UNIVERSITY OF AGDER

The effect of a failure and submaximal blood flow restriction resistance exercise protocol on changes in muscle size, strength and swelling

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This Master's Thesis is carried out as a part of the education at the University of Agder and is therefore approved as a part of this education. However, this does not imply that the University answers for the methods that are used or the conclusions that are drawn.

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ABSTRACT

Introduction: Blood flow restricted resistance exercise (BFRRE) can induce rapid increases in muscle size, strength and swelling. No previous research has investigated the importance of conducting BFRRE to voluntary failure and few studies has been carried out to investigate associations between swelling and muscle size. Therefore, the aim of the present study was twofold (1) compare changes in muscle size and strength between a failure (FA) and submaximal (SU) BFRRE protocol (2) investigate associations between swelling and muscle size.

Methods: Seventeen untrained men had their legs randomized to FA and SU protocols. The intervention consisted of two training periods including seven BFRRE sessions within five days (separated with 10 days' rest) using unilateral knee extension at 20% of one repetition maximum (1RM) (30 s rest between sets). Swelling and muscle size was measured with ultrasound, whereas strength was measured as 1RM and maximal voluntary contraction (MVC).

Results: Cross-sectional area (CSA) of rectus femoris increased significantly in both groups compared to baseline (FA: 7.9 ± 7.6%; $p < 0.001$ and SU: 9.1 ± 10.8%; $p = 0.003$), where no differences in muscle size were observed between groups. Strength (1RM) increased significantly in both groups (FA: 9±8%; $p < 0.001$ and SU: 11±7%; $p < 0.001$) at 24 days' post intervention, whereas no group differences were found. Swelling increased CSA of rectus femoris (12.0±9.72%, $p<0.001$) compared to ultrasound measurement obtained right before BFRRE.

Conclusion: FA and SU induced similar gains in muscle size and strength. Acute swelling increased, whereas no associations was observed between swelling and muscle size

Keywords: ultrasound, blood flow restriction resistance exercise, concentric failure, submaximal, muscle thickness, cross-sectional area, swelling

SAMMENDRAG

Introduksjon: styrketrening med redusert blodstrøm (BFRRE) kan indusere hurtige økninger i muskelstørrelse, styrke og svelling. Ingen tidligere forskning har undersøkt viktigheten av å utføre BFRRE til utmattelse og få studier har undersøkt sammenhengen mellom svelling og muskelvekst. Derfor er målet til denne studien todelt (1) sammenligne endringer i muskelstørrelse og styrke mellom en protokoll til utmattelse (FA) og en submaksimal (SU) BFRRE protokoll (2) Undersøke sammenhengen mellom svelling og muskelstørrelse

Metode: Sytten utrente menn hadde benene randomisert til FA og SU protokoller. Intervensjonen besto av to treningsperioder som inkluderte 7 BFRRE økter på 5 dager (separert med 10 dagers hvile) i kneekstensjon apparat på 20% av 1 repetisjon maksimum (1RM) (30 s pause mellom sett). Svelling og muskelstørrelse ble målt med ultralyd, mens styrke ble målt som 1RM og maksimal voluntær kontraksjon (MVC).

Resultater: Tverrsnitts areal (CSA) av rectus femoris økte signifikant i begge gruppene sammenlignet med baseline (FA: 7.9 ± 7.6%; $p < 0.001$ and SU: 9.1 ± 10.8%; $p = 0.003$), mens ingen signifikante forskjell ble observert mellom gruppene i muskelstørrelse. Styrke (1RM) økte signifikant i begge gruppene (FA: 9±8%; $p < 0.001$ and SU: 11±7%; $p < 0.001$) 24 dager etter siste BFRRE økt, mens ingen gruppeforskjeller ble observert. Akutt svelling (målt med ultralyd) økte CSA av rectus femoris (12.0±9.72%, $p<0.001$) sammenlignet med ultralydmålingen utført rett før BFRRE.

Konklusjon: FA og SU induserte samme økning i muskelstørrelse og styrke. Akutt svelling økte, mens ingen sammenheng ble observert mellom svelling og muskel størrelse.

Nøkkelord: ultralyd, styrketrening med redusert blodstrøm, utmattelse, submaksimal, muskeltykkelse, tverrsnitts-areal, svelling

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PART 1:

THEORETICAL FRAMEWORK AND METHODS

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Contents

1.0 INTRODUCTION

American College of Sports Medicine recommend to use weights of at least 70% of one-repetition maximum (1RM) to gain muscle hypertrophy during strength training (Kraemer et al., 2002). However, increasing amount of research supports the effect of strength training at lower loads on both muscle size and muscle strength (Abe, Kearns, & Sato, 2006; Ogasawara, Loenneke, Thiebaud, & Abe, 2013). Blood flow restricted resistance exercise (BFRRE) at 20-30% of 1 RM has been observed to improve skeletal muscle hypertrophy, strength and endurance (Madarame et al., 2008; Takarada, Sato, & Ishii, 2002; Takarada, Tsuruta, & Ishii, 2004). Furthermore, BFRRE has shown beneficial effects for a wide variety of populations and purposes. Not only has it shown hypertrophy and strength gains in untrained individuals (Kubo et al., 2006; Madarame et al., 2008; Takarada et al., 2004), but also in rugby players (Cook, Kilduff, & Beaven, 2014) and netball athletes (Manimmanakorn, Hamlin, Ross, Taylor, & Manimmanakorn, 2013), as well as in frail elderly (Abe et al., 2006). Blood flow restricted resistance exercise can even be utilized as a tool in attenuating muscle atrophy during immobilization (Kubota, Sakuraba, Sawaki, Sumide, & Tamura, 2008). It is important to emphasize that the potential ischemic muscle pain associated with BFRRE might limit this exercise method to highly motivated individuals (Wernbom, Jarrebring, Andreasson, & Augustsson, 2009a). However, it is likely that BFRRE does not pose a greater risk to the cardiovascular system, muscle damage, oxidative stress or nerve conduction velocity, compared to traditional strength training (Loenneke, Wilson, Wilson, Pujol, & Bemben, 2011).

The mechanisms behind the benefits seen with BFRRE are not well elucidated (Loenneke, Wilson, & Wilson, 2010; Pope, Willardson, & Schoenfeld, 2013). However, several potential mechanisms has been proposed, such as increase in metabolic accumulation, enhanced fiber-recruitment, increased hormone activity, muscle damage, intracellular swelling and intracellular signaling (Pearson & Hussain, 2015; Scott, Slattery, Sculley, & Dascombe, 2014; Wernbom, Augustsson, & Raastad, 2008). In one study (Nielsen et al., 2012) a remarkable 150-300 % increase in the number of satellite cells, 30% increase in the number of myonuclei and 40% increase in muscle fiber area was reported already after one week (7 sessions) of BFRRE performed to voluntary failure in leg extension (20% of 1RM). In this study satellite cells, muscle fiber area and myonuclei adaptations seemed to plateau after the first week of training, showing no further increase the following two weeks of BFRRE. Previous work within our research group attempted to reproduce the remarkable results observed in Nielsen

et al., (2012), but found no changes after one week of training applying similar protocol. It has been speculated whether the failure protocol utilized in our previous research has been too hard compared to the failure protocol in Nielsen et al., (2012), which is the rationale for comparing two different BFRRE protocols (one to failure and one submaximal) in the present study.

Some research has been conducted with respect to compare a failure and submaximal protocol for traditional strength training (i.e. >70% of 1RM), where the results are conflicting (Drinkwater et al., 2005; Izquierdo et al., 2006). Furthermore, a small amount of research has aimed for a direct comparison of a failure and submaximal protocol (Nobrega & Libardi, 2016). Additionally, most of these studies has been aiming to increase muscular strength and not muscle size (Nobrega & Libardi, 2016). Even less research is prevalent in terms of BFRRE and to the authors knowledge no study has investigated the importance of conducting BFRRE to voluntary failure.

Swelling is an increase in cellular hydration status and believed to induce muscle growth (Martin-Hernandez et al., 2013; Pearson & Hussain, 2015). Swelling occurs as a result of strength training and particularly if the muscle is exposed to high metabolic stress, as with BFRRE (Hernandez et al., 2013). Findings in a number of studies refers to enhanced levels of swelling with BFRRE (Hernandez et al., 2013; Yasuda, Loenneke, Thiebaud, & Abe, 2012) and research is also pinpointing the importance of swelling due to its role in cell signaling (Abe et al., 2006; Loenneke, Fahs, Rossow, Abe, & Bemben, 2012; Yasuda et al., 2012). However, few studies has been conducted to investigate associations between muscle swelling and muscle size.

1.1 Overall goals

The primary objective of the present study was to compare changes in muscle size and strength between a failure and submaximal BFRRE protocol. The secondary objective was to investigate associations between muscle swelling and muscle size.

1. Primary hypothesis

Hypothesis:

- *a submaximal protocol will induce a larger increase in muscle size and strength than a BFRRE protocol with four sets to failure*

2. Secondary hypothesis

Hypothesis:

- *Level of muscle swelling after a bout of BFRRE is associated with increases in muscle size*

2.0 THEORETICAL FRAMEWORK

2.1 Background for BFRRE

Blood flow restricted exercise (BFRRE) is a method with origin in Japan. Professor Yoshiaki Sato discovered numbness and swelling in his calf's during a Buddhist memorial in the 1960s. The feeling of increased swelling and being numb was described as somewhat similar to that of performing strenuous calf-raise exercise. In order to transfer this experience to training, he experimented with placement of the pressure cuff of the respective limbs, how much occlusion pressure to use etc. He continued this process approximately six months before he achieved what he described as a significant "pump effect" (Sato, 2005).

BFRRE is known by different synonyms as KAATSU- (ka atsu, meaning added pressure), vascular occlusion-, ischemic- and occlusion training. The technique uses a tourniquet (Shinohara, Kouzaki, Yoshihisa, & Fukunaga, 1998), inflatable cuff (Takano et al., 2005) or elastic band (Loenneke, Kearney, Thrower, Collins, & Pujol, 2010) to reduce arterial blood flow, while occluding the venous reflux. This gives a local hypoxic condition inside the muscle with accumulation of metabolites (Wernbom et al., 2008). Resistance of approximately 20-30% of 1RM is typically used (Fahs et al., 2011), but in some studies loads on 15% of 1RM (Kacin & Strazar, 2011) and 80% of 1RM has been tested out (Laurentino et al., 2008)

The cuff should be placed at the proximal end of the limb (Loenneke et al., 2013). In the original Japanese model, inflatable cuffs with an occlusion pressure of up to 200 mmHg was applied. However, it is possible to achieve muscle adaption with cuff pressure at 50 mmHg (Sumide, Sakuraba, Sawaki, Ohmura, & Tamura, 2009). The width of the Japanese model was markedly smaller (33mm) compared to cuffs used in other studies (up to 180mm; (Loenneke, Wilson, Marin, Zourdos, & Bemben, 2012). It is suggested that the pressure needed for muscle adaption can be relatively low when the cuff is wide (e.g. 100 mmHg with 15 cm cuff; (Nielsen et al., 2012), and needs to be increasingly higher the narrower the cuff is (Loenneke, Wilson, et al., 2012). The pressure should therefore likely be determined on the basis of the cuffs width, as well as the circumference of each individual`s limb (Loenneke, Wilson, et al., 2011). High pressure combined with wide cuffs should probably be avoided because of potential severe occlusion (Wernbom et al., 2008). In summary, there is not a consensus regarding the optimal occlusion pressure or the size of the cuff utilized during

BFRRE. Furthermore, variables such as training load, volume, frequency, whether the cuff pressure is released between sets or not, length of the rest and degree of voluntary exhaustion is of great importance when evaluating the effect of BFRRE (Bird, Tarpenning, & Marino, 2005).

2.2 Resistance training to voluntary failure

Failure can be defined as the point where all accessible motor units have reached fatigue, where the load cannot be moved outside a critical joint angle (also known as the "sticking point") (Van Den Tillaar & Ettema, 2010). The basis for conducting strength training to voluntary failure is found in the theory of maximizing motor unit recruitment (Willardson, 2007). Even though failure is a good option for maximizing motor unit recruitment, there are findings challenging this theory. Sundstrup et al. (2012) observed complete motor-unit activation 3-5 repetitions prior to failure in untrained women. This indicates that performing sets to failure with the aim of maximizing motor-unit recruitment appears to be unnecessary, at least in some cases. Additionally, observations from several studies confirm similar increases in muscle mass and strength without going all the way to failure (Folland, Irish, Roberts, Tarr, & Jones, 2002; Madarame et al., 2008; Sampson & Groeller, 2015).

Izquierdo et al. (2006) randomized 42 basque pelota players in two groups to investigate changes in maximal strength. Group one performed repetition failure (3 sets of 10-RM), while group two performed no repetition failure (~ 6 sets of 3-5 repetitions), where similar intensity (75% of 1RM) and volume was carried out. Results indicated no difference between groups in maximal strength gains. In line with this, Mitchell et al. (2013) conducted a study (10 weeks) on men with no strength training experience within the last year. In this study, the participants leg was randomized into one of three possible training conditions performing unilateral leg extension: one set performed to voluntary failure (80% of 1RM); three sets to the point of fatigue (80% of 1RM); or three sets to the point of fatigue (30% of 1RM). Results were similar between protocols for both maximal strength and total quadriceps volume. Although no significant difference was observed between groups in the degree of quadriceps volume, the mean gain was doubled in favor of the two point to fatigue groups compared to the voluntary failure group. Burd et al. (2010) included 15 males to investigate the effect of three different unilateral leg extension protocols on protein synthesis: 90% of 1RM performed to failure, 30% 1RM work-matched to 90% failure (30WM) or 30% of 1RM performed to failure (30FAIL). Both low-load groups induced a substantial increase in muscle protein synthesis, where 30FAIL protocol induced the largest increases, even when compared to the

high-load group. In one BFRRE study were the subjects performed a submaximal protocol (3 sets of 15 repetitions, 30 sec rest) in squat and leg curl, Abe, Kawamoto, et al. (2005) found a substantial increase in quadriceps, biceps femoris and gluteus maximus. Respectively, 7.7%, 10.1% and 9.1%, whereas the observations in the non-BFRRE group was 1.4%, 1.9% and 0.6%.

However, some studies are pointing in a slightly different direction. Drinkwater et al. (2005) randomized 26 male elite junior basketball players into a failure and non-failure group, where they conducted bench press for a period over 6 weeks. Results showed a superior increase in the failure group (virtually twofold compared to baseline) versus the non-failure group in maximal strength gains. Furthermore, Schoenfeld, Contreras, Willardson, Fontana, and Tiryaki-Sonmez (2014) applied a within subject design, where 18 resistance trained young men conducted two protocols to voluntary failure; one high-load (72% of 1RM) and one low-load (30% of 1RM). Results showed higher peak and mean EMG activity through high-load failure protocol. The authors suggested therefore the high load failure protocol to be superior to the low-load protocol, considering activation of motor-units. Interestingly, the same research group performed another study (Schoenfeld, Peterson, Ogborn, Contreras, & Sonmez, 2015) with comparison of a high-load failure protocol and a low-load failure protocol. Results showed similar increases in muscle mass after 8 weeks of training. Muscle strength, however, increased more in the high-load group. In one BFRRE study where 10 males performed four sets to failure in unilateral knee extension (4 weeks), Kacin and Strazar (2011) observed an increase in cross-sectional area in quadriceps (3.4%).

Although conducting sets to failure can be favorable in some cases, there are several disadvantages as well. Firstly, failure has been related to enhanced risk of injury and/or overtraining (Willardson, 2007) and secondly, failure can impede the possibility to train within a selected repetition range. This may result in lower or higher training volume than indented and thereby give a negative effect in desirable outcome (e.g. muscle size and strength increases) (Krieger, 2010)

A systematic review and meta-analysis (Davies, Orr, Halaki, & Hackett, 2015) sums up important facts considering failure versus non-failure protocols. Results from this review indicate that both failure and non-failure exercise causes increases in maximal strength. Nevertheless, non-failure protocols showed a small, but evidentially higher effect compared to failure groups in maximal strength. However, when volume was calculated for, no

difference was evident between protocols. In summary, muscle size can be equally affected regardless if a failure or non-failure protocol is utilized (Nobrega & Libardi, 2016). Consequently, it might not be necessary to conduct strength training to failure in order to maximize gains in muscle mass and strength (Nobrega & Libardi, 2016; Willardson, 2007). However, it is important to pinpoint that the non-failure protocol probably has to be conducted somewhat close to failure to achieve similar effects in muscle size and strength (Mitchell et al., 2012).

2.3 Time course for gains in muscle mass and strength with BFRRE

Growth rate various lots between muscle size in different studies, but increases between 3-25% in exercised muscle groups are common (Wernbom, Augustsson, & Thomee, 2007). This corresponds to an increase of approximately 0.1-0.5% per training session, while muscle strength tends to increase 1% per bout when measured as 1RM (Raastad, Paulsen, Refsnes, Rønnestad, & Wisnes, 2010). Several studies from traditional strength training refers to increases in muscle size first after 6 weeks with regularly training (Häkkinen et al., 1998; Raastad et al., 2010). A lack of sensitivity and accuracy on prevailing apparatus applied to quantify muscle size, is perhaps the reason for this seemingly late increase (Seynnes, de Boer, & Narici, 2007). However, the degree of uncertainty is still prevalent on this issue (Abe, DeHoyos, Pollock, & Garzarella, 2000).

In this case, BFRRE has been shown to increase muscle size with 0.5-0.55% per day with intense training (Fujita, Brechue, Kurita, Sato, & Abe, 2008) and thereby it is not surprising that increases has been observed already after a few weeks following BFRRE (Abe, Yasuda, et al., 2005). Rapports from a meta-analysis confirm these findings which refers to rapid increases in muscle size (Loenneke, Wilson, et al., 2012). The rapid increases in muscle size might be possible due to the low mechanical tension with BFRRE, which gives the opportunity to include several sessions in a short period of time (Takarada, Nakamura, et al., 2000).

Furthermore, Nielsen et al. (2012) included twenty untrained male subjects who performed 23 BFRRE-sessions over a period off 19 days. One BFRRE group (n=10) performed leg extension to voluntary failure (20% of 1RM) with 30 seconds rest between sets and a pressure cuff with 100mmgh (the exact same cuff was applied in the present study), whereas a work-matched control group exercised without BFRRE (n=8). Some of the findings were a remarkable increase in MFA (~40%) for BFRRE group already after the first training week.

The authors concluded the MFA results to be unique according to the low load combined with the short intervention. Similar findings are observed with traditional strength training, where increases in MFA on 15-20% has been observed with untrained male subjects. However, these increases in MFA is first prevalent after 12-16 weeks (Kadi et al., 2004; Aagaard et al., 2001). Another rapport is consistent with these findings by showing increases of MFA (~37%) after 16 weeks of heavy resistance training on individuals characterized as hypertrophy responders (Petrella, Kim, Mayhew, Cross, & Bamman, 2008).

2.4 Primary mechanisms for muscle growth

Mechanical tension is commonly regarded as the primary mechanisms for muscle growth (Goldberg, Etlinger, Goldspink, & Jablecki, 1974). Mechanically induced tension produced by stretch and force generation is counted as important for muscle growth (Schoenfeld & Contreras, 2014) and the combination of these stimuli seems to have a distinctively effect (Schoenfeld, 2010). Furthermore, mechanical tension has been widely associated with muscle growth in animal experiments (Ronnestad et al., 2007), whereas few studies are carried out in humans (Raastad et al., 2010). Research available today shows some of the secondary mechanisms mechanical tension may be working through such as mechanotransduction (Goldspink, 1998; Schoenfeld, 2013), increased localized hormone production (Adams, 2002), muscle damage (Tatsumi et al., 2006), ROS production (Tatsumi et al., 2006; Uchiyama, Tsukamoto, Yoshimura, & Tamaki, 2006) and increased recruitment of fast twitch muscle fibers (Cook, Murphy, & Labarbera, 2013; Manini & Clark, 2009). It is plausible that these mechanisms increase protein synthesis during activation of signaling pathways (Bodine et al., 2001) and/or satellite cell activation and proliferation (Adams, 2002) to elicit muscle growth.

If mechanical tension was the only primary factor leading to hypertrophy it would be reasonable to assume that pure eccentric strength training was more effective than concentric training, cause of higher force production during eccentric stimuli (Raastad et al., 2010). However, it seems like the *metabolic stress* (i.e. buildup of metabolites), which is higher with concentric training compared to eccentric training, is important for muscle growth (Schoenfeld, 2013). This is clear in experiments where the force development is equal, whereas the metabolic stress is different (Raastad et al., 2010). Several studies confirm the hypothesis that BFRRE gives a larger stress on the muscle compared to corresponding training without blood flow restriction (BFR) (Suga et al., 2009; Takarada, Nakamura, et al.,

2000). In the literature, metabolic stress is described as an essential primary mechanism for muscle growth (Loenneke & Pujol, 2009; Schoenfeld, 2013; Suga et al., 2009), where some studies are going as far as to suggest this mechanism as more important for the induction of muscle growth than mechanical tension (Loenneke & Pujol, 2009; Suga et al., 2009).

Metabolic stress are thought to mediate muscle growth through several secondary mechanisms, including elevated systemic hormones production (Reeves et al., 2006), increased recruitment of fast-twitch fibers (Takarada et al., 2002), swelling (Loenneke, Fahs, et al., 2012), muscle damage (Schoenfeld, 2013) and increased production of ROS (Pope et al., 2013; Schoenfeld, 2013). Mechanical tension and metabolic stress works through specific mechanisms to induce signaling processes and/or satellite cell proliferation to elicit muscle growth (Pearson & Hussain, 2015). When it comes to BFRRE, metabolic stress is believed to be the dominant primary mechanism which influence associated secondary mechanisms (Pearson & Hussain, 2015). However, it is likely that some of these secondary mechanisms have a stronger relationship with mechanical tension (Pearson & Hussain, 2015). Therefore it is plausible that mechanical tension possesses a certain amount of influence with BFRRE. However, it is important to emphasize that the influence of mechanical tension is probably low with BFRRE (<50% of 1RM) (Pearson & Hussain, 2015), but it does not automatically follow that its potential contribution is of no importance. Therefore it is suggested that these mechanisms work together, with main contribution from metabolic stress, and acts synergistically to induce the benefits seen with BFRRE (Pearson & Hussain, 2015).

2.5 Mechanisms behind BFRRE

Several underlying mechanisms for the increase in muscle size and strength following BFRRE are proposed (figure 1), but not yet well established (Wernbom et al., 2008). In the following section some of the most important mechanisms will be discussed. In that case, it is important to highlight that the effects of BFRRE are probably not dependent upon one single mechanism, but rather a combination of all the mechanisms (Loenneke, Wilson, et al., 2010)

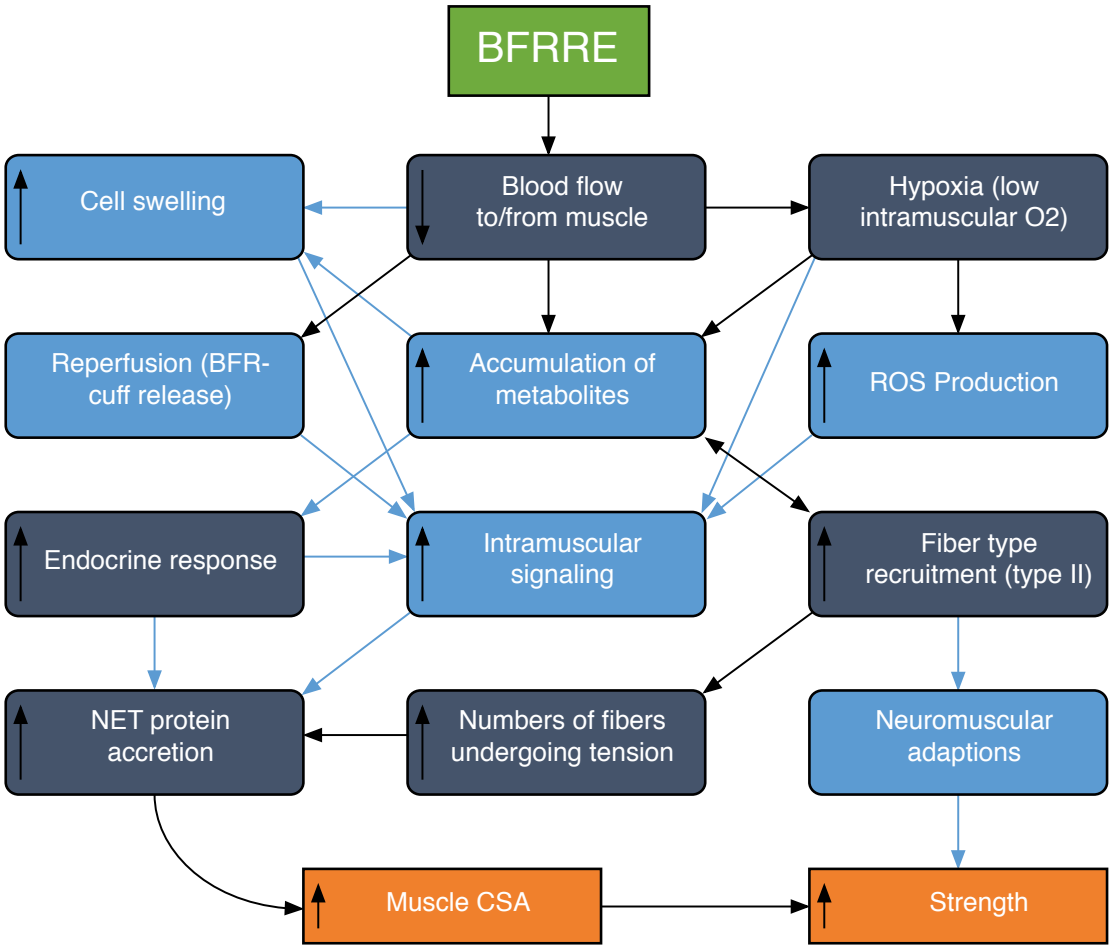

Figure 1: Simplified overview over the suggested interaction between potential mechanisms that may induce the adaptive responses to BFRRE. Modified after Scott, Slattery, Scullery & Dascombe (2014). Likely mechanisms are presented in boxes with dark blue, while possibly mechanisms are presented in bright blue boxes. Outcomes of training are represented in orange boxes. *Black arrows* indicate a likely link between suggested mechanisms, whereas *bright blue arrows* indicate a possible link between suggested mechanisms.

2.5.1 Fiber type recruitment

Muscle fibers are being recruited in a hierarchy, starting with slow-twitch type 1 fibers and as the workload increases larger motor units (fast twitch, type 2 fibers) gradually activates and contributes (Henneman, Somjen, & Carpenter, 1965). Only muscle fibers recruited during training are adapting as a result of the strength training conducted (Wernbom et al., 2008). To achieve increases in muscle mass and strength, it is crucial to activate type 2 fibers which possesses the largest potential for hypertrophy (Loenneke, Fahs, Wilson, & Bemben, 2011). In that case, it is recommended to perform strength training with heavy loads (60%< of 1RM) to recruit fast twitch fibers (Takarada, Nakamura, et al., 2000). Nevertheless, BFRRE studies at 20% of 1RM rapport recruitment of fiber type 2 (Moritani, Sherman, Shibata, Matsumoto, & Shinohara, 1992). In this regard, it is possible that fiber type 1 fatigue at a faster pace than normally with BFRRE, because of the hypoxic conditions and accumulation of metabolites, which forces larges motor units to engage early (Meyer, 2006; Moritani et al., 1992). Furthermore, literature substantiates the importance off BFRRE by showing higher increases in muscle fiber recruitment/firing frequency measured with electromyography (EMG) during BFRRE compared to a work-matched group without BFR (Takarada, Nakamura, et al., 2000; Takarada et al., 2002). Takarada et al. (2004) observed 1.8 times higher muscle stimulation with BFRRE compared to control group without BFRRE (same force and mechanical work produced).

However, enhanced recruitment of fiber type 2 is not observed in all studies conducted on BFRRE. Studies have reported similar EMG-activation between BFRRE versus non-BFRRE conditions in unilateral leg extension (Kacin & Strazar, 2011; Wernbom, Jarrebring, Andreasson, & Augustsson, 2009b). In addition, it is likely that high-intensity resistance training evokes higher activation of fiber type 2 compared to BFRRE, when both are conducted to voluntary failure (Cook et al., 2013; Manini & Clark, 2009). For that reason, mechanical tension might have a greater impact on fiber type 2, than BFRRE induced metabolic stress. Nonetheless, it is still possible that BFRRE enhanced recruitment acts as one of the possible mechanisms behind BFRRE (Pearson & Hussain, 2015)Increase in MFA of type 1 fibers seems to increase more with BFRRE compared to traditional strength training (Nielsen et al., 2012). In one study (Nielsen et al., 2012) equally increases in both type 1 and 2 fibers were observed (McCall, Byrnes, Dickinson, Pattany, & Fleck, 1996). Interestingly, previous work within our research group observed significant higher increases in type1, than type 2 fibers. Further, stress response in type 1 following BFRRE has been observed to

exceed that off type 2 fibers (Cumming, Paulsen, Wernbom, Ugelstad, & Raastad, 2014). Consequently, this might explain the robust increase in type 1 fibers observed in Nielsen et al., (2012) and our previous work.

2.5.2 Cell swelling

A number of studies have shown increases in cellular hydration state after BFRRE (Abe et al., 2012; Hernandez et al., 2013; Yasuda, Fukumura, Iida, & Nakajima, 2015). This increase is thought to be responsible for some of the benefits seen with BFRRE (Pearson & Hussain, 2015). Muscle swelling is caused by the accumulation of blood in the extracellular matrix surrounding the muscle fiber, as well as intracellular accumulation (Schoenfeld & Contreras, 2014). The extent of swelling is dependent on the exercise performed. With intense muscle-work the veins are compressed, while arteries supply the working muscle with blood. Thence, the blood starts to seep out of the capillaries and into the interstitial places (Schoenfeld & Contreras, 2014). This fluid buildup triggers the extracellular pressure gradient, which in turn release plasma flow back and into the muscle (Schoenfeld & Contreras, 2014). This phenomenon is commonly called "the pump", while its terminology is cellular swelling, muscle swelling or intramuscular swelling. Swelling is primarily influenced by training aiming for a high quantity of repetitions combined with short rest periods (Schoenfeld & Contreras, 2014). This method prevents blood escaping the musculature, leading to enhanced levels off swelling and is therefore typically related to metabolic stress. Nevertheless, it is currently unclear whether swelling is solely induced by metabolic stress or if mechanical tension also plays a part (Pearson & Hussain, 2015).

Muscle swelling has previously been shown to increase protein synthesis and reduce protein breakdown in a spectrum of cell types (Dangott, Schultz, & Mozdziak, 2000; Pearson & Hussain, 2015; Schoenfeld & Contreras, 2014), namely hepatocytes, osteocytes, breast cells and muscle cells (Lang et al., 1998). Muscle fiber type 2 has been observed to be specifically sensitive with osmotic changes, possibly due to their large content of water transport channels (AQP4) and therefore it is more likely that these fibers respond better to BFRRE induced swelling than type 1 fibers (Schoenfeld & Contreras, 2014)

Swelling seems to appear in activated and not inactive cells (Sjogaard & Saltin, 1982). Measurements of acute swelling has shown an increase in leg circumference by 2.5±0.6cm immediate upon cuff release after BFRRE compared to a non-BFRRE group who increased leg circumference by 1.3±0.3 cm (Fry et al., 2010). Umbel et al. (2009) are also showing enhanced levels of swelling in vastus lateralis (5.5%) 24 hours after training in BFRRE-leg

versus a non-BFRRE leg (2.2%). Hernandez et al. (2013) observed 16.9% increases in muscle thickness of rectus femoris measured after BFRRE. Interestingly, findings within our previous work showed an even higher increase on 22±6.0% in muscle thickness of rectus femoris.

Several causes to why swelling may be so beneficial for muscle growth are proposed, but the mechanisms are still not fully elucidated. One reason may be the rapid reperfusion after cuff release resulting in pressure of the cells cytoskeleton and/or cell membrane, which ultimately may lead to augmenting of the cells ultrastructure, possibly via osmosesensors (Schoenfeld, 2010). Another reason is the extracellular fluid and metabolite buildup, which causes a change in concentration gradient of water, leading water into the muscle cell to stabilize the osmotic gradient (Loenneke, Fahs, et al., 2012). When water fluctuates into the cell, the above mentioned osmosesensors in the cell-membrane recognize this and gives further activation in different anabolic signaling pathways such as mTOR and MAPK (Low, Rennie, & Taylor, 1997), with latter as the strongest mediator of swelling-induced anabolism (figure 2) (Clarke & Feeback, 1996). Swelling might also have an effect in activating satellite cells (Dangott et al., 2000) as well as a direct effect on amino acid transport system, primarily on glutamine and alpha-(methyl) aminoisobutyric transport (Low et al., 1997)

However, Gundermann et al. (2012) investigated whether swelling was important for muscle protein synthesis with comparison of BFRRE versus similar training without BFR, where hyperemia was stimulated by a pharmacological vasodilator. The group performing BFRRE showed increased rapidity off protein synthesis, whereas the vasodilator group showed no increase. However, the hyperemia response was higher in the BFRRE group and for that reason it is timely to speculate if the group with vasodilation did not reach the threshold necessary for stimulating anabolic processes. Based upon this study, it may not be likely that reperfusion is of great importance concerning gains in muscle mass with BFRRE. Nevertheless, further investigations are required to uncover the potential benefits off swelling. Measurement of swelling is conducted indirectly through measurement of acute variations in muscle thickness and/or muscle volume (Hernandez et al., 2013). The increased levels of swelling has been observed to last for 48 hours (Farup et al., 2015), which could make it difficult to ascertain actual muscle growth.

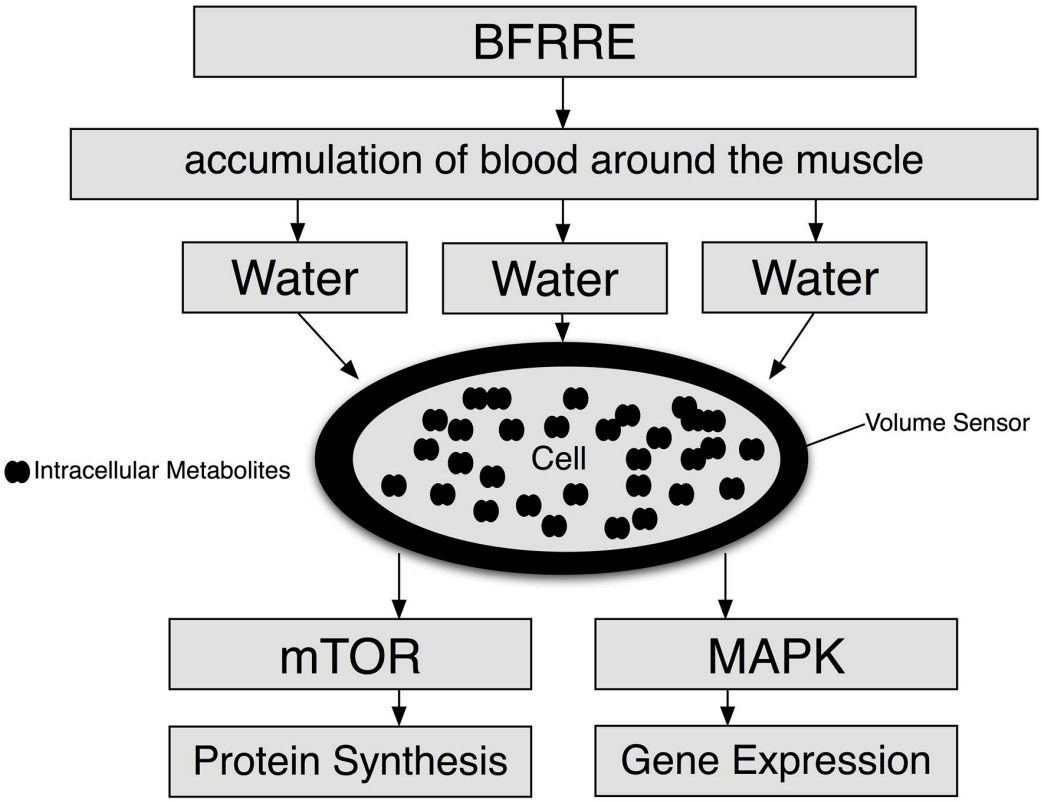

Figure 2: the figure shows the potential course for muscle swelling and its further effect on signaling pathways. Modified after Loenneke (2012) and Haussinger (1996)

2.5.3 Intramuscular signaling

Mechanical disruptions of muscle fibers through contractile processes and stretching are participating in stimulating signaling pathways regardless of growth factors and hormones (Hornberger et al., 2004).

The most important intracellular signals leading to enhanced protein synthesis runs probably during *mammalian target of rapamycin (mTOR*) and *mitogen activated protein kinases (MAPK)* (Dickinson et al., 2011; Kramer & Goodyear, 2007). mTOR elevates muscle protein synthesis by increasing translational efficiency (Spiering et al., 2008) and is therefore important for consecutive hypertrophy (Bodine et al., 2001). Two different mTOR complexes have been observed (mTORC1 and mTORC2), where mTORC1 is considering the most important regulator of protein synthesis (Proud, 2007) during downstream effectors as p70S6K, 4E BP's and eEF2 (Wernbom, 2011). In one study (Wernbom et al., 2013) observed increases in the p-p70S6K (at site Thr389) after 1-hour post exercise in the BFR leg. Authors suggest that increased mTOR signaling partially could explain the fortified hypertrophic effects mediated by BFRRE. Importantly, Gundermann et al. (2014) augments the conclusion of Wernbom et al., (2011) with observations that protein synthesis stalled when inhibiting complex 1 mTORC1 with BFRRE, suggesting this signaling pathway to be of greatest significance to induce muscle growth.

There is a direct link between how intense the tension a muscle is exposed to and the potential activation of selected MAPKs. Activation of these kinases is related to the size of the tension and time under tension (Martineau & Gardiner, 2001). MAPK branches are stimulated by cytokines, cellular stress and growth factors, and regulates gene expression and metabolism relative to energetic, oxidative and mechanical stress in the muscle (Force & Bonventre, 1998; Kramer & Goodyear, 2007). Wernbom et al. (2013) detected increased phosphorylation of p38MAPK (site Thr180/Tyr182) after 1-hour post-exercise in the BFR-leg, compared to no change in the free-flow leg (30% of 1RM).

2.5.4 Muscle damage

Muscle damage is allegedly thought to play an important role as a regulator of satellite cells, where a rapid proliferation is initiated leading to successive muscle growth (Pearson & Hussain, 2015). Muscle damage has typically been associated with heavy eccentric training (Newham, McPhail, Mills, & Edwards, 1983; Vissing, Overgaard, Nedergaard, Fredsted, & Schjerling, 2008) and is evident throughout protracted loss in muscle strength, muscle soreness, enhancement in serum intramuscular enzymes and water retention in the subsequent days after training (Takahashi et al., 1994). Preliminary a large part off the literature is unclear whether muscle damage is important relative to BFRRE, due to contradictory findings in various studies (Pearson & Hussain, 2015). Additionally, it is currently unclear whether the underlying mechanisms causing damage with BFRRE are somewhat similar to that observed after eccentric exercise (Sieljacks et al., 2016). In one study (Thiebaud, Yasuda, Loenneke, & Abe, 2013) BFRRE was observed to elicit muscle damage lasting less than 1 day, whereas another study (Umbel et al., 2009) reported considerable larger damaging effect, lasting 48 hours post exercise. Importantly, in one recent study (Sieljacks et al., 2016) researchers aimed to compare the muscle-damaging effect off a single bout of BFRRE performed to failure versus a bout of maximal eccentric exercise. In this study substantial damage in both the BFRRE group as well as in the eccentric group was observed. Interestingly, BFRRE induced similar magnitude in muscle damage as eccentric training, where two subjects got rhabdomyolyse in the BFRRE group. In addition, the muscle damage observed in Sieljacks et al. (2016) is in line with other studies conducted on eccentric training (Foley, Jayaraman, Prior, Pivarnik, & Meyer, 1999; Newham, Jones, & Clarkson, 1987; Vissing et al., 2008). Hence, it follows that BFRRE can elicit substantial muscle damage and possibly mediate muscle growth through similar mechanisms as eccentric training.

2.5.5 Hormonal responses

Several systemic hormones have been observed to increase in response to BFRRE, such as *growth hormone* (Takano et al., 2005; Takarada, Nakamura, et al., 2000; Takarada et al., 2004) and *insulin-like growth factor 1 (IGF-1)* (Takano et al., 2005). Interestingly, BFRRE shows hormonal increases in line with traditional strength training (Kraemer, Kilgore, Kraemer, & Castracane, 1992). Although the prominent hormonal increase is evident, it is important to pinpoint that enhanced levels off systemic hormones do not appear to be associated with increase in muscle protein synthesis (McCall, Byrnes, Fleck, Dickinson, & Kraemer, 1999; West et al., 2009) or long term adaptive hypertrophy gains (Mitchell et al., 2013). Some studies even proposes systemic hormones not to have any evidence based material to show for in the link between increased hormone response and muscle growth (West, Burd, Staples, & Phillips, 2010; West & Phillips, 2010)

Conversely, local hormones are considered as way more essential for the induction of muscle growth than systemic hormones (Loenneke, Fahs, et al., 2011). *Mechano-growth factor (MGF)* is one of several isoforms of IGF-1 localized in the muscle tissue (Philippou et al., 2009). Interestingly, it seems to be the only one of these isoforms responding to mechanically stimuli or cellular damage (Goldspink, Wessner, & Bachl, 2008). Mechano-growth factor is shown to expedite the post-exercise hypertrophic response and facilitating in local repair of damaged tissue (Goldspink, 2005), activate hypertrophy signaling through different cascades such as mTOR (Sandri, 2008b) and mitogen-activated protein kinase (MAPK) (Sandri, 2008a) as well as mediate growth during satellite cell activation, proliferation and differentiation (Yang & Goldspink, 2002). However, to which extent MGF is associated with BFRRE is to date not well understood (Pearson & Hussain, 2015). Finally, even though systemic hormones appears to be irrelevant, it may have an amplified effect on local hormones (Wernbom et al., 2008).

2.5.6 Other possible mechanisms

Reactive oxygen species (ROS) potential effect on skeletal muscles are uncertain (Takarada, Takazawa, et al., 2000) and even though ROS is stimulated in hypoxic conditions (Korthuis, Granger, Townsley, & Taylor, 1985), observations from previous research shows no increase in markers of ROS (lipid peroxide and protein carbonyl) following BFRRE (Takarada, Nakamura, et al., 2000). *Nitric oxide (NO)* is a variant of ROS linked to hypertrophy (Nakane, Schmidt, Pollock, Förstermann, & Murad, 1993). Nevertheless, mechanical forces primarily stimulate this molecule (Tatsumi et al., 2006) and thereby it is unlikely that the contribution in BFRRE induced muscle growth is of great importance. There are essentially two *heat shock proteins* (HSP70 and HSP72) discussed in literature with respect to BFRRE (Pearson & Hussain, 2015), where HSP72 is regarded as the most important by which occlusion increases muscle size and attenuates atrophy (Yudai Takarada, Takazawa, & Ishii, 2000). *Myostatin* has been observed to decrease following BFRRE (Loenneke, Wilson, et al., 2010). In one other study (Gundermann et al., 2014) no decrease in protein breakdown following BFRRE was documented. However, the mismatch between these studies may be due to differences in measurement time points.

3.0 METHODS

The present study was a part of a main study called *occlusion 5* and was conducted in the southern part of Norway at University of Agder, Kristiansand in September and October 2015. The intention was to investigate differences between a failure and a submaximal protocoll on various variables such as muscle-activation, satellite cells, myonuclei, muscle thickness, MFA, CSA, 1RM and MVC.

3.1 Study design

The study was carried out as a randomized controlled trial and consisted for a period of 9 weeks, starting with familiarization and baseline testing for 2 weeks, blood flow restricted resistance exercise intervention for 3 weeks (interspersed by 10 days of rest) and a final 4-week period of post-testing (figure 3). All participants included in the study went through familiarization to the leg extension exercise (without BFRRE), ultrasound, 1RM and MVC two weeks before the first training week. Baseline measurements were conducted in the week prior to the first training week and consisted of ultrasound, 1RM and MVC. The participants had their legs randomized to one of two BFRRE protocols: one leg performed four sets to voluntary failure, whereas the submaximal leg aimed for four sets with 30-, 15-, 15- and 15 repetitions. The intervention consisted of two training periods including seven BFRRE sessions within five days (separated with 10 days' rest) using unilateral knee extension machine (G200 Knee extension, DMS/EVE Electronic Version, David Health Solutions' LTD, Helsinki, Finland). For logistical reasons, half of the participants trained from Monday to Friday, while the other half trained from Tuesday to Saturday. In both periods participants performed 2 sessions in the last two days of their training week (separated with at least 4 hours). The first half of the participants underwent ultrasound measurements on Mondays, Wednesdays and Fridays, whereas the other half was measured on Tuesdays, Thursdays and Saturdays in both training weeks. On the first day in each training week, ultrasound measurement prevailed before and after BFRRE to detect acute muscle cell swelling.

The first day in training week one contained breakfast (2 hours before baseline biopsies and collectives of blood, appendix: 5) consisting of oatmeal, as well as a fixed dose of sugar and oil based on participant's weight, 1 BFRRE-bout, 1 EMG during BFRRE, 2 ultrasound measurements (pre- and 15 min post BFRRE), 2 biopsies (pre and 2 hours' post), 2 MVC tests (pre and 3h post) and 3 collectives of blood (pre, 2 h and 4 h post). The first day in the second training week was conducted in a similar manner, but excluding biopsies and collectives of

blood. In the resting week there was only one day of testing with ultrasound, biopsy, 1RM and MVC. After the BFRRE intervention, 4 weeks with post-testing followed (3-, 10-, 17- and 24 days' post BFRRE), were the test battery contained ultrasonography, 1RM and MVC (post 3-, 10-, 17-, and 24). The only difference between the four post-test time points was the addition of muscle biopsies at post 10.

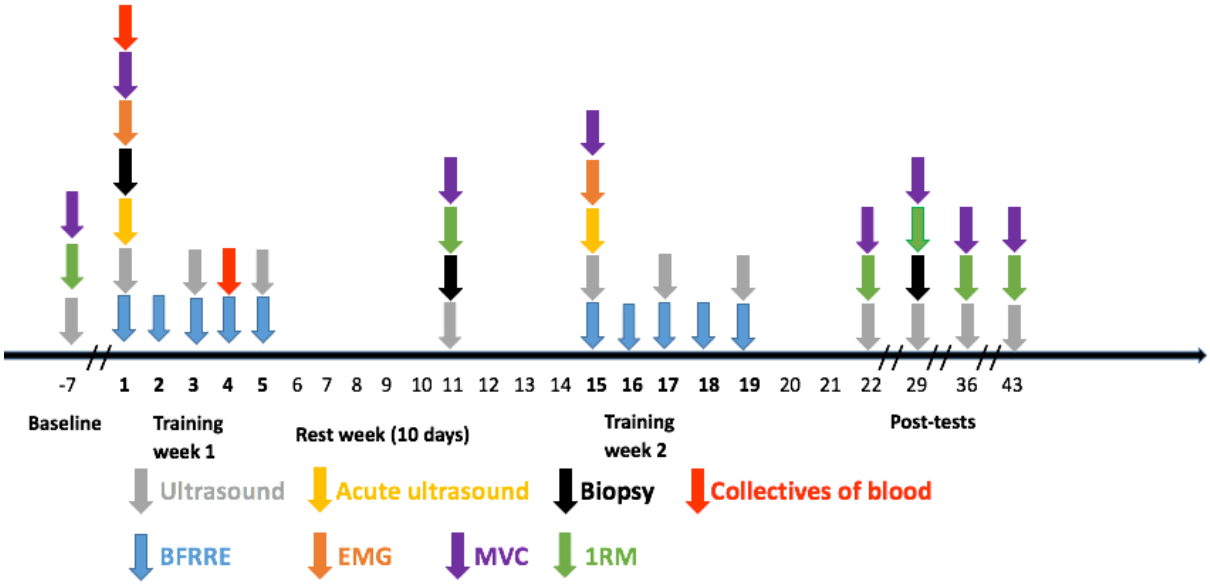

Figure 3. Timeline for tests and training for the present study (*occlusion 5*). One *arrow* is equivalent to one type of measurement

3.2 Participants

Twenty-two male subjects were originally recruited from University of Agder by use of presentations in lectures, stands in cafeteria, student TV, social media (facebook) as well as posters (appendix 1) placed around campus and student residences. The subjects had not conducted systematic strength training the last six months (< 1 session per week the last 6 months). Four subjects were excluded prior to the intervention mainly because of sickness (cold). During the intervention one subject dropped out for reasons unrelated to the study, which lead to 17 subjects whom completed the study.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
- Men between 18-45 years' old - The participants should not have trained the leg muscle on a regular basis within a period of 6 months before the study (<1 session per week)	- Injuries that could prevent the participants from completing the study - Participants should not use any form of drugs or supplement under the study (protein supplementation, vitamins, creatine or similar) - No former experience with blood flow restricted resistance exercise (BFRRE)

In our first meeting with potential participants they were given a short overview of the upcoming study and asked if they were interested. Those who showed interest were placed on a list with some contact information (e.g. mail, phone) and contacted again a few days later. Subjects who decided to participate were invited to one of two meetings (voluntary) where information concerning advantages, disadvantages and completion of the study was given. After the meeting we arranged the remaining familiarization and baseline testing (pre-test) as well as the first acute day for both groups. Not everybody included in the study showed up and was therefore followed up and given the same information. In addition, they were given an oral presentation regarding BFRRE. The study complied with the standards set by the Declaration of Helsinki and was approved by the Norwegian center for research data. The

nature and goals of the study were thoroughly explained, and all subjects provided a written informed consent (appendix 2). Furthermore, no significant differences between groups was observed in any variables measured at baseline ($p<0.01$) (table 2).

Table 2. Baseline characteristics

	All (n=17)	Failure (n=17)	Submaximal (n=17)
Age	25.0 (5.6)		
Height (cm)	181.7 (11.6)		
Weight (kg)	79.9 (13.2)		
1RM (kg)		74.1 (13.3)	75.8 (15.6)
MVC (nm)		226.7 (39.5)	226.7 (40.9)
CSA of rectus femoris (mm)		7.3 (2.1)	6.8 (1.7)
Thickness of rectus femoris (mm)		18.4 (3.6)	17.9 (2.9)
Thickness of vastus lateralis (mm)		25.6 (3.5)	25.3 (3.7)

Data is presented as mean (SD).

3.3 Training protocols

Both protocols where carried out at 20% of 1RM with 30 seconds rest between sets and 5 minutes' rest between each leg; were the participants always started exercising the right leg first. The pressure cuff (9-7350-003, Delfi Medical, Vancouver BC, Canada) stayed on during all four sets and was inflated to 100 mmHg (15cm wide with a 13,5 cm pressure zone). Cuff pressure was first released after last repetition in last set. The pneumatic cuff was coupled to a computerized tourniquet system (Zimmer A.T.S.750, Warsaw, IN, USA) and was placed at the proximal part of the thigh. Velocity of repetitions was set to 1 second concentric and 1 second eccentric, complied by a metronome (Korg Metronome, MA-30, China). Test personnel assisted participants when the first repetition in set 3 and/or 4 was hard to accomplish. Range of motion from 90 to 10 degrees (0 degrees=full extension) in the knee extension had to be conducted in order for the repetition to be approved. Verbal and non-verbal motivational methods were used to encourage participants during training, especially

when it started to get heavy. After every BFRRE-session the participants were asked how painful it was (Borg CR10 scale; appendix 3) and ratings of perceived exertion (Borg 6-20; appendix 4). Both scales have been shown to be reliable and valid (Chen, Fan, & Moe, 2002)

3.4 Test protocols

3.4.1 Muscle size

Ultrasonic-measurements was conducted using a brightness mode (B-mode) ultrasonography device (Logic Scan 128 CEXT-1Z kit, Telemed, LT). Different settings in Echo Wave 2 (3.4.1) such as focus, depth, dynamic range, power, gain and frequency was fine tuned to best identify collagenous tissue that defines the outlying part of the muscle. One trained ultrasound examiner performed all the measurements. Muscle size was measured as muscle thickness of rectus femoris, vastus lateralis, vastus intermedius and cross-sectional-area (CSA) of rectus femoris.

In the first ultrasound session for each participant (familiarization) transparent, acetate paper was positioned over the thigh, to mark scars, birthmarks, moles as well as the marks from the transducer, to ensure reliable positioning with re-testing (Bjornsen et al., 2015). Thus the measurement site could be rapidly located on the upcoming ultrasound sessions. In addition, participants number, depth and leg was noted on this sheet. The participants were instructed to lie supine on an examination bench with their knees fully extended and strapped into position to ensure stability. Before the investigation took place participants were told not to do any muscle-contractions in the lower limbs, due to the flaws this could cause on the pictures. Measurements were conducted distally, at a distance similar to 40% of the femur length. Thereafter, two measurement sites were rapidly located with the transparent, acetate paper. Then, the researcher applied transmission gel to the transducer and took six pictures of rectus femoris (three with panoview and three with still picture-function) as well as three pictures of vastus lateralis (stillpicture). In total, 9 pictures per leg each time was obtained (15 time points per participant).

ImageJ (version 1.46r, National Institutes of Health, USA) is widely applied to analyze ultrasound pictures and was used in the present study (N. D. Reeves, Maganaris, & Narici, 2004). Two different investigators were responsible for ultrasound analysis (one for CSA and one for thickness). Firstly, all pictures from all measurement time points were collected in one folder for each participant, before analyzing. Several spot checks for pictures of each subject

was performed to investigate possible errors (e.g. wrong pictures, too few pictures). Thereafter, all images for each subject was first opened in preview to investigate potential errors, depth differences, and determine how one should draw the vertical lines. Then pictures were opened in imageJ, where all pictures for each participant were analyzed together in random order to ensure accuracy of measurement sites within the images. Muscle thickness was measured with the average of 3 vertical lines per picture (3 pictures) between the inner edge of the superficial and deeper aponeurosis. For CSA analysis freehand function was selected to draw a line around the muscle, where the average of 3 pictures determined CSA. Changes in depth often occurred in the different pictures and therefore needed to be converted to mm, something that often had to be done considering the various depth ranging from 40-100mm. The test-retest analysis demonstrated intraclass-class correlation (ICC) ranging from 0.94 to 0.99 ($p<0.001$, in all cases). Coefficient of variation (CV) was 2.91% for CSA of rectus femoris, 2.05% for thickness of rectus femoris, 0.98% for vastus lateralis and 2,36% for vastus intermedius.

3.4.2 Muscle fiber area

Biopsy area was first washed using disinfectant liquid and further local sedated (Xylocain-adrenaline, 10 mg*ml-1 + 5 µg *ml-1, AstraZeneca, Södertälje, Sverige). Then a scalpel was applied to cut 15-20 millimeter through the skin and muscelfascien. Muscle tissue was extracted by use of a six millimeter sterile "Bergstrømneedle" connected to a 50 millimeter injector, with 200-300 mg muscle tissue per biopsy. Muscle tissue was then being washed clean of blood, before potential fat and connective tissue was dissected. However, this was not the case for muscle tissue to immunohistochemistry (not washed before cutting). Tissue to IHC was cut perpendicular with razorblade and thereafter placed in a form of stabilizing glue (Tissue-tek, O.C.T. compound, Sakura, USA). All biopsies were immediately frozen down in pre cooled (~ -140° C) isopetan and forms with the frozen IHC pieces was placed in cryostat (CM 3050, Leica Microsystems, Nussloch, Tyskland) (~ -22° C). Then biopsies were cut out of the forms using scalpel and loaded in eppendorf tubes as further was placed in an ultra freezer (~ -80° C). Quantifying muscle fiber area was done in the image software TEMA (CheckVision, Hadsund, Danmark).

3.4.3 One repetition maximum

Two instructors were responsible for supervising the 1RM tests. Seat length was first adjusted to fit every individual, where participants back should rest against the chairs backrest and the lateral epicondyle of the knee aligned with rotational axis of the machine. This was noted at the first test and used for upcoming tests in resting week, as well as the four post-tests. Then a seatbelt was wrapped around participant's waist, hands placed on handles alongside the chair and foot pedal positioned right over the ankle joint. Warm up consisted of 5 minutes cycling (100 watt) and a standardized procedure in knee extension starting with 10 repetitions (50% of 1RM), 6 repetitions (70% of 1RM), 3 repetitions (80% of 1RM) and 1 repetition (90% of 1RM) on both legs with 1-minute rest between each warm up-set. In addition, MVC testing was conducted prior to 1RM testing. Then 1RM was found with gradually increase in heavier loads (minimum weight: 1.25 kg) until concentric failure was reached. The lift was accepted when the knee joint reached an angle of 10 degrees (0 degrees=full extension). To ensure this, marks was made on the leg extension machines-display, apparent for both the test personnel and participants. Between 1RM attempts participants had 2 minutes' pauses and at least 30 seconds rest between legs. Right leg was always exercised before left leg and strong verbal communication was given to motivate participants during each 1RM attempt.

3.4.4 Maximal voluntary contraction

Test was conducted in the same machine as the 1RM test (locked in 90 degrees' position). In similarity to 1RM test procedure, seat was adjusted for, hands placed on the handles, seatbelt fastened and the foot pad positioned right over the ankle joint. A general warm up session consisted of 5 min cycling (100 watt), while the specific warm up was conducted with four sets with 5 seconds contraction (perceived 50%, 60%, 80% and 90%) on both legs with 30 seconds rest between each warm-up set. Thence, participants had 3 attempts for each leg and 2 minutes' rest between attempts as well as at 30 seconds rest between right and left leg (right leg was always tested first). The highest value for each leg was noted by one of the two test instructors (same personnel as for 1RM).

3.5 Preparation and pilot study

Several test-sessions were performed prior to the intervention on random subjects not included in the study. In total, 18 subjects volunteered for ultrasound, where some of these performed test-sessions with the two BFRRE protocols as well. In addition to a few other random volunteers who did not perform ultrasound, which lead to 12 random subjects testing the BFRRE protocols. Only one of these participants did not manage to complete the submaximal protocol. Several subjects also underwent test-procedures with EMG, 1RM and MVC. The pilot study was conducted with test-battery consisting of ultrasound, 1 RM, EMG and BFRRE on personnel from a fitness center.

3.6 Statistical analysis

Data in figures are presented as mean with 95% confidence interval (CI) for muscle size (CSA and thickness), maximal strength (1RM and MVC), acute swelling and MFA. All data analyzed was found to be satisfactory normal distributed (Gaussian distribution) according to skewness, mean, median and visual confirmation. For that reason, parametrical tests were chosen as the best option for statistical analysis. To analyze differences between failure and the submaximal protocol an independent sample t-test was used, while paired sample t-test was utilized too investigate changes from baseline. Pearson's correlation was chosen to examine relationship between muscle swelling and muscle size as well as muscle size and maximal strength. Statistics were conducted with IBM SPSS statistics 22.0 (version 22, IBM, Chicago, IL, USA). Level of approved significance was set to ≤1% due to multiple testing with CSA and muscle thickness, whereas significance level was set to ≤5% for maximal strength, acute swelling and MFA.

4.0 METHOD DISCUSSION

4.1 Design

It is a necessity to have a well-designed experiment in order to investigate causality, and experimental design is a good option for illuminating causal relationships (Polit & Beck, 2013). The present study was conducted as a randomized controlled trial (RCT) and was done within subjects longitudinally. Randomized control trial studies are ranked second to systematic review on the evidence hierarchy: levels of evidence and regarded as the "gold-standard" for investigation of hypothesis concerning causal relationships (Polit & Beck, 2013). Causality was the case for the present study considering the primary and secondary objective, where the effect from an independent variable (BFRRE and swelling) was investigated on the dependent variable (muscle size and strength).

Although RCT is considered the "gold-standard" for examining causal relationships, there are limitations associated with this type of experiment as well. For instance the *hawthorne effect* (Polit & Beck, 2013). However, this effect might not influence the present study in appreciably degree, due to the within subject design, where participant's legs functioned as control relative to each other. The within subject method was suitable cause the effects from two different BFRRE protocols could be compared directly within the present study, whereas compared such protocols indirectly (Nobrega & Libardi, 2016).

We attempted to take as many confounders into consideration as possible (e.g. running, bicycling, football: ≤ 1 per week) by informing participants to minimize endurance similar activities, not to begin any new training form, or to perform any kind of strength training while the study was in progress. Nevertheless, it is difficult to control all factors affecting the dependent variable. Independent variables such as energy consumption, protein consumption and sleep were more difficult to control. Although every participant was getting a fixed dosage (30 gram) of protein supplementation after every BFRRE-session to ensure sufficient protein consumption.

4.2 Study sample

Our previous experience with variables such as MFA, satellite cells and myonuclei per myofiber suggests that a standard deviation of 10-20% is probable. Thereby, the main study would require 15 subjects in each group to uncover group differences of 20% with 80% power and alpha level at 5%. Study sample in the present study consisted off 17 participants that completed the intervention. Nielsen et al. (2012) recruited 10 subjects (BFRRE-group) with no strength training experience within the last year. In addition, these subjects were not performing any additional activities without this study. This is somewhat different from the present study, where the inclusion criteria allowed less previous training prior to the intervention than Nielsen et al. (2012). Additionally, several subjects in the present study participated in regular activities. Thereby it can be speculated if the population off the present study was more fit than participants in Nielsen et al. (2012). Conversely, participants in Nielsen et al. (2012) lifted in average 20 kg more (1RM) than participants in the present study. For that reason, it seems like the participants in Nielsen et al. (2012) was better strength trained than the participants in the present study. Furthermore, when attempting to generalize this to a population lying within this age group, it is important to consider whether a selected population would differ significantly from the participants in the present study. In this case, it would be reasonable to assume that participants interested in a strength training intervention, would be more active and healthy in comparison to other individuals within this age group (18-45). Therefore, the participants in the present study could have been in better physical health than the average individual, which might complicate generalization.

4.3 Preparatory work

In the weeks prior to intervention several test-sessions with ultrasound, BFRRE, 1 RM, MVC and EMG was conducted on 12 random volunteers not included in the study, which underwent the same procedures as the participants included in the study. All these subjects participated in failure and submaximal bouts of BFRRE. This testing was crucial in order to ensure that every participant managed to conduct all repetitions required in the submaximal protocol without going to failure. Thus it could be considered that the protocol intended to be submaximal, in fact was submaximal. Results for this trial was that 11 out of 12 subjects managed to complete the submaximal alternative.

Preparatory work was carried out to best prepare test personnel, to ensure validity and reliability with effective and correct routines. To achieve validity and reliability, every test-instructor was guided by a previous trained instructor. When the current task was mastered several test-sessions were performed. In that case, reliability measures in the form of coefficient of variation (CV) and intraclass correlation (ICC) was conducted prior to intervention to investigate quality of measurements (see methods: muscle size). Quality of procedures combined with rapid execution was of significance for the present study due to several training and test-sessions in a short period of time. Unfortunately, rapid execution of the various procedures was given lower priority than the validity aspect, and perhaps too few sessions with several test-participants after one another was carried out. This could have made the first days in both training weeks even more effective as well as the other BFRRE days. To exemplify this: participants were told that every BFRRE session would last for only 15 minutes, which did not correspond with reality in the intervention, where a little more time per session asserted itself (latency).

In a two-week period prior to the intervention participants included in the present study underwent familiarization and baseline testing, where ultrasound, 1RM and MVC was carried out. This was conducted due to several reasons. Firstly, make necessary adjustments to each individual and use those settings in the forthcoming tests and training. Secondly, calculate training load of 20 % of 1RM, where the highest value of two tests was applied as training load for participants during the whole intervention. Thirdly, rule out potential learning effect, which is difficult, but by applying two tests in both 1RM and MVC prior to the intervention, it was attempted to minimize the prevalence of this effect.

4.4 Training protocol

We attempted to exert range of motion as carefully as possible, although this was difficult to oversee for every repetition. To make this less demanding to comply, a screen indicating approved repetition was viewable for both participants and instructors. Controlling for velocity of the repetitions could have been better instructed in the present study. Even though we attempted to control this by applying a metronome, several participants did not follow this rhythm to a satisfactory degree. Typically by including a pause in the end of a repetition, which could have lead to lack in muscle tension, which in turn could have lead blood to escape the muscle (Schoenfeld & Contreras, 2014). Controlling for repetition velocity may have been a difference in comparison to Nielsen et al. (2012). It can also be speculated whether the power exchange of the machine complicates comparison to Nielsen et al. (2012) and our previous work. Particularly cause the leg extension machine used in the present study was a different model than applied in our previous work and in Nielsen et al. (2012).

The submaximal protocol of the present study was harder than expected, with the last set sometimes going to failure for some participants. This was primarily the case in the first training week. To exemplify this, more than half of the participants failed to complete the submaximal protocol without going to failure on the first day in the first training week. Nevertheless, similar cases prevailed in the second training week as well, but in less magnitude. This was interpreted as somewhat surprising, due to the 11 out of 12 random volunteers not included in the study, who managed to complete the submaximal protocol in the preparatory weeks. In this case, it can be speculated if the 1RM test was conducted less accurate for the random volunteers compared to the subjects included in the study. Hence, it is possible that the submaximal protocol was less demanding for these random volunteers, which lead to the discrepancies between the random volunteers not included in the study versus the included subjects.

The present study did not apply different pressures to each individual, which is a weakness if achieving maximized individual results is the aim (Loenneke et al., 2013). However, the present study aimed for a group comparison to Nielsen et al. (2012) and our previous work, making this less important. Eventually, some of the important strengths was the carefully supervision of pauses between sets (30 sec) as well as between legs (5 min). Additionally, cuff placement, range of motion (as already mentioned) and collecting info from participants regarding pain (Borg CR 10, appendix 3) and perceived exertion (Borg 6-20, appendix 4) was substantial strengths.

4.5 Measurements

Testing should be conducted with high quality considering good validity and reliability, with standardized procedures (Thomas, Silverman, & Nelson, 2015). The tests sensitivity must also be high enough to detect small changes in progress (Raastad et al., 2010). Furthermore, the equipment should be accurate and fine-tuned for its respective task (Thomas et al., 2015). In the present study, protocols for each test was carefully complied for every test-session with competent supervisors for each test.

4.5.1 Muscle size

In the present study a brightness mode (B-mode) ultrasound apparatus was used to measure and quantify skeletal muscle size assessed as muscle thickness and CSA. This apparatus has proved to be a good option for measurement of both muscle thickness and CSA in legs of healthy adult subjects (Rankin & Stokes, 1998; Reeves et al., 2004; Weiss & Clark, 1985). Ultrasound has also been shown to be a good alternative compared to other measurement methods such as magnetic resonance imaging (MRI) and computed tomography (CT) (English, Fisher, & Thoirs, 2012; Reeves et al., 2004; Thomaes et al., 2012). MRI and CT possess the highest level of accuracy and are considered the "gold-standard" for measuring muscle size (Mitsiopoulos et al., 1998; Sanada, Kearns, Midorikawa, & Abe, 2006). However, these methods are expensive as well as time consuming and were not used in the present study cause of unavailability. Ultrasound was therefore the best alternative and has several advantages such as portability, fairly inexpensive, non-invasive and safe for use *in vivo* (Koppenhaver et al., 2009).

Validity of ultrasound measurements depends largely upon the personnel conducting them and the existence of an automatic procedure capable of minimizing measurement errors is lacking (Barber, Barrett, & Lichtwark, 2009). The ultrasound examiner in the present study underwent therefore several weeks of regularly training with the apparatus. Firstly, on colleagues involved in the main study and thereafter on random volunteers not included in the study. In total, 18 test-subjects volunteered for the pilot study including ultrasound imaging and some of these also underwent this procedure up to several times. Purpose was to perform the procedure as correct as possible and conduct a substantial number of repetitions with this approach and thereby facilitate for valid and reliable measurements considering the forthcoming study. Right and left leg was measured and the same amount of pictures was taken as in the training intervention. After every test-session pictures were evaluated and

potential flaws detected. Some pictures were also analyzed with supervisor, which gave advices on how to improve ultrasonic skillset.

In a two-week period prior to start of the intervention subjects included in the study underwent familiarization and baseline testing. This was favorable for a multitude of reasons. Firstly, to create the transparent acetate sheets, which were important due to several reasons (1) to ensure that the examination was performed as equally as possible from time to time (2) control for prevailing participants muscle depth, which leg being measured as well as which participant being measured and (3) improve effectiveness by rapid localization of measurement site. Secondly, it gave extra training with the apparatus in addition to extra baseline measurements (three including the first day in the first training week). Thirdly, it improved effectiveness of the measurements, which was favorable because of a tight schedule.

When the intervention started, the routine from time to time was identical, with detecting similar measurement site as before by positioning transparent, acetate sheets over partcipants thigh. Further, another computer was utilized which contained pictures from the first measurement of each participant making it easier for the examiner to get reliable measurements. Excessive use of gel combined with minimal pressure was applied to the transducer to avoid tissue compression and thereby get proper pictures of the muscle. Tissue compression has previously been shown to be a prominent error in ultrasound imaging (Reeves et al., 2004). In that case, the test-retest analysis with intraclass correlation (ICC) and coefficient of variation (CV) showed good reliability in the present study (see methods: muscle size). Other potential flaws can be the muscle bellies lack of homogeneity in growth (Noorkoiv, Nosaka, & Blazevich, 2010; Raastad et al., 2010). For that reason, it is possible that potential growth alongside the muscle was overlooked in the present study. Furthermore, muscle cell swelling could make it difficult to ascertain actual hypertrophy, especially cause this phenomenon is known to last for several days (Farup et al., 2015). Differences in hydration levels of participants could also affect the measurements, according to rapports from cadavers (Ward & Lieber, 2005)

4.5.2 One repetition maximum

A standard method applied in several studies for testing of maximal strength is 1RM (Raastad et al., 2010). This test has been found valid and reliable for measuring skeletal muscle strength in adults when using a carefully protocol (Levinger et al., 2009) and is considered the "gold-standard" for assessing maximal muscle strength in non-laboratory settings (Levinger et al., 2009). Two instructors were responsible for performing the tests. For reliability purposes perhaps one instructor would have suited best, but due to practical reasons it was difficult to accomplish. Participants was told not to perform any exercise at least one day prior to 1RM test and consume a normal diet as well as continue a normal sleep pattern. This was done in order to ensure satisfactory recovery (Knight & Kamen, 2001) and prevailing procedure was communicated to participants before every 1RM-test. The unilateral leg extension machine had a weight interval of 5 kg, which was considered too large. Therefore additional weights (2-. 2.5-. 1.25 kg) were included to adjust load within this interval. Rest-interval between 1RM attempts was set to 2 minutes, which should be enough recovery between sets (Weir, Wagner, & Housh, 1994). Although, this can be discussed and perhaps longer recovery time (e.g. 3-5 minutes) would have been more expedient (de Salles et al., 2009). Participants did not possess any familiarity with 1RM in the current exercise and showed therefore naturally a lack of opinion regarding their own level. Hence, participants could have increased rapidly in the test-exercise without any training, which could have had a negative impact on the reliability of the test (Raastad et al., 2010). In the present study it was attempted to minimize this effect with familiarization.

4.5.3 Maximal voluntary contraction

Another test for quantifying skeletal muscle strength in the present study is MVC. This test is considered the "gold-standard" for assessment of muscle strength in laboratory settings (Verdijk, van Loon, Meijer, & Savelberg, 2009). In the present study, force development (newton meter) was measured in a 90-degree position. This is a reliable approach for measuring strength, but it is not a specific test compared to exercise intervention programs (Verdijk et al., 2009). This is mainly the reason why 1RM is chosen before MVC in most studies (Verdijk et al., 2009). Nevertheless, it is an exercise ideal for untrained individuals cause of the simplicity and low demands for technique (Raastad et al., 2010). In similarity to 1RM, participants were told not conduct any training at least one day before test as well as eat and sleep normally. Test procedure also consisted of the same test personnel as 1RM and rest-interval (2 minutes between each attempt).

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PART 2:

PAPER

The effect of a failure and submaximal blood flow restriction resistance exercise protocol on changes in muscle size, strength and swelling

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Effect of two different blood flow restriction resistance exercise protocols on muscle size

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ABSTRACT

Purpose: (1) compare changes in muscle size and strength between a failure (FA) and submaximal (SU) BFRRE protocol (2) investigate associations between swelling and muscle size

Methods: Seventeen untrained men had their legs randomized to one of two BFRRE protocols: one leg performed four sets to voluntary failure, while the submaximal leg aimed for four sets with 30-,15-,15- and 15 repetitions. The intervention consisted of two training periods including seven BFRRE sessions within five days (separated with 10 days' rest) using unilateral knee extension at 20% of one repetition maximum (1RM) (30 s rest between sets). The pressure cuff stayed on during all four sets and was inflated to 100mmHg (15 cm width). Swelling and muscle size was measured with ultrasound, whereas strength was measured as 1RM and maximal voluntary contraction (MVC).

Results: Cross-sectional area (CSA) of rectus femoris increased significantly 17 days' post BFRRE in both groups compared to baseline (FA: 7.9 ± 7.6%; $p < 0.001$ and SU: 9.1 ± 10.8%; $p = 0.003$), whereas no significant difference between groups were observed. 1 RM increased significantly in each group (FA: 9±8%; $p < 0.001$ and SU: 11±7%; $p < 0.001$) 24 days' post intervention compared to baseline, whereas no group differences were found. Swelling increased CSA of rectus femoris (12.0±9.72%, $p<0.001$) compared to ultrasound measurement obtained right before BFRRE.

Conclusion: FA and SU induced similar gains in muscle size and strength. Acute swelling increased, whereas no associations was observed between swelling and muscle size

Keywords: ultrasound, concentric failure, submaximal, muscle thickness, cross-sectional area, swelling

INTRODUCTION

A widespread theory is to apply weights of at least 70% of one repetition maximum (1RM) to achieve gains in muscle mass and strength [1]. This theory is highly challenged by blood flow restricted resistance exercise (BFRRE), where increasing amount of research supports muscle growth and maximal strength using loads as low as 20-30% of 1RM for untrained [2], recreational trained [3] and well-trained subjects [4]. Furthermore, BFRRE can be utilized in attenuating muscle atrophy during immobilization [5] or enhancing recovery during rehabilitation after knee surgery [6]. The mechanisms behind the benefits seen with BFRRE are currently unclear. However, a diversity of possibilities has been suggested such as increase in metabolic accumulation, enhanced fiber-recruitment, increased hormone activity, muscle damage, intracellular swelling and intracellular signaling [7-9].

In one study Nielsen & co-workers [2] observed a remarkable 150-300 % increase in the number of satellite cells, 30% increase in the number of myonuclei and 40% increase in muscle fiber area already after one week (7 sessions) of BFRRE performed to voluntary failure in leg extension (20% of 1RM). In this study satellite cells, muscle fiber area and myonuclei adaptations seemed to plateau after the first week of training, showing no further increase the following two weeks of BFRRE. Previous work within our research group attempted to reproduce the remarkable results observed by Nielsen & co-workers, but found no changes after one week of training applying similar protocol. It has been speculated whether the failure protocol applied in our previous research has been too hard compared to the failure protocol with Nielsen & co-workers, which is the rationale for comparing two different BFRRE protocols (one to failure and one submaximal) in the present study.

Some research has been conducted with respect to investigate the differences between a failure and a submaximal protocol for traditional strength training (>70% of 1RM), where the results are conflicting [10, 11]. Furthermore, a small amount of research has aimed for a direct comparison of a failure and submaximal protocol directly [12]. Additionally, the majority of research investigating a failure and submaximal protocol has aimed to increase muscle strength, not muscle size [12]. Even less research is prevalent in terms of BFRRE and to the authors knowledge no study has investigated the importance of conducting BFRRE to voluntary failure.

Swelling is an increase in cellular hydration status and believed to induce muscle growth [9, 13]. Swelling occurs as a result of strength training and particularly if the muscle is exposed to high metabolic stress, as with BFRRE [9]. Findings in a number of studies refers to enhanced levels of swelling with BFRRE [13, 14] and research is also pinpointing the importance of swelling due to its role in cell signaling [14-16]. However, few studies have aimed to investigate associations between muscle swelling and muscle size.

Therefore, the aim of the present study is twofold (1) investigate changes in muscle size and strength between a failure and submaximal BFRRE protocol after two training weeks with 7 sessions per week (interspersed by 10 days) and (2) investigate association between muscle swelling and muscle size.

MATERIALS AND METHODS

Participants

Eighteen untrained male subjects between 18-45 years were recruited on the southern part of Norway by use of advertising around university campus, Kristiansand. Subjects was invited to a meeting where information concerning advantages, disadvantages and completion of the study was given. Exclusion criteria was injuries that could prevent the participants from completing the study, drugs or supplement (protein powder, vitamins, creatine or similar) and former experience with blood flow restriction resistance exercise (BFRRE). During the intervention one subject dropped out due to reasons unrelated to the study. Subjects was instructed to minimize training activities other than performed in the study as well as avoid starting with any form of new exercise, while the study was in progress. The study complied with the the standards set by the Declaration of Helsinki and was approved by Norwegian Centre for Research data. The goals of the study were carefully explained and all participants signed a written informed consent.

Study design

The study was carried out as a randomized controlled trial and consisted for a period of 9 weeks, starting with familiarization and baseline testing for 2 weeks, BFRRE intervention for 3 weeks (interspersed by 10 days) and a final 4-week period of post-testing. Participants had their legs randomized to one of two BFRRE protocols: one leg performed four sets to voluntary failure, whereas the submaximal leg aimed for four sets with 30-, 15-, 15- and 15 repetitions. The intervention consisted of two training periods including seven BFRRE sessions within five days (separated with 10 days' rest) using unilateral knee extension machine (G200 Knee extension, DMS/EVE Electronic Version, David Health Solutions' LTD, Helsinki, Finland). For logistical reasons, half of the participants trained from Monday to Friday, while the other half trained from Tuesday to Saturday. In both periods participants

performed 2 sessions in the last two days of their training week (separated with at least 4 hours). The first day in training week one contained breakfast (2 hours before baseline biopsies and collectives of blood, appendix: 5) consisting of oatmeal, as well as a fixed dose of sugar and oil based on participants weight, 1 BFRRE-bout, 1 EMG during BFRRE, 2 ultrasound measurements (pre- and 15 min post BFRRE), 2 biopsies (pre and 2 hours post), 2 MVC tests (pre and 3h post) and 3 blood samples (pre, 2 h and 4 h post). The first day in the second training week was conducted in a similar manner, but excluding biopsies and collectives of blood. In the resting week there was only one day of testing with ultrasound, biopsy, 1RM and MVC. Ultrasound measurements was conducted on Mondays, Wednesdays and Fridays for the first half of the participants, whereas the other half underwent measurements on Tuesday, Thursday and Saturday. On the first day in each training week, ultrasound measurements prevailed before and after BFRRE to detect acute muscle cell swelling. After the BFRRE intervention, 4 weeks off post-testing followed (3-, 10-, 17- and 24 days' post BFRRE), were the test battery contained ultrasonography 1RM and MVC (post 3-, 10-, 17-, and 24). The only difference between the four post-test time points was the addition of muscle biopsies at post 10.

Training protocols

Both protocols where carried out at 20% of 1RM with 30 seconds rest between sets and 5 minutes' rest between each leg; were the participants always started exercising the right leg first. The pressure cuff (9-7350-003, Delfi Medical, Vancouver BC, Canada) stayed on during all four sets and was inflated to 100 mmHg (15cm wide with a 13,5 cm pressure zone). Cuff pressure was first released after last repetition in last set. The pneumatic cuff was coupled to a computerized tourniquet system (Zimmer A.T.S.750, Warsaw, IN, USA) and was placed at the proximal part of the thigh. Velocity of repetitions was set to 1 second concentric and 1 second eccentric, complied by a metronome (Korg Metronome, MA-30, China). Test-

personnel assisted participants when the first repetition in set 3 and/or 4 was hard to accomplish. Range of motion from 90 to 10 degrees (0 degrees=full extension) in the knee extension had to be conducted for the repetition to be approved. Verbal and non-verbal motivational methods were used to encourage participants during training, especially when it started to get heavy. After every BFRRE-session the participants were asked how painful it was (Borg CR10 scale; appendix 3) and ratings of perceived exertion (Borg 6-20; appendix 4). Both scales have been shown to be reliable and valid [17]

Muscle size

Ultrasonic-measurements was conducted using a brightness mode (B-mode) ultrasonography device (Logic Scan 128 CEX [17]T-1Z kit, Telemed, LT). Different settings in Echo Wave 2 (3.4.1) such as focus, depth, dynamic range, power, gain and frequency was fine tuned to best identify collagenous tissue that defines the muscle aponeurosis. One trained ultrasound examiner performed all the measurements. Muscle size was measured as muscle thickness of rectus femoris, vastus lateralis, vastus intermedius and cross-sectional-area (CSA) of rectus femoris.

In the first ultrasound session for each participant (familiarization) transparent, acetate paper was positioned over the thigh, to mark scars, birthmarks, moles as well as the marks from the transducer. Thus the measurement site could be rapidly located on the upcoming ultrasound sessions. In addition, participants number, depth and leg was noted on this sheet. Participants were instructed to lie supine on an examination bench with their knees fully extended and strapped into position to make sure of stability when the analysis were in progress. Thereafter, two measurement sites were rapidly located with the transparent, acetate paper. Measurements were conducted distally, at a distance similar to 40% of the femur length. Excessive use of gel was applied to the transducer when pictures of CSA of rectus femoris as

well as muscle thickness of rectus femoris and vastus lateralis were obtained. In total, 12 pictures per leg each time (15 time points per participant).

ImageJ (version 1.46r, National Institutes of Health, USA) is widely applied to analyze ultrasound pictures and was used in the present study [18]. Two different investigators were responsible for ultrasound analysis (one for CSA and one for thickness). Muscle thickness was measured as the average of 3 vertical lines per picture (3 pictures) between the inner edge of the superficial and deeper aponeurosis. For CSA analysis freehand function was selected to draw a line around the muscle, where the average of 3 pictures determined CSA. The test-retest analysis demonstrated intraclass-class correlation (ICC) ranging from 0.94 to 0.99 ($p<0.001$, in all cases). Coefficient of variation (CV) was 2.91% for CSA of rectus femoris, 2.05% for thickness of rectus femoris, 0.98% for vastus lateralis and 2.36% for vastus intermedius.

Muscle fiber area

Biopsy area was first washed using disinfectant liquid and further local sedated (Xylocain-adrenaline, 10 mg*ml-1 + 5 µg *ml-1, AstraZeneca, Södertälje, Sverige). Then a scalpel was applied to cut 15-20 millimeter through the skin and muscelfascien. Muscle tissue was extracted by use of a six millimeter sterile “Bergstrømneedle” connected to a 50 millimeter injector, with 200-300 mg muscle tissue per biopsy. Muscle tissue was then being washed clean of blood, before potential fat and connective tissue was dissected. However, this was not the case for muscle tissue to immunohistochemistry (not washed before cutting). Tissue to IHC was cut perpendicular with razorblade and thereafter placed in a form of stabilizing glue (Tissue-tek, O.C.T. compound, Sakura, USA). All biopsies were immediately frozen down in pre cooled (~ -140° C) isopetan and forms with the frozen IHC pieces was placed in cryostat

(CM 3050, Leica Microsystems, Nussloch, Tyskland) (~ -22° C). Then biopsies were cut out of the forms using scalpel and loaded in eppendorf tubes as further was placed in ultra freezer (~ -80° C). Quantifying muscle fiber area was done in the image software TEMA (CheckVision, Hadsund, Danmark).

One repetition maximum

Two instructors were responsible for supervising the 1RM tests. Seat length was first adjusted to fit every individual, where their back should rest against the chairs backrest and the lateral epicondyle of the knee aligned with the rotational axis of the machine. This setting was noted in the familiarization period and applied in the other test-sessions (rest week and four post tests). Furthermore, a seatbelt was wrapped around participant's waist, hands placed on handles alongside the chair and foot pedal positioned right over the ankle joint. Warm up consisted of 5 minutes cycling (100 watt) and a standardized procedure in knee extension starting with 10 repetitions (estimated 50% of 1RM), 6 repetitions (70%), 3 repetitions (80%) and 1 repetition (90%) on both legs with 1-minute rest between each warm up-set. In addition, MVC testing was conducted prior to1RM testing. Thereafter, 1RM was found with gradually increase in heavier loads (minimum weight: 1.25 kg) until concentric failure was reached. Lift was approved when the knee joint reached an angle of 10 degrees (0 degrees= full extension). In this case, a mark was made on the display off the leg extension machine, which was apparent for both instructors and participants. Between 1RM attempts participants had 2 minutes' pauses and at least 30 seconds rest between legs. Right leg was always exercised before left leg and strong verbal communication was given to motivate participants during each 1RM attempt.

Maximal voluntary contraction

Test was performed in the same machine as 1RM (locked in 90 degrees' position). In similarity to 1RM procedure, seat was adjusted for, hands placed on the handles, seatbelt fastened and the foot pad positioned right over the ankle joint. A general warm up session consisted of 5 min cycling (100 watt), whereas the specific warm up comprised off four sets with 5 seconds contraction (perceived 50%, 60%, 80% and 90%) on both legs with 30 seconds rest between each warm-up set. Thence, participants had 3 attempts for each leg and 2 minutes' rest between attempts as well as at 30 seconds rest between right and left leg (right leg was always tested first). The highest value for each leg was noted by one of the two test instructors (same personnel as for 1RM).

Statistical analysis

Data in figures are presented as mean with 95% confidence interval (CI) for all variables, which includes muscle size (CSA and thickness), maximal strength (1RM and MVC), acute muscle swelling and MFA. All data analyzed was found to be satisfactory normal distributed (Gaussian distribution) according to skewness, mean, median and visual confirmation. For that reason, parametrical tests were chosen as the best option for statistical analysis. To analyze differences between failure and submaximal protocol an independent sample t-test was applied, while paired sample t-test was utilized too investigate changes from baseline. Pearson's correlation was chosen to examine relationship between swelling and muscle size as well as associations between muscle size and strength. Statistics were conducted with IBM SPSS statistics 22.0 (version 22, IBM, Chicago, IL, USA). Level of approved significance was set to $\leq$1% due to multiple testing with CSA and muscle thickness, whereas significance level was set to $\leq$5% for maximal strength, acute swelling and MFA. Our previous experience [19] with variables such as MFA, satellitecells and myonuclei per myofiber suggests that a

standard deviation of 10-20% is probable. Thereby, the main study would require 15 subjects in each group to uncover group differences of 20% with 80% power and alpha level at 5%.

RESULTS

Seventeen subjects completed the study and all 14 training sessions, whereas one subject dropped out due to reasons unrelated to the study. A tendency for difference in training volume between the two groups was present in the first training week ($p=0.07$), whereas a significant difference was present in the second week of training (Failure: 10 010±3361kg and Submaximal: 7760±1421kg, $P=0.02$). The subjects reported the submaximal protocol to be less demanding than the failure protocol (average for both training weeks) with respect to pain (Failure: 7.0±1.7 vs. Submaximal; 5.7±2.1, $p=0.02$, Borg CR10; appendix 3) and perceived exertion (Failure: 18.1±1.4 vs. Submaximal: 15.5±2.5, $p<0.001$, Borg 6-20; appendix 4). There was no significant difference between groups in any variables measured at baseline $p<0.01$ (table 1).

Muscle size

There was no significant difference between the failure and submaximal group in any measurement time points for CSA of rectus femoris as well as in thickness of rectus femoris, vastus lateralis and vastus intermedius (figure 4). However, almost all measurement time points for CSA of rectus femoris as well as muscle thickness in rectus femoris and vastus lateralis increased significantly compared to baseline. No significant increases were observed between groups in vastus intermedius relative to baseline. Interestingly, CSA of rectus femoris in the failure group increased significantly on training day 3 (0.53±0.91mm; $p<0.001$), whereas no increase was observed in the submaximal group (0.43±0.8mm; $p=0.12$). All measurements increased significantly for both groups in rectus femoris compared to baseline. Training day 3, 5 and the resting week (day 10) increased significantly for failure group in vastus lateralis, while only tendencies were observed in the submaximal group

(p=0.02, 0.03, 0.02; respectively). Besides this, all measurements were found to increase significantly in vastus lateralis for both groups relative to baseline. Muscle fiber area decreased significantly in fiber type 1 on post 10 for failure group (-1094±1856 μm2, p= 0.02) (figure 4), whereas the submaximal group remained unchanged (-459±1953 μm2, p=0.82). Additionally, no significant change was observed for MFA in fiber type 2 at post 10 (Failure: -973±2900 μm2, p=0.97 and Submaximal: -762±2041, p=0.27).

One repetition maximum

We did not observe significant difference between groups in 1RM for any of the measurement time points (figure 2). However, a tendency (p=0.07) was observed in the resting week, as the failure group decreased significantly (71.3±7.9kg; p=0.02) compared to baseline, whereas no changes were detected for the submaximal group (72.2±13.0kg; p=0.66). Increases in strength first occurred for both groups at post 17, with failure group (79.8±14.8kg; p=0.004) and the submaximal group (78.3±14.2kg; p=0.002). Peak in maximal strength was observed at post 24 for each failure (82.6±15.6kg; p<0.001) and submaximal group (80.9±14.6kg; p<0.001). Furthermore, no correlation was observed between muscle size (CSA and thickness) and 1RM. Nevertheless, tendencies were found between thickness of rectus femoris and 1RM for failure group on post 17 (r=0.45, p=0.07) and post 24 (r=0.49, p=0.06). A tendency was also observed between thickness in vastus lateralis and 1RM for the submaximal group on post 24 (r=0.45, p=0.07).

Maximal voluntary contraction

One significant difference was observed between groups at post 3 (Failure: 202.25±33.32Nm and Submaximal: 215.59±40.61Nm; p=0.03), where each of the groups decreased significantly (figure 2). Both groups were still decreased significantly at post 10, unchanged at post 17, but increased significantly at post 24 for failure (237.24±44.28; p=0.02) and the submaximal group (240.41±53.85; p=0.01), compared to baseline. Correlation was found between thickness off vastus lateralis and MVC for failure group on post 17 (r=0.60; p=0.012) as well as for the submaximal group on post 17 (r=0.68; p=0.003) and 24 (r= 0.66; p= 0.004). Besides this, tendencies were found between thickness of vastus lateralis and MVC. For CSA of rectus femoris and thickness of vastus lateralis no significant relationship with MVC was observed.

Cell swelling

There was no significant difference in acute muscle swelling between groups measured 15 minutes' post BFRRE in CSA of rectus femoris or in thickness of rectus femoris and vastus lateralis (average of first day in both training weeks) (figure 3). However, each of the groups increased significantly compared to measurement obtained right before BFRRE in CSA of rectus femoris (failure: 0.84±0.59mm; p<0,001 and submaximal: 0.89±0.90; p=0.001), thickness of rectus femoris (failure: 2.60±1.30mm; p <0,001 and submaximal: 2.19±1.38mm; p<0,001) and in thickness of vastus lateralis (failure: 0.83±0.97mm; p<0.001 and submaximal: 0.51±1.57mm; p<0.001). There was no significant correlation between cell swelling and muscle size.

DISCUSSION

The aim of the present study was to investigate changes in muscle size and strength between a failure and a submaximal blood flow restriction resistance exercise protocol after two training weeks (interspersed with 10 days) consisting of 7 sessions per week. Thereafter, to investigate the potential relationship between acute muscle swelling and changes in muscle size. We did not observe any differences between protocols with respect to muscle growth and strength. However, rapid increases in muscle size for both protocols were found compared to baseline in the first week of training, with further increases in second training week, before a slightly decrease occurred at the four post-tests. Maximal strength (1RM and MVC) peaked 24 days' post BFRRE compared to baseline. We observed a robust muscle swelling after a bout of BFRRE for both protocols. Nevertheless, no relationship between swelling and muscle size was observed.

To this authors knowledge no study conducted on BFRRE has directly investigated differences between a failure and a submaximal protocol on changes in muscle size and strength. However, there are studies using traditional strength training that have compared a failure to a non-failure group. In one study Burd & co-workers [20] included 15 males to investigate the effect of three unilateral leg extension protocols on protein synthesis: 90% of 1RM until volitional failure, 30% 1RM work-matched to 90% failure (30WM), or 30% of 1RM performed to volitional failure (30 FAIL). Superior increases in protein synthesis was observed with respect to the low load-failure resistance exercise group (30FAIL) in comparison to the high-load failure group or work-matched (30WM) on muscle protein synthesis. Although Burd & co-workers had different outcomes than the present study (proteins synthesis versus muscle size), increases in protein synthesis has been observed to be highly associated with muscle size [21]. Therefore, it is interesting that the low load-failure group (30FAIL) induced superior increases compared to the high load-failure group on

protein synthesis, whereas the increases in the work matched group (30WM) was solid as well. These findings are somewhat in line with the findings of the present study, where substantial increases were observed in both protocols. Nevertheless, the present study observed no differences between protocols on muscle size and strength, where Burd & co-workers' rapport superior increases to the low load-failure protocol.

Previous studies conducted on BFRRE rapport substantial increases in both muscle size and strength, applying a failure protocol [2, 22] and a non-failure protocol [16, 23, 24]. Moreover, in a meta-analysis [25] it is emphasized that a non-failure protocol can be as effective as a failure protocol for the induction off muscle growth and strength during traditional strength training. Even though performing sets to failure can give considerable increases in muscle mass and strength, it can be speculated if performing sets to failure is unnecessary with respect to traditional strength training and BFRRE [12, 25]. Particularly when the non-failure alternative is less demanding and more feasible, as reported from participants in the present study (Borg CR10 and 6-20 scales). Additionally, a non- failure approach is preferable due to reduced risk off injury and overtraining [25]. However, the non-failure alternative must likely be performed to mediate a certain amount of fatigue, to induce gains in muscle mass and strength in line with a failure protocol [26]. This is consistent with the findings of the present study, where the submaximal protocol induced some degree off fatigue, even failure for some participants in the last set.

The findings of the present study with decrease in MFA of fiber type 1 (-10%) for failure group, do not confirm the findings of Nielsen & co-workers, who observed ~40% increase in both type 1 and 2 fibers after one training week. Rapports from traditional strength training indicates 15-20% increase in MFA, but after 12-16 weeks of regularly training [27, 28], whereas rapports from another study [29] shows ~37% in subjects considered as hypertrophy

responders. Therefore, the results from Nielsen & co-workers are unique and can even be compared to increases in MFA and myonuclei observed after supplementing with anabolic steroids [30, 31]

Previous work within our research group has attempted to reproduce the results from Nielsen & coworkers. However, our previous work did not correspond with Nielsen & co-workers, with no increase in MFA after one week applying a similar protocol. In similarity to our previous work, the present study observed no increases in MFA after the first week of training as well, using a failure and submaximal protocol. Several possible reasons for the conflicting findings between these studies has been suggested. Firstly, the number of repetitions in the failure group of the present study was 82 (average per session) and 69 for the submaximal group in the first week. This number corresponds with our previous work, where 85 repetitions per session was carried out. However, the number of repetitions in the present study's failure group are not consistent with the failure group with Nielsen & co-workers, where 66 repetitions was performed. This mismatch might be threefold (1) participants may have been pushed harder in the present study and our previous work (Bjørnsen and Nielsen, personal communication), (2) we speculate if the training load might have been overestimated with Nielsen & co-workers. These speculations are anchored in the considerably higher training load for participants regarded as having a lower training status than the participants of the present study and our previous work and finally (3) the present study might have had a slightly less supervision of the velocity in repetitions.

Interestingly, the submaximal group in the present study performed almost equivalent number of repetitions as performed in the study of Nielsen & co-workers. The repetition pattern between the present study and Nielsen & co-workers was also somewhat similar (40-, 12-, 8-, 7 versus 30-,15-,15-,15). In addition, training volume between the discussed studies was fairly

similar. For that reason, we speculate if the submaximal protocol in the present study, to a certain extent, recreated the failure protocoll in Nielsen & co-workers. Furthermore, disparities between power exchanges in machines might have impacted the results due to each of these studies applying different leg extension machines.

The present study observed a robust increase in acute cell swelling levels after a bout of BFRRE. These findings confirm the findings from our previous research as well as other studies, where acute cell swelling has been observed [32, 33]. Cell swelling is believed to be important for the induction off muscle growth with BFRRE by mediating several mechanisms [9]. Firstly, cell swelling might activate intramuscular signaling (MAPK and mTOR), due to osmosesensors who register water penetrating the cell membrane [34]. Secondly, cell swelling can possible enhance satellite cell activation [35] and the amino acid transport system [34]. Although several studies substantiate the importance of swelling, the present study did not observe any relationship between swelling and muscle size. This is somewhat in line with findings from our previous research, which did not observe any relationship between cell swelling and muscle size in vastus lateralis. Nevertheless, in our previous research we observed a moderate negative correlation between cell swelling and muscle size in CSA of rectus femoris.

The absence of a distinct correlation in our previously work has been explained by disparities in magnitude of the two variables. It seems like the measurement time points for swelling can have been extra favorable with respect to the percentage of increase (first day in both training-weeks). This might possibly be the case for the present study as well. Particularly cause the measurement time points matched those in our previous work. Therefore, it could be reasonable to deduce that the result would look differently with other measurement time points. Nevertheless, acute swelling has a solid foundation in the literature as a possible

mechanism for the anabolic benefits observed with BFRRE. Hence, it can be interesting to speculate if swelling might have had some degree of influence on muscle size in the present study after all.

The present study observed delayed increases in both muscle size and maximal strength. These delayed increases were particularly prominent for 1RM and MVC, which peaked 24 days' post BFRRE, whereas peaks in muscle size preceded the peaks observed in maximal strength. To this authors knowledge, few studies can point to such delayed increases. However, in one study Zory & co-workers [36] detected increases in MVC (21.5%) after 4 weeks of detraining. This is twice the increase in MVC compared to the increases in the present study. The differences in MVC between these studies might be due to various training methods, where Zory & co-workers applied electrical stimuli in their training regime.

Basically, BFRRE is believed to elicit low mechanical tension and therefore low muscle damage compared to traditional strength training, which makes it possible to include several sessions in a short period of time [37]. Surprisingly, rapports from a recent study [38] suggests that BFRRE can induce the same magnitude of muscle damage and protection against following heavy eccentric strength training (repeated bout effect), as a bout of heavy eccentric strength training (2 subjects got rhabdomyolyse). Furthermore, the researchers in this study suggests an overlap of the mechanisms inducing muscle damage in both BFRRE and eccentric training. In this case, it can take several weeks (even months) to recover from heavy eccentric training for untrained subjects due to substantial muscle damage [39]. The present study observed signs of necrosis, which can indicate considerable muscle damage [39]. This scope of muscle damage might lead to a process requiring long recovery time [39]. Hence, it follows that BFRRE is capable of inducing similar magnitude of muscle damage as

386 eccentric training, which might have caused a “superdelayed supercompensasation” in the
387 present study.

388 In conclusion, the present study observed no differences in muscle size and maximal strength
389 between a failure and submaximal blood flow restriction resistance exercise protocol after two
390 weeks of training (interspersed by 10 days). However, muscle size increased at all four post
391 tests compared to baseline (except failure group on post 10: CSA of rectus femoris). We
392 observed a delayed increase in maximal strength, where both protocols first increased 17
393 days’ post BFRRE and peaked 24 days’ post BFRRE. Eventually, the present study did not
394 observe any relationship between muscle swelling and muscle size. Nevertheless, acute
395 swelling increased the first day in each training week.

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TABLE AND FIGURE LEGENDS

Table 1. Baseline characteristics for the participating subjects.

The values are presented as mean ± standard deviation (SD) *

Figure 1.

Muscle size

Percent changes in CSA of rectus femoris (A) and thickness in rectus femoris (B) as well as vastus lateralis (C) for the whole study between the failure and submaximal group compared to baseline. Data is presented as mean with 95% confidence intervals. * Significantly different from baseline ($p \leq 0,01$)

Figure 2.

Maximal muscle strength

Percent changes from baseline between groups for all 1RM (A) and MVC (B) measurements. Data is presented as mean with 95 CI. * Significantly different from baseline ($p < 0,05$)

Figure 3.

Acute swelling

Overall and individual changes in acute muscle swelling (percent) for thickness in rectus femoris (A), thickness in vastus lateralis (B) and CSA of rectus femoris (C). Data is presented as mean (average of two acute swelling measurements) with 95% CI. * Significantly different from measurement obtained right before BFRRE ($p < 0,001$)

Figure 4.

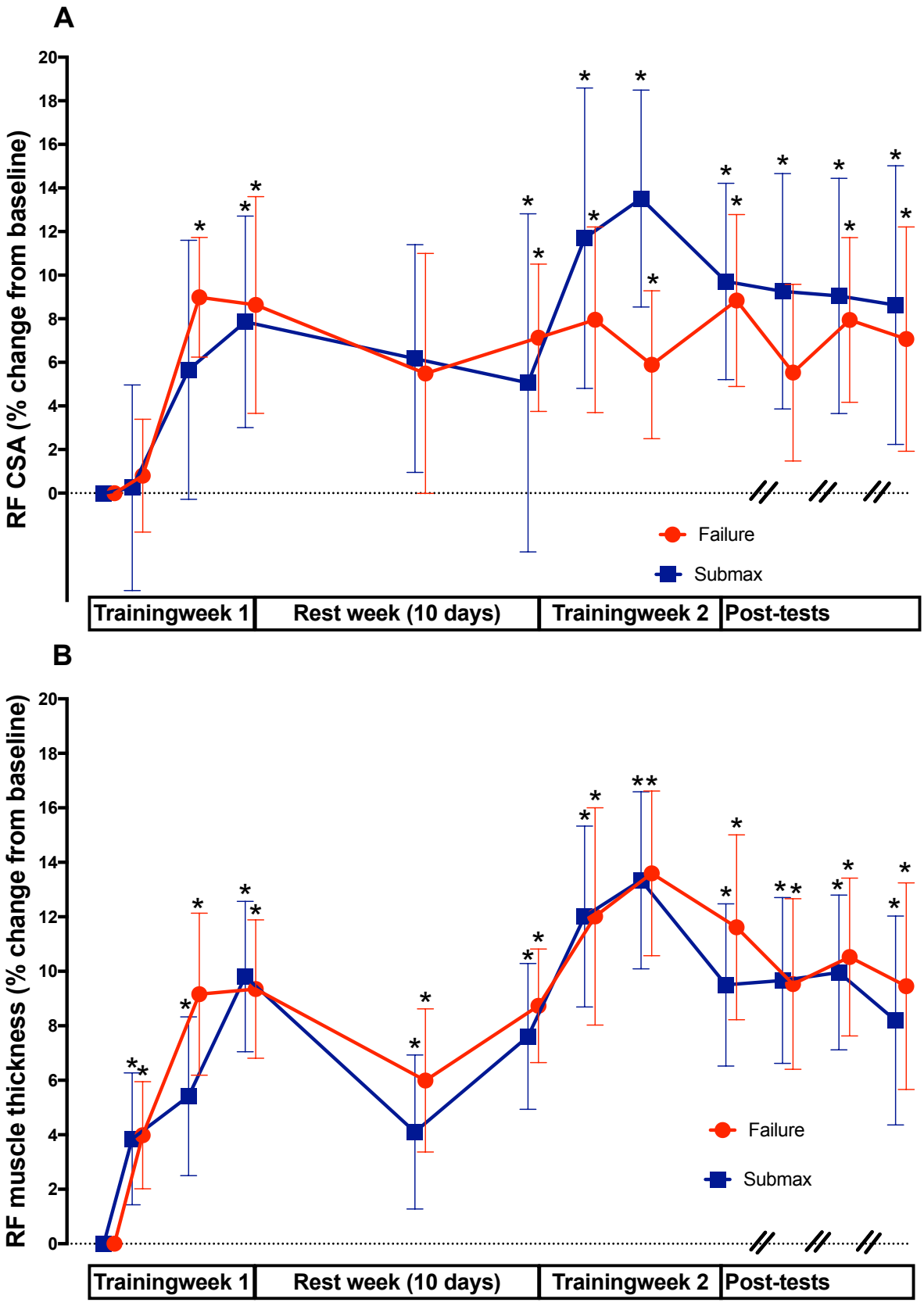
CSA of myofiber

Percent changes from baseline between groups in myofiber 1 (A) and myofiber 2 (B) for the whole study. Data is presented as mean with 95% CI. * Significantly different from baseline

Table 1

	All (n=17)	Failure (n=17)	Submaximal (n=17)
Age	25.0 (5.6)		
Height (cm)	181.7 (11.6)		
Weight (kg)	79.9 (13.2)		
1RM (kg)		74.1 (13.3)	75.8 (15.6)
MVC (nm)		226.7 (39.5)	226.7 (40.9)
CSA of rectus femoris (mm)		7.3 (2.1)	6.8 (1.7)
Thickness of rectus femoris (mm)		18.4 (3.6)	17.9 (2.9)
Thickness of vastus lateralis (mm)		25.6 (3.5)	25.3 (3.7)

Figure 1


A
RF CSA (% change from baseline)
20
18
16
14
12
10
8
6
4
2
0
Failure
Submax
Trainingweek 1
Rest week (10 days)
Trainingweek 2
Post-tests
B
RF muscle thickness (% change from baseline)
20
18
16
14
12
10
8
6
4
2
0
Failure
Submax
Trainingweek 1
Rest week (10 days)
Trainingweek 2
Post-tests

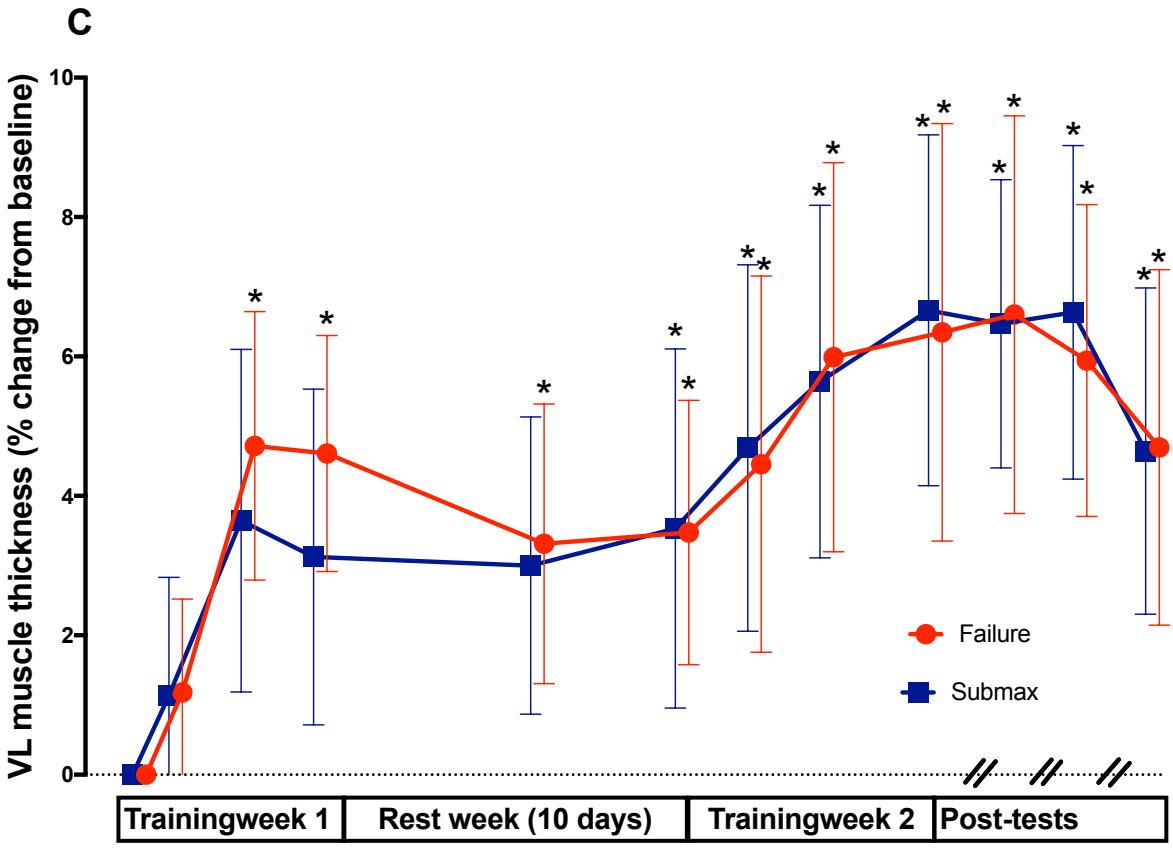

C
VL muscle thickness (% change from baseline)
10
8
6
4
2
0
Failure
Submax
Trainingweek 1
Rest week (10 days)
Trainingweek 2
Post-tests

FIGURE 2

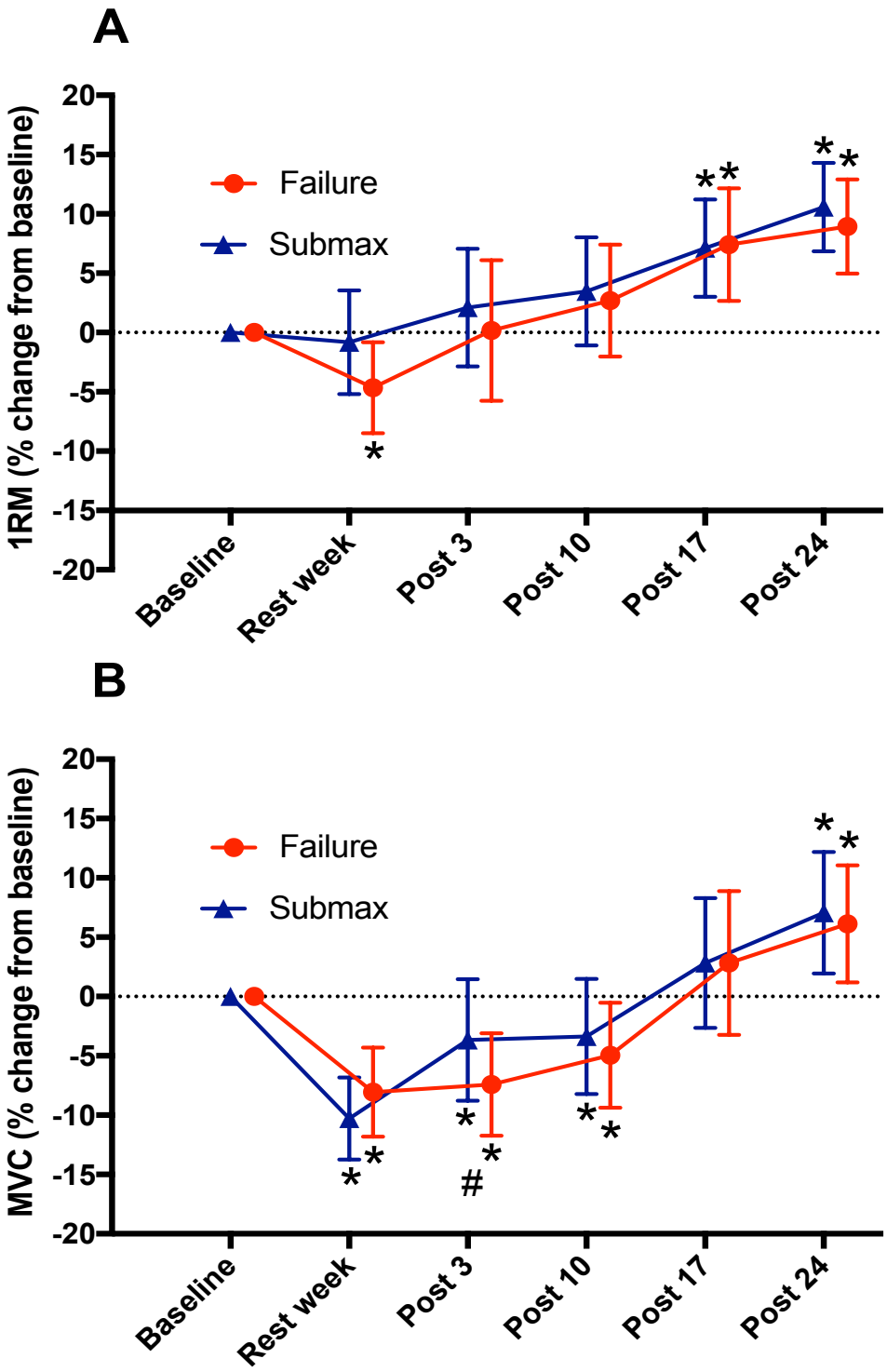

A
1RM (% change from baseline)
20
15
10
5
0
-5
-10
-15
-20
Failure
Submax
Baseline
Rest week
Post 3
Post 10
Post 17
Post 24
B
MVC (% change from baseline)
20
15
10
5
0
-5
-10
-15
-20
Failure
Submax
Baseline
Rest week
Post 3
Post 10
Post 17
Post 24

FIGURE 3

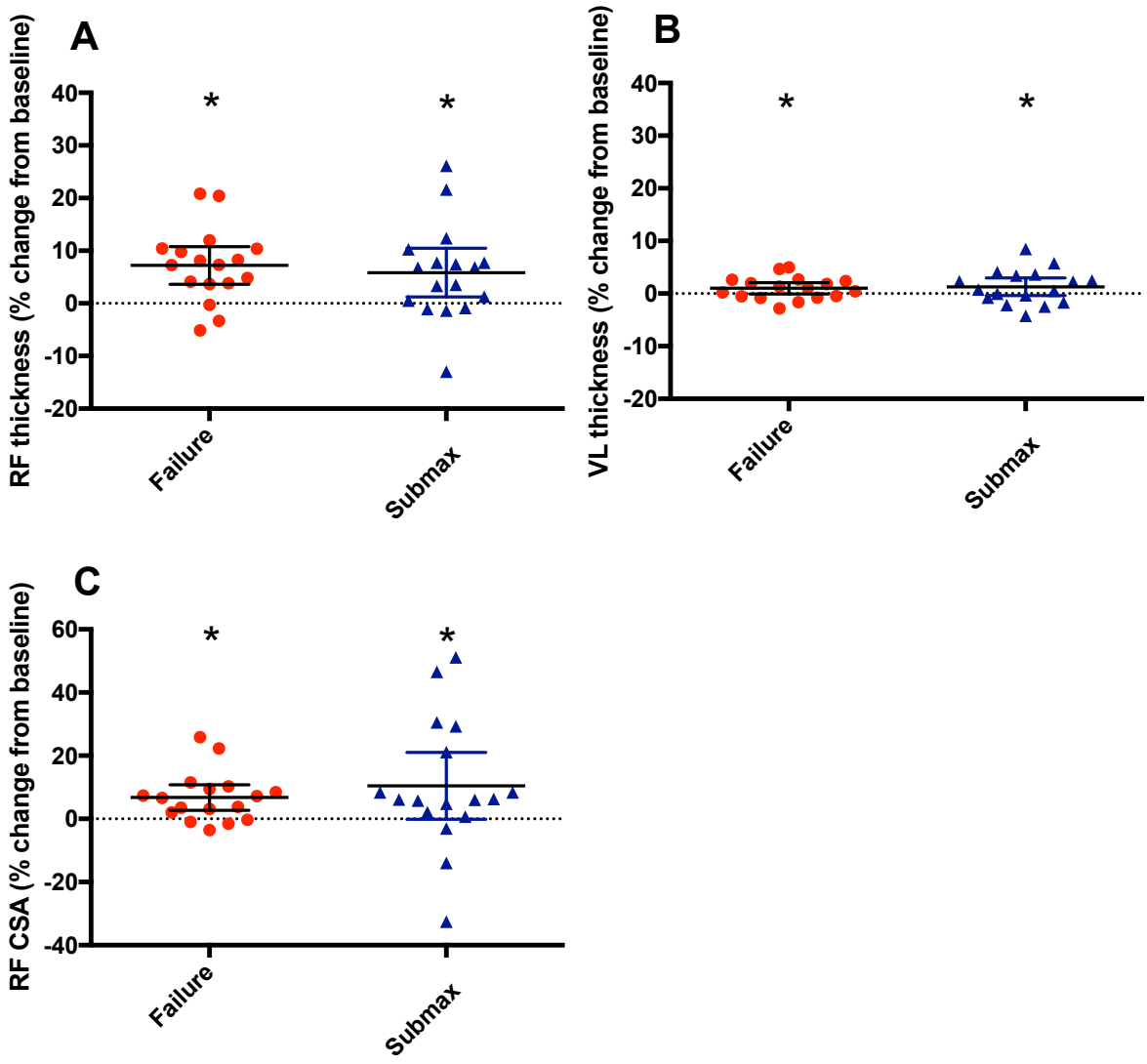
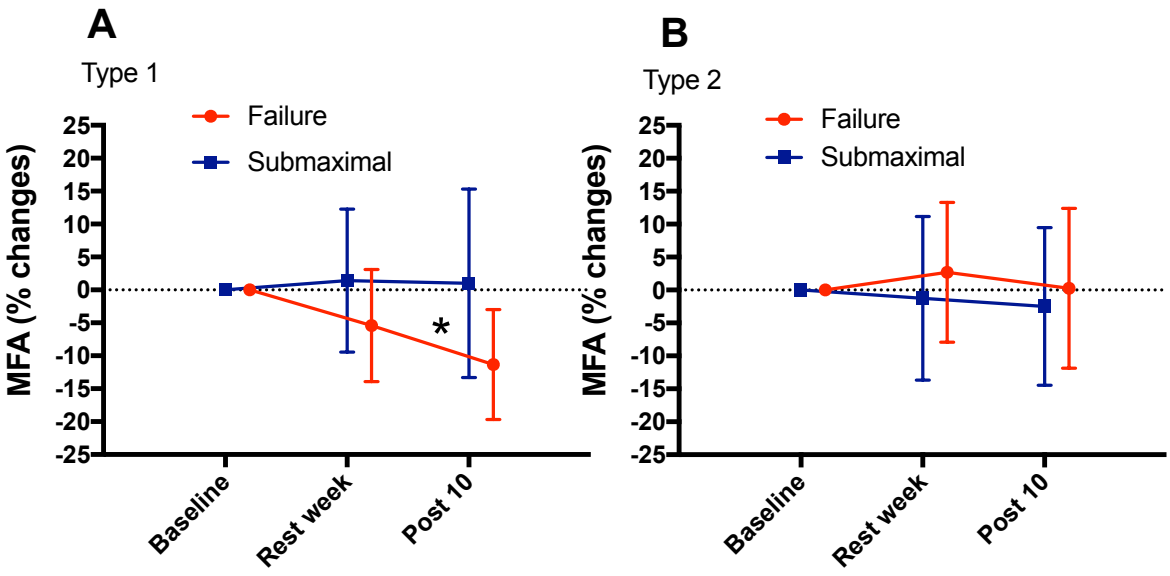

A
RF thickness (% change from baseline)
40
30
20
10
0
-10
-20
*
*
Failure
Submax
B
VL thickness (% change from baseline)
40
30
20
10
0
-10
-20
*
*
Failure
Submax
C
RF CSA (% change from baseline)
60
40
20
0
-20
-40
*
*
Failure
Submax

FIGURE 4


A
Type 1
Failure
Submaximal
MFA (% changes)
25
20
15
10
5
0
-5
-10
-15
-20
-25
*
Baseline
Rest week
Post 10
B
Type 2
Failure
Submaximal
MFA (% changes)
Baseline
Rest week
Post 10

Part 3:

Appendix

Contents:

Joakim Sundnes

University of agder

May, 2016

APPENDIX 1:

Forsøkspersoner søkes

Kom i gang med beintrening!

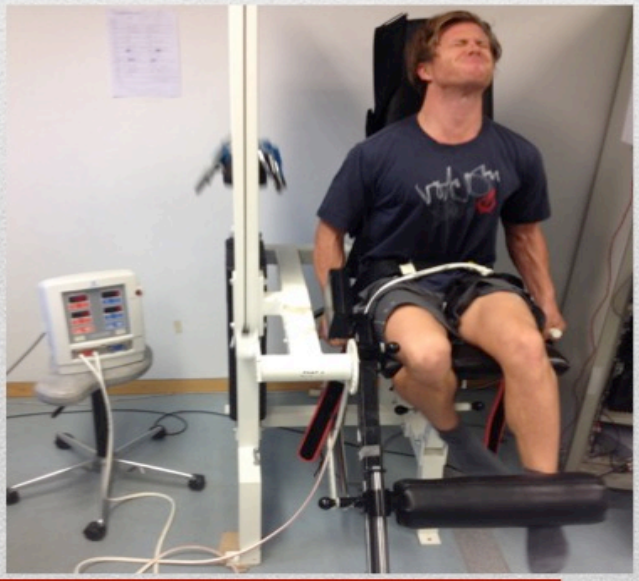
Okklusjonstrening – styrketrening med redusert blodstrøm

Bakgrunn for studien:
Tidligere studier har vist kraftig muskelvekst på få dager selv med relativ lett motstand om blodtilførselen til muskelen reduseres med en trykkmansjett under trening (okklusjonstrening).

Som forsøksperson:
Deltagelse innebærer to treningsperioder med syv økter (15 min per økt) fordelt på fem ukedager, adskilt med ti dagers hvile i mellom. Det vil bli utført en rekke tester under hele forsøket. Blant annet: kroppssammensetning (Inbody), kostholdsoppfølging, muskeltverrsnitt (ultralyd), muskelstyrke og biopsier (muskelfibertype, antall cellekjerner, satellittceller). Som forsøksperson vil du få tilgang til dine resultater, lærer om en ny treningsmetode og et innblikk i forskningsverden.

Kriterier for å være med:
- Mann mellom 18-40 år
- Ingen bruk av kosttilskudd under testperioden.
- Ikke regelmessig styrketrening på bein de siste 6 måneder (>1 økt i uka).

Eventuelle ulemper med å være med:
- Treningen som gjennomføres kan medføre en følelse av sårhet/stølhet i muskulaturen.



Interesserte bes ta kontakt med doktorgradsstipendiat:

Thomas Bjørnsen, 98619299 thomas.bjornsen@uia.no
Eller masterstudent:
Robert Brankovic 97751984 rob.branko@gmail.com

APPENDIX 2:

Informasjonsskriv

Forespørsel om deltakelse som forsøksperson

Styrketrening med redusert blodstrøm

Dette skrivet er til alle potensielle forsøkspersoner. Vi ber om din deltakelse i prosjektet, så fremt du oppfyller kriteriene:

1) Du må være mann i alderen 18 - 40 år.

2) Du skal *ikke* ha drevet regelmessig styrketrening på lårmusklene under de siste 6 måneder (dvs. >1 økt hver uke).

3) Du må være frisk og uten skader i kneleddene eller lårmusklene som gjør at du ikke kan trene i en knestrekk øvelse.

4) Du kan ikke bruke noen form for medikamenter eller benytte deg av kosttilskudd under treningsperioden (proteinpulver, vitaminer, kreatin eller lignende).

5) Du kan ikke delta om du er allergisk mot lokalbedøvelse (tilsvarende det man får hos tannlegen).

Bakgrunn og hensikt med forsøket

Tidligere studier har vist kraftig muskelvekst selv med relativ lett motstand (20-50 % av maksimal styrke) om blodtilførselen til muskelen reduseres med en trykkmansjett under trening («okklusjonstrening»). Det interessante med denne metoden er at muskelveksten synes å være målbare etter bare få dager med trening. I denne studien ønsker vi å sammenligne to forskjellige treningsprotokoller, samt studere denne treningsformen nærmere, hvor vi er spesielt interessert i å avdekke de cellulære mekanismene. En av hoved-mekanismene bak denne treningsformen er tenkt til å være at muskelcellene permanent øker antall cellekjerner (som inneholder arvematerialet); dette gjør at selv om muskelen svinner om man reduserer treningen, vil muskelen raskt gjenvinne størrelsen ved re-trening.

Treningsmetoden med redusert blodstrøm kan ha viktige implikasjoner for en bred målgruppe, fra idrettsutøvere til eldre med kraftig redusert muskelmasse (sarkopeni) og pasienter som skal gjennom en kneoperasjon.

Gjennomføringen av forsøket

Forsøket går ut på at du trener 7 treningsøkter på 5 dager i 2 runder. De to treningsperiodene er avskilt med 10 dager hvile. Treningen består av sittende kneekstensjoner (forsiden av lårene), mens en trykkmansjett er plassert øverst på låret (i lysken).

Du vil bli trene begge bena, men med forskjellige protokoller. Det ene benet vil trene med 4 sett til utmattelse, mens det benet vil trenes sub maksimalt nært utmattelse, tilfeldig valgt bein. Vi ønsker å se hvilke protokoll som er mest effektiv for muskelvekst, maksimal styrke og økning av cellekjerner i muskel.

For at vi skal kunne studere cellulære mekanismer i musklene, må vi ta prøver av musklene dine. Slike muskelprøver (biopsier) vil tas ved tre tidspunkt (se under). Vi vil maksimalt ta 4 prøver fra hvert lår. Blodprøver vil også tappes fra en vene i armen (vanlig blodprøvetakning).

Muskel-styrke og -størrelse vil registreres ved flere tilfeller før, underveis og etter treningsperiodene. Til dette benytter vi styrketester der du tar i alt du kan, og vi bruker ultralyd til å studere muskeltykkelsen. Alt i alt vil du møte i laboratoriet vårt i overkant av 20 ganger i løpet av 1,5 måneder. Treningsøktene er derimot gjennomført på svært kort tid (15 min). Vi gjør individuelle avtaler.

Før forsøket

Du skal møte på Universitetet i Agder (2. etasje Spicheren) 2-3 ganger for tilvenning til tester og treningsøvelser, samt måling av muskelstørrelse med ultralyd. Hver seanse varer i 1-2 timer (se skjema for oppmøter). Tidspunkter avtales individuelt. Du kan ikke drive krevende fysisk aktivitet (trening) i 2 dager før tester og biopsitakning.

Styrketrening med redusert blodstrøm

Du vil gjennomføre 7 treningsøkter på 5 dager under første og tredje uke av forsøksperioden. På mandag, tirsdag og onsdag har du én treningsøkt, mens torsdag og fredag har du en morgen/formiddagsøkt og ettermiddags/kvelds-økt. Treningen vil foregå i styrkelaboratoriet ved Universitetet i Agder, som er lokalisert i andre etasje over Spicheren treningssenter, og du vil få assistanse med trykkmansjetten og gjennomføringen av selve treningen.

Treningsøkten består av 4 serier med 20 % av maksimal motstand til utmattelse på et ben, eller 4 sett med 30-, 15, 15 og 15 repetisjoner på det andre benet, i et kneekstensjonsapparat. Det vil være 30 sekunder pause mellom seriene. Blodstrømmen til arbeidende muskulatur vil være begrenset med ca. 50 % pga. trykkmansjetten.

Første treningsdag vil kreve det lengste oppmøtet. Her blir det tatt diverse tester (styrke, ultralyd, kroppssammensetning, blodprøve, biopsi, elektromyografi,

Du vil på første treningsdag (14- eller 15. september) teste maksimal isometrisk styrke før og etter treningen, samt 3 timer senere. Det vil også tas ultralyd, blodprøve og en muskelprøve før og to timer timer etter trening som nevnt ovenfor. Videre vil en muskelprøve tas på dag 9 (24- eller 25. september) og 29 (12- eller 13. oktober).

Biopsier: Det vil tas 4 biopsier fra hvert lår. Biopsiene tas ut på følgende måte:

- Huden og bindevevet lokalbedøves der prøven skal tas.
- Et snitt på ca. 1 cm gjøres gjennom hud og muskelfascien.
- En nål med diameter på 5-6 mm føres inn (2-3 cm) og 1-3 små biter av muskulaturen, på størrelse med et fyrstikkhode, tas ut.
- Snittet lukkes med tape.

Eventuelle ulemper ved å delta

- Deltakelse i prosjektet vil kreve mye tid og oppmerksomhet i treningsukene. Du må møte ved Universitetet/Spicheren totalt 14-16 dager denne høsten (september – oktober).
- Trening skal gjennomføres vil medføre en viss risiko for muskelskader, og følelse av sårhet/stølhet i muskulaturen vil du oppleve.
- Trening med redusert blodstrøm kan oppleves som meget ubehagelig, men det er ikke knyttet stor risiko til denne typen trening.
- Vevsprøvetakninger (biopsier) medfører en liten infeksjonsfare, og ubehag/smerter kan oppleves under inngrepet. Du kan også oppleve lette til moderate smerter i 1-2 døgn etter inngrepet.
- Du vil få et lite arr etter snittet i huden; arret vil sakte bli mindre tydelig. Enkelte personer vil kunne få en fortykning av huden i arrområdet.
- Blodprøvetakning (veneprøve) medfører en liten infeksjonsfare og det kan oppleves ubehagelig.

Personvern

Vi vil kun lagre informasjon om deg under ditt forsøkspersonnummer. Undervis 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. Representanter fra kontrollmyndigheter i inn- og utland kan få utlevert studieopplysninger og gis innsyn i relevante deler av din journal. Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. Alle som får innsyn i informasjon om deg har taushetsplikt. Innsamlet data vil bli anonymisert etter 15 år (kodelisten destrueres).

Alle prøver vil analyseres ”blindet”, det vil si at forskerne som utfører den enkelte analysen ikke vet hvilken forsøksperson prøven kommer fra.

Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Biobank

Biopsiene og blodprøvene 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 biobanken. Prøvene vil bli lagret til år 2028. Ansvarlig for biobanken er Prof. Truls Raastad ved Seksjon for fysisk prestasjonsevne ved Norges idrettshøgskole. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk.

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

Du som er deltaker i prosjektet er forsikret dersom det skulle oppstå skade eller komplikasjoner som følge av forskningsprosjektet. Universitetet i Agder er en statlig institusjon og er således selvassurandør. Dette innebærer at det er Universitetet i Agder som dekker en eventuell erstatning og ikke et forsikringsselskap.

Finansiering

Prosjektet er finansiert av Universitet i Agder, Norges idrettshøgskole, Olympiatoppen Norge, og Universitet i Gøteborg.

Publisering

Resultatene fra studien vil offentliggjøres i internasjonale, fagfellevurderte, tidsskrift. Du vil få tilsendt artiklene hvis du ønsker det.

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.

Det er frivillig å delta og du kan når som helst trekke deg fra prosjektet uten videre begrunnelse. Alle data vil, som nevnt ovenfor, bli avidentifisert før de blir lagt inn i en database, og senere anonymisert.

Dersom du ønsker flere opplysninger kan du ta kontakt med

Thomas Bjørnsen på tlf: 98619299, eller på mail: thomas.bjornsen@uia.no

Vennlig hilsen

Thomas Bjørnsen (doktorgradsstipendiat)

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)

APPENDIX 3:

Borg CR10 Scale (1982)[12]

0	Nothing at all
0.5	Extremely weak (just noticeable)
1	Very weak
2	Weak (light)
3	Moderate
4	Somewhat strong
5	Strong (heavy)
6	
7	Very strong
8	
9	
10	Extremely strong (almost max)
•	Maximal

Borg CR10 Scale® (2010)[20]

0	Nothing at all	
0.3		
0.5	Extremely weak	Just noticeable
0.7		
1	Very weak	
1.5		
2	Weak	Light
2.5		
3	Moderate	
4		
5	Strong	Heavy
6		
7	Very strong	
8		
9		
10	Extremely strong	"Maximal"
11		
∫		
•	Absolute maximum	Highest possible

APPENDIX 4:

rating	description
6	NO EXERTION AT ALL
7	EXTREMELY LIGHT
8	
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

APPENDIX 5:

Frokost (kcal)	Kcal havregryn	Gram havregryn	Kcal sukker (6 g)	Kcal olje	Gram olje	Antall ts (1 ts=5 g)
400	256	68	24	120	13	3
440	284	76	24	132	15	3
480	312	83	24	144	16	3
520	340	90	24	156	17	3
560	368	98	24	168	19	4
600	396	105	24	180	20	4
640	424	113	24	192	21	4
680	452	120	24	204	23	5
720	480	128	24	216	24	5
760	508	135	24	228	25	5
800	536	143	24	240	27	5
840	564	150	24	252	28	6
880	592	157	24	264	29	6
			6 gram			