING

Utvalget består av friske menn og kvinner i alderen 20-35 år som trener styrketrening jevnlig. Rekruttering vil skje gjennom rekrutteringsplakater som henges opp på trenings- og samlingssteder for studenter i Oslo, samt via NIHs nettside og Facebookside. Informasjonsskriv sendes deretter til de som melder sin interesse. Personvernombudet har ingen innvendinger til dette.

METODE

Innsamling av personopplysninger vil skje gjennom papirbasert spørreskjema. I tillegg vil effekten av treningen bli vurdert på følgende målinger før og etter en ti uker lang treningsintervensjon:
A) Muskelvekst målt som størrelse på lårsmuskulatur med hjelp av MR-skann
B) Muskelvekst målt som størrelse på type I og type II fibre målt på snitt fra muskelbiopsier.
C) Styrke målt som 1 RM i knebøy
D) Muskelstyrke i knestrekkerne
E) Muskulær utholdenhet
F) Oksygeninnhold i lårmuskelen ved NIRS.

SPESIFIKK FORSKNINGSBIOBANK

Det fremgår av meldeskjema at det skal opprettes en spesifikk forskningsbiobank i prosjektet for oppbevaring av biologisk materiale. Helseforskningsloven § 25 regulerer opprettelse av forskningsbiobanker. For opprettelse av en spesifikk forskningsbiobank forutsetter dette at REK har vurdert prosjektet som fremleggingspliktig etter helseforskningsloven. På bakgrunn av at dette ikke er tilfellet for dette prosjektet, vil REK heller ikke ha hjemmel til å godkjenne opprettelsen av omsøkt biobank.

Att. 2: Approval from the Norwegian Centre for Research Data

Truls Raastad
Postboks 4014
0806 OSLO

Vår dato: 06.07.2018 Vår ref: 61071 / 3 / TAL Deres dato: Deres ref:

Tilrådning fra NSD Personvernombudet for forskning § 7-27

Personvernombudet for forskning viser til meldeskjema mottatt 10.06.2018 for prosjektet:

61071	*Effekten av okklusjonstrening med lav motstand på muskelmasse, styrke og funksjon hos godt trente friske unge menn*
Behandlingsansvarlig	*Norges idrettshøgskole, ved institusjonens øverste leder*
Daglig ansvarlig	*Truls Raastad*
Student	*Tore Chrsitian Næss*

Vurdering

Etter gjennomgang av opplysningene i meldeskjemaet og øvrig dokumentasjon finner vi at prosjektet er unntatt konsesjonsplikt og at personopplysningene som blir samlet inn i dette prosjektet er regulert av § 7-27 i personopplysningsforskriften. På den neste siden er vår vurdering av prosjektopplegget slik det er meldt til oss. Du kan nå gå i gang med å behandle personopplysninger.

Vilkår for vår anbefaling

Vår anbefaling forutsetter at du gjennomfører prosjektet i tråd med:
- opplysningene gitt i meldeskjemaet og øvrig dokumentasjon
- vår prosjektvurdering, se side 2
- eventuell korrespondanse med oss

Meld fra hvis du gjør vesentlige endringer i prosjektet

Dersom prosjektet endrer seg, kan det være nødvendig å sende inn endringsmelding. På våre nettsider finner du svar på hvilke endringer du må melde, samt endringsskjema.

Opplysninger om prosjektet blir lagt ut på våre nettsider og i Meldingsarkivet

Vi har lagt ut opplysninger om prosjektet på nettsidene våre. Alle våre institusjoner har også tilgang til egne prosjekter i Meldingsarkivet.

Vi tar kontakt om status for behandling av personopplysninger ved prosjektslutt

Ved prosjektslutt 15.08.2023 vil vi ta kontakt for å avklare status for behandlingen av personopplysninger.

Dokumentet er elektronisk produsert og godkjent ved NSDs rutiner for elektronisk godkjenning.

NSD – Norsk senter for forskningsdata AS Harald Hårfagres gate 29 Tel: +47-55 58 21 17 nsd@nsd.no Org.nr. 985 321 884
NSD – Norwegian Centre for Research Data NO-5007 Bergen, NORWAY Faks: +47-55 58 96 50 www.nsd.no

Se våre nettsider eller ta kontakt dersom du har spørsmål. Vi ønsker lykke til med prosjektet!

Vennlig hilsen

Marianne Høgetveit Myhren

Trine Anikken Larsen

Kontaktperson: Trine Anikken Larsen tlf: 55 58 83 97 / Trine.Larsen@nsd.no
Vedlegg: Prosjektvurdering
Kopi: Tore Chrsitian Næss, tore.christian.93@gmail.com

Personvernombudet har ved tidligere anledning forhørt seg med REK sør-øst og NEM om hvilket regelverk som regulerer opprettelse av en spesifikk biobank til forskningsformål som ikke innebærer medisinsk og helsefaglig forskning på mennesker. Slik vi forstår det, er tilbakemeldingene at regelverket er noe uklart og at Helsedepartementet finner at det kan være hensiktsmessig å se nærmere på praktiseringen av lovverket knyttet til forskningsprosjekter som befinner seg i gråsonen av helseforskningslovens virkeområde.

Frem til en nærmere avklaring og for at disse prosjektene ikke skal havne i et lovtomt rom, har personvernombudet mottatt e-post fra NEM datert 18.12.2014. Her opplyser NEM at helseforskningslovens § 25 annet ledd ikke kommer til anvendelse, og at de legger til grunn at ved opprettelse av en spesifikk biobank til annet forskningsformål, vil personopplysningsloven gjelde. De skriver blant annet at "det opprettes et forskningsregister. Biologisk materiale oppfattes som informasjonsbærende, og analyserbart materiale blir "opplysninger" i registeret. Dette gjør registeret med tilhørende biobank til et konsesjonspliktig register."

Personvernombudet har tatt tilbakemeldingen fra NEM til etterretning og er enig i at dette en akseptabel løsning frem til spørsmålet er nærmere avklart. Det kan med dette opprettes en spesifikk forskningbiobank i tilknytning til prosjektet.

UTFØRING AV MUSKELBIOPSI OG MEDISINSKE TESTER
Personvernombudet forutsetter at kompetent helsepersonell utfører muskelbiopsi og medisinske tester i prosjektet.

INFORMASJON OG SAMTYKKE - INNFØRING AV NY PERSONVERNLOVGIVNING
Det er opplyst i meldeskjema at utvalget vil motta skriftlig og muntlig informasjon om prosjektet, og samtykke skriftlig til å delta. Vår vurdering er at informasjonsskrivet til utvalget er godt utformet etter gjeldende lovverk.

I løpet av 2018 vil ny personopplysningslov med skjerpede krav til informasjon og samtykke. På grunn av prosjektets varighet oppfordrer personvernombudet at det gjøres enkelte tilføyinger i informasjonsskrivet for å imøtekomme disse endringene:

- at samtykke er det lovlige behandlingsgrunnlaget for behandling av personopplysninger
- hvilke typer opplysninger som innhentes
- hvem som skal ha tilgang til personopplysninger, og hvilke opplysninger de får tilgang til
- hvilke tiltak dere gjør for å sikre at ikke uvedkommende får tilgang til personopplysningene
- kontaktopplysninger til institusjonens personvernombud (personvernombudet@nsd.no, +47 55 58 21 17)
- deltakernes rettigheter, herunder rett til innsyn i hvilke opplysninger som er registrert om deltakeren, rett til å få slettet eller rettet de opplysningene som er registrert, eventuelt motsette seg at opplysningene registreres, rett til å få utlevert en kopi av opplysningene som er registrert (dataportabilitet), samt rett til å sende klage til personvernombudet eller Datatilsynet angående behandlingen av personopplysninger.

På våre nettsider finnes en ny mal for informasjonsskriv vi anbefaler at det tas utgangspunkt i:
http://www.nsd.uib.no/personvernombud/hjelp/informasjon_samtykke/informere_om.html

Vi ber om at det reviderte informasjonsskrivet sendes til personvernombudet@nsd.no Husk å oppgi prosjektnummer. Prosjektet kan deretter starte.

INFORMASJONSSIKKERHET
Personvernombudet forutsetter at alle data behandles i tråd med Norges idrettshøgskole sine retningslinjer for datahåndtering og informasjonssikkerhet.

PROSJEKTSLUTT
Prosjektslutt er oppgitt til 15.08.2023. Det fremgår av meldeskjema og informasjonsskriv at datamaterialet anonymiseres ved prosjektslutt. Anonymisering innebærer vanligvis å:
- slette direkte identifiserbare opplysninger som navn, fødselsnummer, koblingsnøkkel
- slette eller omskrive/gruppere indirekte identifiserbare opplysninger som bosted/arbeidssted, alder, kjønn

Vi forutsetter det biologiske materialet som er lagret i den spesifikke forskningsbiobanken destrueres ved prosjektslutt.

For en utdypende beskrivelse av anonymisering av personopplysninger, se Datatilsynets veileder:
https://www.datatilsynet.no/globalassets/global/regelverk-skjema/veiledere/anonymisering-veileder-041115.pdf

Att. 3: Health declaration form

Etternavn:	Fornavn:	Født:
Studentadresse:		
Hjemmeadresse:		
Tlf.:	E-mailadresse:	
Idrettsbakgrunn (angi idrettsgrener og omtrent hvor mange timer du trener pr. uke):		

EGENERKLÆRING FOR FORSØKSPERSONER

Takk for at du vurderer å delta som forsøksperson ved Norges idrettshøgskole! Før du kan delta, må vi imidlertid kartlegge om din deltakelse kan medføre noen form for helserisiko. Vær snill å lese gjennom alle spørsmålene nøye og svar ærlig ved å krysse av for JA eller NEI. Hvis du er i tvil, bør du be om å få snakke med legen som er ansvarlig for forsøket.

Hvis du krysser av for JA på ett eller flere av disse spørsmålene, må du gjennomgå en legeunder-søkelse før forsøksstart. Ved enkelte typer forsøk vil du uansett bli innkalt til legeundersøkelse.

JA	NEI	
☐	☐	1. Kjenner du til at du har en hjertesykdom?
☐	☐	2. Hender det du får brystsmerter i hvile eller i forbindelse med fysisk aktivitet?
☐	☐	3. Kjenner du til at du har høyt blodtrykk?
☐	☐	4. Bruker du for tiden medisiner for høyt blodtrykk eller hjertesykdom (f.eks. vanndrivende tabletter)?
☐	☐	5. Har noen av dine foreldre, søsken eller barn fått hjerteinfarkt eller dødd plutselig (før fylte 55 år for menn og 65 for kvinner)?
☐	☐	6. Røyker du?
☐	☐	7. Kjenner du til om du har høyt kolesterolnivå i blodet?
☐	☐	8. Har du besvimt i løpet av de siste 6 måneder?
☐	☐	9. Hender det du mister balansen på grunn av svimmelhet?
☐	☐	10. Har du sukkersyke (diabetes)?
☐	☐	11. Kjenner du til noen annen grunn til at din deltakelse i prosjektet kan medføre helse- eller skaderisiko?

Gi beskjed straks dersom din helsesituasjon forandrer seg fra nå og til undersøkelsen er ferdig, f.eks. ved at du blir forkjølet, får feber, eller blir gravid.

____

Sted - dato

____

Underskrift

Att. 4: Informed consent form

Forespørsel om deltakelse som forsøksperson i prosjektet

" Effekten av okklusjonstrening med lav motstand på muskelmasse, styrke og funksjon hos godt trente friske unge menn"

Dette skrivet er til alle potensielle forsøkspersoner. Før du bestemmer deg er det viktig at du forstår hvorfor studien gjøres og hva den vil innebære. Ta deg tid til å lese følgende informasjon nøye og diskuter informasjonen med andre om du ønsker det. Spør oss gjerne om det er noe som er uklart eller om du ønsker mer informasjon. Ta deg tid til å bestemme om du ønsker å være med eller ikke.

Takk for at du leser dette.

Hva er formålet med studien?
Hovedformålet med studien er å undersøke om okklusjonstrening med lav motstand fører til like god muskelvekst og styrkeøkning som vanlig tung styrketrening. Videre tror vi, basert på våre tidligere studier, at de to treningsformene vill skille seg ut ifra hvilke fibertyper de påvirker mest. Effekten av treningen vil bli vurdert på følgende målinger før og etter en ti uker lang teningsintervensjon:
A) Muskelvekst målt som størrelse på lårsmuskulatur med hjelp av MR-skann og DXA-skann
B) Muskelvekst målt som størrelse på type I og type II fibre målt på snitt fra muskelbiopsier tatt fra den ytre lårmuskelen.
C) Styrke målt som 1 RM i knebøy
D) Muskelstyrke i knestrekkerne målt i en isometrisk (statisk) kneekstensjon
E) Muskulær utholdenhet målt som totalt arbeid og en tretthetsindeks under 50 maksimale konsentriske kneekstensjoner.
F) Oksygeninnhold i lårmuskelen ved NIRS (en probe holde på huden over lårmuskelen og ved hjelp av infrarødt lys som sendes inn i muskelvevet kan man estimere oksygeninnhold i muskelen)
G) Hopphøyde målt i et svikthopp på kraftplattform

Hvem kan delta?
Vi ser etter godt styrketrente menn og kvinner i alderen 18-35 år som er/har:

- Friske og frie for skader
- Erfaring med styrketrening

Du kan dessverre ikke delta hvis noe av det følgende gjelder for deg:

Hva er de mulige ulempene og risikoene ved å delta?
Du kan oppleve fysisk ukomfortabelhet under testing og de harde treningsøktene:

- Svimmelhet og utmattelse etter maksimale anstrengelser både på testene til utmattelse og de tøffeste styrkeøktene
- Muskelbiopsien kan oppleves som ubehagelig og du vil føle en sårhet/stølhet i muskulaturen 1-2 dager etter inngrepet.

Risikovurderinger for alle prosedyrer har blitt gjennomført og vil bli fulgt gjennom hele studien.

Hva er de mulige fordelene ved å delta?
Vi håper at å delta i studien vil hjelpe deg. Likevel kan ikke dette garanteres. Informasjonen vi får gjennom studien kan hjelpe oss å vurdere om trening med redusert blodstrøm kan gi noen tilleggseffekter til vanlig tung styrketrening hos godt trente. Per dags dato er det lite dokumentasjon om effekten av slik trening på godt trente og spesielt når okklusjonstreningen gjennomføres i øvelser som knebøy. Resultatene fra denne studien vil derfor potensielt øke den forskningsbaserte kunnskapen om effekten av okklusjonstrening. Det følgende er fordeler for deg som deltaker ved å delta i studien:

- Gratis analyse av kroppssammensetning.
- Omfattende testing av din muskelstyrke og funksjon i beina.
- Tilbakemelding på dine testresultater og sammenligning før og etter treningsperioden.
- Tilbakemelding på din individuelle respons på treningen.
- Innsikt i spennende vitenskapelig forskning

Blir min deltakelse holdt konfidensielt?
Forskningsgruppen har gjort flere tiltak for å beskytte taushetsplikten ovenfor deg som deltaker. Du blir tildelt en deltaker-ID som vil bli brukt i alle registreringer av data du gir. Navnet ditt og annen personlig informasjon vil ikke bli assosiert med dine date, for eksempel, blir samtykkeskjemaet ditt holdt separat fra resten av dataene dine. Alle papirregistreringer blir oppbevart i et låst arkiv kun tilgjengelig for forskningsgruppen, og all elektronisk data blir oppbevart på passordbeskyttet PC.

Hva vil skje med resultatene fra studien?
Dataene og informasjonen som registres under testingen, skal brukes i henhold til formålet og hensikten med studien. Alle opplysningene vil bli behandlet uten direkte gjenkjennende opplysninger, som navn og fødselsnummer. Du vil ved forsøksstart få utdelt et forsøkspersonnummer (ID-nummer) som skal brukes under studien og det er bare dette nummeret som vil være tilknyttet til dine data. Det betyr at alle data vil bli behandlet anonymt og det vil ikke være mulig å identifisere deg i resultatene. Underveis i forsøket vil vi oppbevare en kodeliste med navn og forsøkspersonnummer. Denne kodelisten vil fysisk være låst inne, slik at det er kun forskerne tilknyttet studien som har adgang til den. Alle som får innsyn i informasjon om deg har taushetsplikt. Kodelisten lagres i 5 år etter prosjektslutt, og destrueres etter dette.

Muskelprøvene som tas av deg vil bli oppbevart i en forskningsbiobank uten kommersielle interesser. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i denne biobanken. Prøvene vil

- Skader som påvirker din treningsevne eller gjennomføring av tester
- Kardiovaskulær sykdomsdiagnoser eller annen sykdom som påvirker treningsevne eller som diskvalifiserer fra å ta muskelbiopsi
- Røykere
- Nåværende eller tidligere narkotikabruk (siste 3 måneder)
- Bruk av medikamenter/kosttilskudd som kan påvirke testresultatene

Må jeg delta?

Det er opp til deg å bestemme om du skal delta eller ikke. Hvis du bestemmer deg for å delta får du dette informasjonsskrivet og du blir bedt om å signere et samtykkeskjema. Du kan likevel trekke tilbake samtykke om å delta når som helst uten å oppgi noen grunn. Hvis du trekker deg fra deltakelse i studien, vær vennlig å informer en av studielederne så raskt som mulig. Hvis du, av hvilken som helst grunn, ønsker å trekke tilbake data vi har registrert på deg, vær vennlig å kontakte en av studielederne innen en måned etter deltakelse. Etter dette er det mulig at dine resultater allerede har blitt publisert. All data blir uansett anonymisert og dine individuelle data kan ikke bli identifisert på noen måte.

Hva innebærer deltagelse i studien?

Deltagelsen i prosjektet skjer over 12 uker og innebærer i denne perioden regelmessige oppmøter ved Norges idrettshøgskole på Sognsvann for testing og trening. Studien består av en tilvenningsdag og en testdager før treningsintervensjonen starter, i tillegg til en dag hvor vi tar en muskelprøve fra en lårmuskel. Treningsintervensjonen går over ni uker og det vil være tre økter per uke.

Ønsker du å delta på treningsintervensjonen blir du tilfeldig trukket inn i gruppen som skal gjennomføre styrketreningen med lav motstand og redusert blodstrøm (okklusjon) eller gruppen som gjennomfører tradisjonell tung styrketrening. I gruppen som skal trene med redusert blodstrøm vil man bruke en trykkmansjett som er 10 cm bred og som festes høyt opp på låret før første serie starter. Trykket i mansjetten vil være på 80-120 mmHg, avhengig av størrelsen på lårene dine, og vil halvere blodstrømmen til beina. Begge gruppene vil gjennomføre 4 serier i knebøy og 4 serier i kneekstensjon på økt 1 og 3 i hver uke, og det samme i ettbeinsknebøy og kneekstensjon i økt 2. Havner du i gruppen som skal trene med lav treningsmotstand og redusert blodstrøm vil treningsmotstanden være 30% av 1 RM og seriene kjøres med 30-15-15-15 reps, med 45 sek pause mellom hver serie. Havner du i gruppen som skal gjennomføre tung styrketrening vil treningsmotstanden være på ca. 75% av 1 RM og du gjennomfører 8-10 repetisjoner i hver serie med 120 sek pause mellom hver serie.

I forbindelse med den siste treningsøkten vil vi ta muskelbiopsier 2, 24 og 48 timer etter økta. Dette for å studere mekanismene som fører til muskelvekst ved å måle aktivitet i viktige enzymer som er med på å regulere proteeinsyntesen og ved å studer aktiveringen av satellittceller som også bidrar til muskelvekst ved styrketrening.

Alle styrketester og måling av kroppssammensetning og størrelse på lårmusklene vil bli gjennomført før og etter treningsintervensjonen.

Besøk 1: Tilvenning til tester

Det første besøket skal gi deg informasjon og tilvenne deg testene du skal gjennom på testdagene. Dagen vil ta omtrent 2 timer og inkluderer:

- Signering av samtykkeskjema (kun besøk 1)
- Utfylling av helsespørreskjema (kun besøk 1)
- Vekt og høyde måling
- Kroppsanalyse med DXA scan
- Gjennomføring av tester i følgende rekkefølge:
  - Oppvarming
  - Spensthopp på kraftplattform
  - 1 RM i beinpress knebøy
  - Isometrisk styrke i kneekstensjon
  - Test av muskulær utholdenhet i kneekstensjon
- Informasjon og instruksjon av forberedelser du trenger å gjøre før testdagen

Besøk 2: Testdagen

Testdagen er helt identisk før og etter treningsintervensjonen og består av de samme fysiske tester som beskrevet over. Før oppvarmingen vil du gjennomgå målingen av oksygeninnhold i lårmuskelen. Selve testdagen varer ca. 30 min kortere enn tilvenningsdagen

Besøk 3: MR av lårmuskler

MR skann av lårene gjennomføres på Curato røntgeninstitutt i Oslo sentrum. Du ligger på ryggen i en magnetisk trommel i ca. 15 min mens skanningen pågår.

Trening

Alle treningsøktene gjennomføres i styrkerommet på Norges idrettshøgskole. De som skal trene med redusert blodstrøm vil bruke en trykkmansjett som er 10 cm bred og som festes høyt opp på låret før første serie starter. Trykket i mansjetten vil være på 80-120 mmHg, avhengig av størrelsen på lårene dine, og vil halvere blodstrømmen til beina. Begge gruppene vil gjennomføre 4 serier i knebøy og 4 serier i kneekstensjon på økt 1 og 3 i hver uke, og det samme i ettbeinsknebøy og kneekstensjon i økt 2. Havner du i gruppen som skal trene med lav treningsmotstand og redusert blodstrøm vil treningsmotstanden være 30% av 1 RM og seriene kjøres med 30-15-15-15 reps, med 45 sek pause mellom hver serie. Havner du i gruppen som skal gjennomføre tung styrketrening vil treningsmotstanden være på ca. 75% av 1 RM og du gjennomfører 8-10 repetisjoner i hver serie med 120 sek pause mellom hver serie.

Muskelbiopsier

Muskelprøven tas fra den ytre lårmuskelen (m. vastus lateralis). Du får lokalbedøvelse i hud og i bindevevet til muskelen før det lages et ca. 1 cm langt snitt gjennom huden og bindevevshinnen til muskelen. Deretter tas muskelprøven med en spesialtilpasset nål. Du kjenner et trykk i muskelen når nålen går inn og et lite napp når en liten muskelbit kuttes inne i nålen. En prøve tilsvarer ca. 200 mg vev (0,2 g). Hele prosedyren tar ca. 20 min. Etter biopsien lukkes såret med teip og plaster som skal sitte på i 1 uke. I muskelbiopsiene vil vi størrelse på type I og II muskelfibre, antall satellittceller og cellekjerner, og sentrale signalveier som regulerer proteinsyntese.

bli lagret i biobanken til år 2023. Ansvarlig for biobanken er Professor Truls Raastad ved Seksjon for fysisk prestasjonsevne ved Norges idrettshøgskole.

Hvem har gjennomgått studien?
Studien har fått etisk godkjenning fra Norges Idrettshøgskoles etiske komité.

Innsynsrett og oppbevaring av materiale
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Informasjon om utfallet av studien
Etter at data er innsamlet og analysert vil vi avholde et møte for alle forsøkspersonene der vi presenterer resultatene fra studien.

Forsikring
Deltakere i prosjektet er forsikret dersom det skulle oppstå skade eller komplikasjoner som følge av deltakelse i forskningsprosjektet. NIH er en statlig institusjon og er således selvassurandør. Dette innebærer at det er NIH som dekker en eventuell erstatning og ikke et forsikringsselskap.

Finansiering
Prosjektet er finansiert av Norges idrettshøgskole. Det er søkt om midler fra Quensland Academy of Sport for å dekke kostnadene knyttet til MR analysene av lårmuskler.

Samtykke
Hvis du har lest informasjonsskrivet og ønsker å være med som forsøksperson i prosjektet, ber vi deg undertegne "Samtykke om deltakelse" og returnere dette til en av personene oppgitt nedenfor. Du bekrefter samtidig at du har fått kopi av og lest denne informasjonen.

Kontakt for videre informasjon
Hvis du trenger videre informasjon, har spørsmål eller ønsker å trekke deg fra studien, vær vennlig å kontakte:

Masterstudenter:
Tore Chrsitian Næss: Tlf: 48 21 92 33, epost: tore.christian.93@gmail.com
Maria Moen: Tlf: 98 43 78 81, epost: m-mooen@live.no

PhD-student Charlie Davids: tlf: 48 63 62 96, epost: c.davids@uq.edu.au

Prosjektleder:
Truls Raastad, Norges idrettshøgskole, Sognsveien 220, 0863 Oslo, +47 23 26 23 28, truls.raastad@nih.no

Takk for at du deltar i denne studien. Du bør beholde dette informasjonsskrivet da det inneholder viktig informasjon og kontaktdetaljer til forskningsgruppen.

Husk å

- Avstå fra alkohol 48 timer før testdagene
- Avstå fra hard trening 48 timer før testdagene (følg anvist trening siste dage før siste test)

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Att. 5: Questionnaire regarding screening of training history

Participant no:

Resistance & endurance training history questionnaire

Resistance training

1. Have you resistance trained your lower body in the last 6 months? — Yes / No
2. Has your training been *regular* e.g. 2-3 times a week on average, or more *sporadic* e.g. 1-2 a fortnight? — Regular / Sporadic
3. How would you generally rate the load(s) used in your sessions? — Heavy / Moderate / Light or bodyweight
4. If regular, how many months have you approximately regularly (2-3 times a week) trained in this manner for? — ………. Months
5. Also if regular, what's been your aim for the training? — Strength / Muscle mass / Body fat decrease / Sport-specific
6. If sporting specific, please state which sport(s) — ……………………… ……………………… ………………………

Endurance training

1. Are you currently do any form of endurance training? — Yes / No
2. If yes, what sport(s)/activity do you train for? — ………………………
3. If yes, how many hours on average would you say you train for? — ………. hours
4. If yes, what would your average training intensity be? — Low / Moderate / Intense
5. If yes, what modality or modalities do you typically undertake? — ……………………… ……………………… ………………………

Att. 6: Questionnaire for female participants regarding menstrual cycle and contraception

Participant no:

Menstruation and oral contraceptive use questionnaire

1. **Please enter today's date; ______ day ______ month**

2. **Do you currently, and will you over the course of this study continue to use some form of oral contraception? Please check a box.**

   Yes ☐ No ☐

   - If you answered **Yes** to the above, please **only** complete question 3
   - If you answered **No** to the above, please proceed to question 4

3. **What type of oral contraceptive pill are/will you use? Please check a box and enter the brand name.**

   - **Monophasic** e.g. Microgynon, Levelen, Nordette or Monofeme ☐
   - **Mini/progesterone only** e.g. Microval, Microlut or Micronor ☐
   - **Triphasic** e.g. Triphasal, Trifeme or Triquilar ☐
   - **Combined** - Yasmin or Yaz ☐
   - **Combined** - Norimin or Brevinor ☐

   What is the brand name of the pill? ____________________

4. **Are you using, and will continue to use a method of contraceptive that is different to oral contraception? E.g. IUD or implant**

   Yes ☐ No ☐

   If **YES**, please state the type ________________________________

   If **NO**, please answer questions 5, 6, 7 and 8 below

5. **Approximately how many days does your cycle usually last for; from the first day of bleeding in one month, to first day of bleeding in the next month?**

   _________ days

6. **Approximately what was the date of your previous first day of bleeding?**

   ______ day ______ month

7. **From your usual cycle duration, approximately how many days does bleeding occur for?**

   _________ days

8. **To today's date, approximately how many days have passed since bleeding stopped?**

   _________ days

Att. 7: Diet recording log during the training intervention

Dietary record sheet

Please use this template to record *all* the food and drink you consume in **three complete days each week. Choose two weekdays and one weekend day.** These don't have to be consecutive days.

We'll use this information to work out your calorie intake, so the more information you record, the more accurate we'll be.

Important points to remember:

- **AIM TO CONSUME 1.5g OF PROTEIN PER KG OF BODY MASS DAILY, THROUGHOUT THE TRAINING PERIOD!!!**
- Include details of the types of food and beverages (for example brand names if known, or describe the dish if you are eating out)
- Record all food and beverages as soon as you eat or drink them (you will remember and record more this way)
- Aim to record portion sizes for each food and beverage (use the portion guide sheet to help you estimate the portion)
- Record the type of food or dish and list each ingredient if you can.
- Don't forget to record the little 'extras and details' like types and amount of dressings, condiments, the kind of spreads on toast, the type of milk (full fat, semi or skim) and any added sugar or honey, and the protein provided to you for your sessions.
- Use theses abbreviations if you like: Tbsp = tablespoon; tsp = teaspoon
- Please ask one of the research team members if you have any questions about filling in your food diary

Example:

Time of day	**FOOD TYPE** Type of food, ingredients, method of cooking and portion size	**BEVERAGE** Type of beverage and portion size	**EXTRAS** e.g. milk, sugar, spreads, condiments and portion size
Breakfast	2 boiled eggs 2 slices Wholegrain toast 1 handful Avocado	1 cup of tea 1 cup of fresh orange juice	1 Tbsp butter 1 Tbsp strawberry jam 1 Tbsp full fat milk 1 tsp white sugar
Morning Snack	1 cup of cashew nuts	1 cup of coffee	1 Tbsp full fat milk 2 tsp white sugar

Page 1

DAY AND DATE: ____________________

Time of day	**FOOD TYPE** Type of food, ingredients, method of cooking and portion size	**BEVERAGE** Type of beverage and portion size	**EXTRAS** e.g. milk, sugar, spreads, condiments and portion size

Page 2

Att. 8: Exercise recording log during the training intervention

Date	Exercise type	Duration	Intensity / sets
Example Mon 22nd May	Example Soccer match Quick chest and back session	Example 90 min 30 min	Example N/A Bench Press – 5 x 5 @ 80Kg Rack pull – 5 x 5 @ 150Kg

Att. 9: Project cost/funding

C1 **What is the proposed budget for your project? (Please add additional lines if needed)**

		Costing		
Source of funds	**ESSA**	**Investigators**	**Collaborative organisations**	**Total**
Direct costs				
Consumables				
Muscle biopsy consumables (120 biopsies x 200 NOK per biopsy, 0.16 exchange rate)		$3,860.17		$3,860.17
Protein supplementation			$1,400.00	$1,400.00
Muscle tissue analysis (antibodies, chemicals and buffers for 120 biopsies/time points)			$27,115.07	$27,115.07
Blood sample collection and analysis (needles, vacutainers, kits, calibrators, quality controls)		$2,125.00		$2,125.00
Biopsy collection practitioner (120 biopsies, 0.5hr per biopsy (60 hours total)). 350 NOK per hour, 0.16 exchange rate.			$3,360.00	$3,360.00
Equipment				
Dual X-ray absorptiometry scanning (24 participants, pre- and post-training scan; 250 NOK per scan, 0.16 exchange rate)			$1,920.00	$1,920.00
MRI scanning (24 participants, pre- and post-training scan; 2,200 NOK per scan, 0.16 exchange rate)	$16,000.00	$896.00		$16,896.00
Laboratory training and testing space usage (in-kind). Estimated 150 hours, 350 NOK per hour, 0.16 exchange rate			$8,400.00	$8,400.00
Resistance training and testing equipment (in-kind). Estimated 300 hours, 175 NOK per hour, 0.16 exchange rate			$8,400.00	$8,400.00
Travel (directly related to research)				
Return flight to Oslo		$2,300.00		$2,300.00
Oslo accommodation		$4,039.40		$4,039.40
Oslo sustenance		$4,000.00		$4,000.00
TOTAL DIRECT COSTS	**$16,000.00**	**$17,220.57**	**$50,595.07**	**73,476.24**

Att. 10: Western blotting procedure, NuPAGE Invitrogen

Norges idrettshøgskole **Muskellaboratorium, SFP**

TITTEL	**Elektroforese og Western Blot, NuPAGE (Invitrogen)**								
SOP NR.	DATO	UTSKREVET	GODKJENT	TYPE	VEDLEGG	REFERANSE	ERSTATTER	KOPI	SIDE
ML M-8	12.07.2018	HNØ		M-bilag	1		M-AL 14	Muskellaboratorium	1 av 6

1. **Invitrogen**
   - 1.1. Levert av Thermo Fisher Scientific.
   - 1.2. Invitrogen systemet separerer proteiner fra muskelhomogenat vha. SDS-PAGE elektroforese og immunoblotting (Western Blot).
   - 1.3. Bildebehandlingen blir gjort på ChemiDoc MP, Imaging System fra BioRad.

2. **Reagenser**
   - 2.1. Sample Reducing Agent (10x), NuPAGE, NP0009, Invitrogen *(Kjøleskap)*
   - 2.2. LDS Sample buffer (4x), NuPAGE, NP0007, Invitrogen
   - 2.3. MES/(MOPS) SDS Running Buffer (20x), NuPAGE, NP0002/(NP0001), Invitrogen
   - 2.4. Transfer Buffer, NuPAGE, NP00061, Invitrogen
   - 2.5. Antioxidant, NuPAGE, NP0005, Invitrogen *(Kjøleskap)*
   - 2.6. Tris buffered saline, 10 x TBS, Cat #170-6435, BioRad
   - 2.7. Tween 20, 437082Q, VWR
   - 2.8. Skim milk powder, 1.15363.0500, Merck KGaA
   - 2.9. Methanol, 1.06007.2500, Merck KGaA *(Avtrekksskap/Brannsikkertskap)*, ***les SDS***
   - 2.10. SuperSignal, 34076, Thermo Scientific
     - Luminol/Enhancer Solution, 1859024
     - Stable Peroxide Buffer, 1859025
   - 2.11. NuPAGE 4-12% Bis-Tris Gel, 10 well, NP0321BOX, Invitrogen
   - 2.12. Immun-Blot PVDF Membranes for Protein Blotting, Cat. #1620177, BioRad

3. **Utstyr**
   - 3.1. Elektroforese/Blottekammer m/lokk (Novex Mini-Cell, XCell SureLock, Invitrogen)
   - 3.2. Gelholder
   - 3.3. Blottekassett (XCell II Blot Module, Invitrogen)
   - 3.4. Lås/klemme til elektroforese/blottekammeret
   - 3.5. Power supply (Power Pac 200, BioRad)
   - 3.6. Buffer dam (ved analyse av en gel i et kammer)
   - 3.7. Metallredskap og rulle til montering av blottesandwich
   - 3.8. Diverse utstyr (pipetter, pinsetter, glassflasker, målekolber, målesylinder m.m.)

4. **Farer**
   - 4.1. Kan irritere huden ved kontakt.
   - 4.2. Kan gi alvorlig øyeskade.

5. **Førstehjelpstiltak**
   - 5.1. Ved kontakt med øynene.
     - 5.1.1. Kontakt umiddelbart ***Giftinformasjonssentralen*** eller ***lege***.
     - 5.1.2. Skyll forsiktig med vann i flere minutter.
   - 5.2. Kontroller sikkerhetsdatablad (SDS) for løsninger og kjemikaler du benytter.
     - 5.2.1. Logg inn i ecoonline.no for å få tilgang til SDS:
       - Firmakode: **2145**
       - Brukernavn: **biokjemi**
       - Passord: **biokjemi**

6. Løsninger

6.1. Running Buffer, 2 L *(Til 2 geler)*
- 100 ml MES/MOPS SDS Running Buffer + 1900 ml dH_2O *(Kjøleskap)*

6.2. Transfer Buffer, 1 L *(Til 2 geler)*
- 50 ml Transfer Buffer + 100 ml metanol + 850 ml dH_2O + 1 ml antioxidant (tilsettes rett før bruk) *(Kjøleskap)*

6.3. TBS (vaskebuffer), 2 L
- 200 ml TBS + 1800 ml dH_2O *(Kjemikalieskap)*

6.4. TBS-T (vaskebuffer), 2 L
- 200 ml TBS + 1800 ml dH_2O + 2 ml Tween 20 *(Kjemikalieskap)*

6.5. dH_2O, 2 L *(Kjøleskap)*

7. Klargjøring av prøver

7.1. Sett varmeblokk på 59,5 °C.
 7.1.1. Korrekt temperatur er 70 °C, se eksternt termometer.
7.2. Sett prøvene (aliqouter fra homogenisering, ML M-4 eller ML M-5) på is.
 7.2.1. Cytoskjelett-prøver skal stå i romtemperatur.
7.3. Bland prøve, dH_2O og sample buffer i henhold til «Prøveopparbeidelse til WB». (Link)
7.4. Bland prøvene godt (bruk en vortxer).
7.5. Sett merkede eppendorfrør på varmeblokk i 10 minutter ved 70°C.

8. Klargjøring til elektroforese

- Finn frem geler, Running buffer, elektroforesebokser og markør.
- Tilsett 500 µl antioxidant i 200 ml Running buffer, og bland godt.
- Ta gelene ut av plastemballasjen, og fjern kammen og tapen på fremsiden av gelen.
- Bruk en pasteur-pipette til å skylle brønnene med blandingen *Running buffer+antioxidant*.
- Nummerer gelene.
- Sett gelene i boksen med brønnene vendt innover.
- Kontroller at gelene står korrekt før de låses fast.
- Tilsett blandingen *Running buffer+antioxidant* i det indre kammeret.
  - Kontroller for lekkasje.

9. Elektroforese

9.1. Tilsett 5 µl proteinmarkør i de to ytterste brønnene (1 og 10).
9.2. Pipetter mengde prøve etter ønsket loadingvolum i brønn 2-9.
- Kontroller maksvolumet i brønnene på gelene som benyttes.

9.3. Fyll det ytre kammeret med Running buffer (uten antioxidant).
9.4. Sett på lokk, monter ledninger, og skru på power supplyen.
9.5. Juster volt til 200 V, og start elektroforesen.
9.6. Elektroforesen tar 25-45 minutter.
- La tiden telle oppover.
- Noter ampere ved start.

9.7. Kontroller for bobler i elektroforesekammeret når strømmen blir slått på, og se til at proteinene vandrer parallelt nedover i gelen.

10. Klargjøring til blotting

10.1. Tilsett 1 ml antioxidant til 1 L Transfer buffer.

10.2. Klipp opp en PVDF-membran per gel.

- Skriv nummer øverst i venstre hjørne på membranen.

10.3. Finn frem filterpapir og pads.

10.4. Pads legges i Transfer buffer >15 minutter før bruk.

- Klem ut luftbobler.

10.5. Aktiver PVDF-membranen:

- 30 sekunder i metanol
- 30 sekunder i dH_2O
- 1-2 minutter i dH_2O
- 10-15 minutter i Transfer buffer

10.6. Filterpapir fuktes i Transfer buffer rett før bruk.

11. Blotting

11.1. Noter ampere og tid 1-2 minutter før elektroforeseslutt.

11.2. Trykk på stoppknappen og skru av power supplyen.

11.3. Ta ut gelene og skyll elektroforese-/blottekammeret med dH_2O.

11.4. Plasser en pads i den største delen (katoden (-)) av blottekassetten.

11.5. Benytt et metallredskap til å åpne plasten rundt gelen.

11.6. Legg platen i håndflaten og fjern den ene plastplaten.

11.7. Fjern brønnene og lag et kutt øverst i hjørnet over brønn 1 for å markere hvor brønn 1 befinner seg.

11.8. Fukt et filterpapir og plasser den oppå gelen.

11.9. Fjern den andre plastplaten og fjern den uthevede kanten nederst på gelen.

11.10. Plasser filterpapiret med gelen oppå paden i blottekassetten.

11.11. Plasser en membran over gelen.

- Hold i ytterkant av membranen. Ta aldri i midten av membranen.
- Ikke beveg membranen etter at den er lagt på gelen. Dette vil kunne vi utflytende bånd.

11.12. Benytt en fuktet rulle til å fjerne evt. luftbobler mellom gel og membran.

- Rull med et lett trykk mot membranen.

11.13. Blottesandwich med to geler:

- Pad
- Filterpapir
- Gel
- Membran
- Filterpapir
- Pad
- Filterpapir
- Gel
- Membran
- Filterpapir
- Pad

11.14. I et blottkammer er det plass til to geler.
- Dersom du kun skal blotte en gel. Må du fylle på med 2 ekstra pads i ytterkantene.

11.15. Sett på lokket på blottekammeret.
11.16. Fyll blottekassetten med Transfer buffer rett i overkant av sandwichen.
11.17. Fyll 2/3 av kammeret rundt blottekassetten med kaldt dH_2O (4°C).
11.18. Sett på lokk, monter ledninger, og skru på power supplyen.
11.19. Juster volt og tid, og start blottingen.
- 30 volt i 90 minutter.
- Noter ampere etter 1-2 minutter.

11.20. Noter ampere 1 minutt før slutt.
11.21. Skru av power supplyen og trekk ut ledninger.

12. Laging av blokkeringsløsning

12.1. Lag en 5 % melkeløsning (evt. en annen blokkeringsløsning avhengig av proteinene som skal undersøkes, kontroller følgeskrivet til proteinet som skal benyttes).
- 5 g melkepulver + 100 ml TBS-T

13. Blokkering

13.1. Hell av vann og buffer.
13.2. Legg pads i en egen boks for skylling med dH_2O.
- Padsene skal ikke vaskes med vaskemiddel, kun skylles i dH_2O.

13.3. Legg membranene i 5 % melkeløsning eller en annen blokkeringsløsning (avhengig av proteinene som undersøkes).
13.4. Inkuber membranene på en risteplate (rolige bevegelser) i romtemperatur i 2 timer (evt. over natt i 4°C).

14. Laging av løsning til inkubering med antistoff

14.1. Lag en 1 % melkeløsning (evt. en annen inkuberingsløsning avhengig av proteinene som skal undersøkes).
- 1 g melkepulver + 100 ml TBS-T

15. Vasking

15.1. Vask membranen for å fjerne overskudd av blokkeringsløsningen.
15.2. Vask med romtempererte løsninger. Benytt en gyrorocker med hastighet: 65.
15.3. Vaskeprosedyre av membraner etter blokkering:
- Skyll 2 raske ganger med TBS-T.
- Vask 2 x 2 minutter med TBS-T.

15.4. Legg membranen i TBS før primært antistoff tilsettes.

16. Primært antistoff

16.1. Kontroller fortynningen av antistoff i følgeskrivet og velg inkuberingsbeholder.
- Primært antistoff kan inkuberes i rør, små inkuberingskammere og i bokser.

16.2. Eksempel på primært antistoff; ***Antistoff mot HSP70 (ADI-SPA-810-F, Enzo):***
- *Fortynnes 1:4000*; 10 ml 1% melkeløsning + 2,5 µl antistoff.

16.3. Dersom du skal undersøke flere proteiner på en membran. Må membranene deles i flere mindre deler.
16.4. Legg membranen på en glassplate og kutt membranen etter vektmarkørene i brønn 1 og 10.
- Fukt platen før membranen legges på.
- Benytt linjal og en skalpell for å dele membranen.

16.5. Plasser inkuberingsbeholderen med membranen på en rollermikser eller en risteplate (rolige bevegelser) over natt i 4°C.
- Dersom du har blokkert membranen over natt, skal membranen inkuberes med primært antistoff i 2 timer i romtemperatur.

16.6. Nye antistoff skal alltid testes ut med ulike antistoff-fortynninger. For å finne optimal fortynning på antistoffet, se ***ML M-? - Utprøving av nye antistoff til WB***.

17. Vasking

17.1. Vask membranen for å fjerne overskudd av primært antistoff.
17.2. Vask med romtempererte løsninger. Benytt en gyrorocker med hastighet: 65.
17.3. Vaskeprosedyre av membraner etter inkubering med primært antistoff:
- Skyll 2 raske ganger med TBS-T.
- Vask 15 minutter i TBS-T.
- Vask 3 x 5 minutter i TBS.

18. Sekundært antistoff

18.1. Velg sekundært antistoff som er rettet mot vertsdyret i det primære antistoffet.
18.2. Kontroller fortynning av antistoffet i følgeskrivet.
18.3. Eksempel på sekundært antistoff; ***Goat-anti-mouse IgG (Prod: 31420, Thermo Scientific):***
- *Fortynnes 1:30 000*; 30 ml 1 % melkeløsning + 1 µl antistoff.

18.4. Sekundært antistoff kan inkuberes i rør og boks.
18.5. Plasser inkuberingsbeholderen med membranen på en rollermikser eller en risteplate (rolige bevegelser) i 1 time i romtemperatur.

19. Vasking

19.1. Vask membranene for å fjerne overskudd av sekundært antistoff.
19.2. Vask med romtempererte løsninger. Benytt en gyrorocker med hastighet: 65.
19.3. Se ***punkt 17.3***

20. Substratløsning

20.1. La gjerne substratløsningen mens membranene blir vasket, ***punkt 19.3***.
20.2. Substratløsningen Super Signal består av to reagenser.
- Reagens A inneholder en stabil peroxid-løsning
- Reagens B inneholder luminol

20.3. Bland reagensene rett før bruk.
- Benytt gjerne et 15 ml rør.
- Bland 1 del reagens A + 1 del reagens B.
- Løsningen er lyssensitiv, og må oppbevares i et mørkt skap eller dekkes til med aluminiumsfolie.

21. Deteksjon

Etter at det primære antistoffet er bundet til målproteinet, dannes et kompleks sammen med HRP-lenket sekundært antistoff.
En enzymatisk reaksjon starter når membranen inkuberes med chemiluminescenssubstratet.
Enzymet på det sekundære antistoffet (Horseradish peroxidase) er en katalysator som starter en oksidasjon av luminol med peroxid tilstede.
Oksydert luminol sender ut et svakt lys mens det går tilbake til sin grunntilstand.
Lyset som sendes ut fanges opp av kameraet i ChemiDocen og danner proteinbåndene som vi er ute etter å undersøke.

22. Deteksjon på ChemiDoc MP Imaging System

22.1. Skru på PC og ChemiDoc.
22.2. Åpne programmet Image Lab 5.1
- Velg ***Protocols: New Single Channel...***

22.3. ***1. Gel Imaging*** vil åpnes.
- Velg ***Application: Select... → Custom → Membran***

22.4. Legg membranen (proteinsiden opp) på glassplaten i skuffen på ChemiDocen.
- Benytt en belagt pinsett (ligger i øverste skuff på pulten).
- Unngå luftbobler mellom membranen og glassplaten.

22.5. Velg ***Position Gel*** og sentrer membranen.
- Zoom inn til membranen fyller hele ruten.

22.6. Velg ***Run Protocol***.
- Lagre bildet av membranen med de synlige markørene.

22.7. Påfør substratløsning på membranen.
- Det skal legge seg en hinne over hele membranen, unngå luftbobler.
- Lukk skuffen og la løsningen inkubere i 4-5 minutter.

22.8. Velg ***Application: Select... → Blots → Chemi Hi Sensitivity***
- ***Image Exposure: The software will automatically optimize the exposure time for: Intense Bands.***

22.9. Velg ***Run Protocol***.
22.10. Lagre bildene i prosjektmappen tilhørende ditt prosjekt.
22.11. Lukk programmet og slå av ChemiDoc og PC.

23. Analysering

23.1. Proteinbåndene kan kvantifiseres ved hjelp av *Image Lab 5.1 software*.
23.2. Velg ***Volume Tools → Rectangle →*** lag en rektangel rundt et av båndene og marker firkanten → trykk ***Ctrl C*** → trykk ***Ctrl V*** til antallet rektangler stemmer med antallet bånd.
23.3. Plasser rektanglene over båndene.
- Det er viktig at rammen på rektangelet ikke dekker båndene ved siden av båndet du skal måle.

23.4. Velg ***Analysis Table***.
23.5. Velg ***Export analysis table to Excel***.
23.6. Lagre resultatene i prosjektmappen tilhørende ditt prosjekt.

Att. 11: Total protein procedure, DC Protein Assay, Bio-Rad

Norges idrettshøgskole **Muskellaboratorium, SFP**

TITTEL	Totalprotein – DC Protein Assay, Bio-Rad								
SOP NR.	DATO	UTSKREVET	GODKJENT	TYPE	VEDLEGG	REFERANSE	ERSTATTER	KOPI	SIDE
ML M-6	18.01.2018	HNØ		M-bilag	ingen		M-AL 10	Muskellaboratorium	1 av 3

1. DC – detergent compatible

1.1. DC protein metoden er en kolorimetrisk analyse (fargemåling) for måling av proteinkonsentrasjon i en prøve.

1.2. Reaksjonen er liknende Lowry-metoden (Folin fenol reagens) med noen forbedringer.

1.2.1. Reaksjonen når 90% av maksimum fargeutvikling innen 15 minutter.

1.2.2. Fargen endres ikke mer enn 5% i løpet av 1 time, og 10% i løpet av 2 timer etter tilsatt reagens.

1.3. Det er to steg i likhet med Lowry-metoden som fører til fargeraskjonen.

1.3.1. Reaksjonen mellom protein og kobber i en alkalisk medium, og den påfølgende reaksjonen av Folin reagens med kobber-behandlet protein.

1.3.2. Den blå fargereaksjonen oppstår først og fremst på grunn av aminosyrene tyrosin og tryptofan, og i mindre grad på grunn av aminosyrene cystin, cystein og histidin.

1.4. Avleses fotometrisk ved 690 nm (maksimum absorpsjon: 750 nm, minimum absorpsjon: 405 nm).

1.5. Vi benytter fortrinnsvis en Microplate Assay Protocol som dekker måleområdet 0.2-1.5 mg/ml.

2. Reagenser/løsninger

2.1. ***Reagensene*** som benyttes til totalproteinmålingen er:

- DC Reagent A, 500-0113, en alkalisk kobber tartrate løsning.
- DC Reagent B, 500-0114, et fortynnet Folin reagens.
- DC Reagent S, 500-0115.
- Reagensene er holdbare i 6 måneder.
- Reagensene skal lagres i romtemperatur.

2.2. ***Standarder*** som blir benyttet er fortynnet "Bovine Gamma Globulin (BGG) Standard Se", Cat no. 500-0209.

- Kitet inneholder syv standarder.
- Vi benytter std. ***0.125, 0.25, 0.5, 1.0 og 1.5*** mg/ml.
- Det er viktig å bruke samme standarder over tid. Ulike protein gir forskjellig fargedannelse og dermed forskjellig standardkurve.

3. Utstyr

- Cryorør (til fortynning av prøver)
- 96 brønners mikrotiterplate
- Finnpipette (1-10 µl) og Multikanalpipette (25-250 µl og 50-1200 µl)
- Trau
- Plastfilm

4. Førstehjelpstiltak

4.1. Kontakt lege.

4.2. Ved øyekontakt, skyll med store mengder vann i minst 15 minutter.

4.3. Ved hudkontakt, fjern kontaminerte klær og skyll med store mengder vann i minst 15 minutter.

4.4. Kontroller sikkerhetsdatablad (SDS) for ***DC™ Protein Assay Reagent A/B/S***.

4.5. Logg inn i ecoonline.no for å få tilgang til SDS:

- Firmakode: **2145**
- Brukernavn: **biokjemi**
- Passord: **biokjemi**

5. Forbehandling av prøvemateriale og kontroll

5.1. Sett opp et pipetteringsskjema, ***tabell 1***.

- Blank – vann
- STD – standard med en gitt konsentrasjon.
- FP – prøvene som skal analyseres.
- Kontroll – en prøve med en gitt konsentrasjon. Blandet og beregnet ut i fra to kjente standarder.

5.2. Prøvene skal til enhver tid oppbevares på is.

5.2.1. Det er kun cytoskjelettfraksjonen som skal stå på benken i romtemperatur for å bli homogen grunnet innhold av detergent i ekstraksjonsbuffer IV.

5.3. ***Homogenat*** og ***cytoskjelettfraksjoner*** må fortynnes.

5.3.1. ***Homogenat*** fortynnes ***1:4*** med type 1-vann.

- 5 µl prøve + 15 µl H_2O.

5.3.2. ***Cytoskjelettfraksjoner*** fortynnes ***1:6*** med type 1-vann.

- 5 µl prøve + 25 µl H_2O.

5.4. Cytosol-, membran- og nukleærfraksjonene og kontroller skal ikke fortynnes.

Tabell 1, Pipetteringsskjema

Blank	Blank	Blank	FP 3	FP 3	FP 3	FP 11	FP 11	FP 11	FP 19	FP 19	FP 19
STD 0,125	STD 0,125	STD 0,125	FP 4	FP 4	FP 4	FP 12	FP 12	FP 12	FP 20	FP 20	FP 20
STD 0,25	STD 0,25	STD 0,25	FP 5	FP 5	FP 5	FP 13	FP 13	FP 13	FP 21	FP 21	FP 21
STD 0,5	STD 0,5	STD 0,5	FP 6	FP 6	FP 6	FP 14	FP 14	FP 14	FP 22	FP 22	FP 22
STD 1,0	STD 1,0	STD 1,0	FP 7	FP 7	FP 7	FP 15	FP 15	FP 15	FP 23	FP 23	FP 23
STD 1,5	STD 1,5	STD 1,5	FP 8	FP 8	FP 8	FP 16	FP 16	FP 16	FP 24	FP 24	FP 24
FP 1	FP 1	FP 1	FP 9	FP 9	FP 9	FP 17	FP 17	FP 17	FP 25	FP 25	FP 25
FP 2	FP 2	FP 2	FP 10	FP 10	FP 10	FP 18	FP 18	FP 18	**Kontroll**	**Kontroll**	**Kontroll**

6. Fremgangsmåte

6.1. Pipetter 5 µl blank/standard/prøve/kontroll i brønnene på mikrotiterplata.

6.1.1. Det skal alltid analyseres som triplikater (tre av samme; blank/standard/prøve/kontroll).

6.1.2. Analyser alltid prøver som skal sammenliknes på samme plate, f.eks. pre og post fra en FP.

6.2. Tilsett 25 µl av ***reagens A**** (reagent A + reagent S), ***se punkt 7.1***.

6.2.1. Reagent S bør tilsettes reagent A rett før tilsetting for å unngå utfelling.

6.2.1.1. Dersom det forkommer utfelling, varm opp og vortex løsningen.

6.2.2. Benytt en multikanalspipette (25-250 µl) for å tilsette 25 µl reagens A* i hver brønn.

6.2.2.1. Innstilling av multikanalpipetten:

- Trykk på ***M*** frem til d står i displayet, og trykk ***E*** (Enter).
- Antall µl vil komme opp. Juster med ***pilene***, og trykk ***E*** (Enter).
- "Tall"* vil komme opp. Juster til antall kolonner, og trykk ***E*** (Enter).

6.2.2.2. Tilsett reagens A* i et trau, og bland godt.

6.2.3. Unngå kontaminering mellom brønnene.

6.2.4. Unngå luftbobler.

6.3. Tilsett 200 µl ***reagent B, se punkt 7.2***.

6.3.1. Benytt en multikanalspipette (50-1200 µl), ***se punkt 6.2.2.1*** for innstilling av pipetten.

6.4. Sett en plastfilm på mikrotiterplaten.

6.5. Inkuber platen i 15 minutter i et mørkt skap.

7. Reagensforbruk

7.1. **Reagens A*: Reagent A + Reagent S**

- ***Reagent A:*** 25 µl x ... prøver = ... µl ***reagent A***

(Eks: 25 µl x 96 (full plate) = 2400 µl (Benytt 4 ml)

- ***Reagent S:*** 20 µl x ... ml ***reagent A*** = ... µl ***reagent S***

(Eks: 20 µl x 4 ml ***reagent A*** = 80 µl ***reagent S***

7.2. **Reagent B:** 200 µl x ... prøver = ... µl ***reagent B***

(Eks: 200 µl x 96 (full plate) = 19 200 µl (~ 25 ml)

8. Avlesning

8.1. Opprett en ny plate, se prosedyre ***ML I-9, Plateleser - ASYS Expert 96***.
8.2. Legg inn ID på prøvene og eventuelle fortynninger.
8.3. Ta plastfilmen av mikrotiterplaten.
8.4. Kontroller alle brønnene for å unngå luftbobler som kan forstyrre lysveien.
8.4.1. Ved bobler bruk en Pasteur pipette til forsiktig og blåse hull på luftboblene.
8.4.2. Plasser platen i plateleseren.
8.5. La platen mikse i 5 sekunder.
8.5.1. Plateleseren har en egen miksefunksjon.
8.6. Mikrotiterplaten avleses ved 690 nm etter 15 minutter.
8.6.1. Reaksjonen er stabil inntil 60 minutter.

9. Resultater

9.1. Kontroller standardverdienes OD-verdier.
9.2. CV (%-avvik) skal være ≤10% for standarder og prøver.
9.2.1. 1 av 3 ODer kan fjernes dersom den skiller seg veldig ut fra de to andre ODene.
9.3. Konsentrasjonen til kontrollen og dens CV føres på et eget kontrollskjema.
9.4. Lagre fil under prosjekt i *sfp.grp*.
9.5. Skriv ut resultatene.

Att. 12: Wound care of biopsy

Stell av sår etter muskelbiopsi

Du er nå forsøksperson i et prosjekt der vi har tatt muskelprøver (biopsi) fra låret (m. vastus lateralis). Dette er et lite inngrep som ikke har noen negative følger, bortsett fra sår muskulatur noen dager. Det kan gjøre litt vondt/være ømt i kveld, når bedøvelsen går ut, og i morgen. Men dette går over i løpet av en dag eller to.

Imidlertid er det alltid en minimal risiko for infeksjon ved slike inngrep. Vi ber deg derfor å følge rådene under. Om det skulle oppstå noe av medisinsk karakter som du tror kan settes i sammenheng med forsøket må du kontakte oss, uansett tid på døgnet (telefonnummer nederst i skrivet).

Det er nå viktig at du tar de forholdsregler som skal til for at sårene dine skal gro godt.

1. Bandasjen som er surret rundt beinet ditt kan tas av i kveld før du legger deg.
2. Hvit plasterlapp og strips skal sitte på i en uke. Vi anbefaler at stripsene ikke rives av, men tas av når de løsner fra selve såret.
3. Hold sårområdet tørt. Du bør ikke vaske området ved sårene eller dusje slik at tapen rundt såret blir våt. Vann vil øke faren for infeksjon og det vil også føre til at tapen som skal holde sårflatene sammen løsner. Du kan dusje, men sørg for at du ikke får vann i nærheten av sårene. (Dusj forsiktig, bruk evt plastfolie/gladpack, vanntette plaster eller lignende)

Hvis "stripsene" som holder såret sammen løsner før det har gått en uke bør du få på nye strips. Ta i så fall kontakt med oss.

Kontaktpersoner ved Norges idrettshøgskole:

Truls Raastad: 91368896

Charlie Davids: 48636296

Kristoffer Toldnes Cumming: 95146403