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Time course for hypertrophic responses with blood flow restricted resistance exercise

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Abstract

Background: Blood flow restricted resistance exercise (BFRRE) has been shown to increase muscle size rapidly. However, few studies have reported the exact time course for when hypertrophic adaptations with BFRRE occurs. This is challenging because it is difficult to distinguish between the exercise-induced muscle cell swelling associated with BFRRE and permanent functional hypertrophy. BFRRE with high training frequency could be important in settings where individuals need to increase their muscle mass fast, but high forces on the musculo-skeletal system are compromised or even contraindicated. **Objective**: To investigate the time course for increases in muscle thickness of *m. vastus lateralis* (VL) and the cross sectional area (CSA) of *m. rectus femoris* (RF) during two weeks of BFRRE with high training frequency. **Method**: Nine healthy subjects (age 24±2.1) performed a total of 14 bouts of unilateral knee extension (on both legs) at 20% of 1RM. Each bout consisted of 4 sets to failure (a total of +/-80 repetitions). Seven bouts were performed per training week and a 10 day rest period separated the two training weeks. Ultrasound measurements and DOMS-analysis was carried out every day before training bouts. One repetition maximum (1RM) and muscle function-tests were investigated pre, day 1, 9, 15, 22 and 29 day. Both training weeks started with "acute days" (day 1 and 15), consisting of all the aforementioned tests conducted before and after exercise as well muscle biopsy obtained from VL 1 hour after exercise **Results**: Over the entire training period VL muscle thickness increased by 5.7% (right leg) and 6.4% (left leg). RF CSA increased by 7% (right leg) and 8.8% (left leg). A rapid gradual increase was observed in both measures of muscle size during the first weel, followed by a reduction during the rest period, before a similar gradual increase was found in the second training week. The increase in muscle thickness and CSA was stabilized between post-tests on day 22 and 29. Maximal isometric strength (MVC) increased significantly with 17.1%, whereas no significant increase was found in maximal isokinetic torque (+ 2.6%), or 1RM in knee extension (+1.4%). **Conclusion** Our findings indicate that one week of BFRRE is sufficient to increase muscle size. However a large portion of the increases observed in muscle size during the first week was probably a result of exercise-induced cell swelling. A more permanent effect on muscle size was found after the second training week (day 19) and from post-tests on day 22 to day 29, indicated by a plateau observed between these time points. Significant increases was found in MVC, but not in isokinetic strength or 1RM.

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Abbreviations

ACL – Anterior cruciate ligament

BFR – Blood flow restriction

BFRRE – Blood flow restricted resistance exercise

CPK – Creatine phosphokinase

CSA – Cross sectional area

DOMS – Delayed onset muscle soreness

eEF2 - eukaryotic elongation factor 2

EIMD – Exercise induced muscle damage

EMG - Electromypgraphy

ERK1/2 – Extracellular signal regulated kinases 1 & 2

GH – Growth hormone

JNKs – c-Jun N-terminal kinases

LP – Lipid peroxide

MAPKs – Mitogen activated protein kinases

MFA – Myofibre area

mTOR – Mammalian target of rapamycin

mTORC1/2 – mTOR complex 1 & 2

MPS – Muscle protein synthesis

mRNA – messenger ribonucleic acid

MRS - Magnetic Resonance Spectroscopy

MuRF1 – Muscle ring finger protein 1

MVC – Maximal voluntary contractions (usually measured isometrically)

p38MAPK – p38 mitogen-activated protein kinases

p70SK6 – 70-kDa ribosomal protein S6 Kinase

PAX7 – Paired box protein 7

PCr - Phosphocreatine

PKB – Protein kinase B, also known as Akt

REDD1 – Regulated in development and DNA damage responses

RF – Rectus femoris

RM – Repetition maximum, the highest load that can be lifted for a given number of repetitions (1 RM is the highest load that can be lifted once)

ROS – Reactive oxygen species

VI – Vastus intermedius

VL – Vastus lateralis

VM – Vastus medialis

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1.0 Background

The origin of blood flow restricted resistance exercise (BFRRE) stems from the Japanese Professor Yoshiaki Sato who started experimenting with tourniquet cuffs on himself and later on patients during the 1960s (Sato, 2005). He came up with the idea while attending a buddhist memorial where he experienced a numbness in his legs due to his seated position. After some self-massaging on his calf he noticed that the swelling and numb sensation felt similar to the feeling of performing vigorous exercises on the calf muscles. Based on this experience he theorized that the muscle swelling and numbness was caused by, or associated with reduced blood flow to the muscle (Sato, 2004).

There has been a growing interest in BFRRE over the last 10 – 15 years. By now, several studies have shown the efficacy of this training methodology in a wide range of populations including both young and elderly people of both sexes, as well as untrained and also in elite athletes (Abe et al. 2005; Takarada et al. 2002). Several studies have reported increases in muscle size after only 1 - 3 weeks of training (Fujita et al. 2008; Abe et al. 2005; Nielsen et al. 2012). When performing BFRRE, a pressure cuff is a placed around the proximal part of the upper or lower limb performing the exercise. The cuff partially restricts the blood flow to the working muscle so that the muscle is forced to work with a limited amount of oxygen supply.

Research on BFRRE have used loads ranging from 15% of MVC (Kacin et al. 2011) to 80% of 1RM (Laurentino et al. 2008), but normally loads between 20 – 30% of 1RM are used (Fahs et al. 2012). These low loads are traditionally thought not to be an adequate load for achieving muscular hypertrophy (Wernbom et al. 2008; ACSM, 2009). However, BFRRE has been reported to induce muscle strength and hypertrophy to a greater extent than low-load training without BFR (Fujita et al. 2008; Takarada et al. 2004) and to similar extents as heavy load resistance exercise (e.g. Takarada et al. 2000; Clark et al. 2011; Karabulut et al. 2010). Because of the low load, the mechanical stress and subsequent muscle damage is suggested to be low, suggesting that the recovery time needed between training bouts may be short, ensuring the possibility of high training frequencies (Takarada et al. 2000a).

It has been shown that low intensity BFRRE activates many of the same signaling cascades as heavy load resistance training, which leads to increased muscle protein synthesis (Fujita et al. 2007, Fry et al. 2010) or reduced breakdown of muscle proteins (Manini et al. 2011). Researchers has also observed that BFRRE can activate the muscles own stem cells, satellite cells (Nielsen et al 2012). The researchers found that low-load BFRRE induced marked

satellite cell proliferation as well as myonuclei addition in human skeletal muscle. These are important processes for optimal exercise-induced muscle growth in humans (Petrella et al. 2008).

Although the research showing the efficacy of BFRRE is rapidly increasing, few studies have reported the exact time course for when hypertrophic adaptations with BFRRE occurs. This is challenging to establish because of difficulty distinguishing between the exercise-induced muscle cell swelling associated with BFRRE and more permanent functional hypertrophy. Cell swelling is a result of osmotic changes in the skeletal muscle, where lactate accumulation is thought to be of importance (Frigeri et al. 1998; Sjøgard et al. 1985). In order to detect the more functional hypertrophy, this swelling needs to go down to baseline levels. This information could be important for a number of reasons. Firstly, to get a better understanding for the underlying mechanisms of how BFRRE works. But also because this information would be valuable when designing an exercise program in a clinical or rehabilitating setting where high forces on the musculoskeletal system is discouraged.

In order to look closer into the time course of muscle swelling and the concomitant muscle hypertrophy, we designed a high frequency training program consisting of two training periods of 5 days (7 sessions) separated by 10 days of rest in-between. The following research questions were investigated: *What is the time course for increased muscle size with frequent bouts of intensive BFRRE?*
We hypothesized that swelling would occur during the first sessions and gradually increase during the first 5 days. However, because a significant part of the rapid increase in muscle thickness was expected to be caused by swelling, we expected a reduction in muscle thickness to occur during the 10 days of rest. In the second training week we expected a new period of increase in muscle size and thereafter a more permanent increase in muscle thickness and CSA caused by functional hypertrophy. Consequently, we also hypothesized that: *The increased muscle size observed some days after the last training session would be correlated with the increase in muscle strength*. Lastly, because muscle cell swelling is thought to be an anabolic signal, we investigated whether *the acute swelling observed immediately after one session of BFRRE, would be correlated with long term training induced change in VL muscle thickness*.

2.0 Theory

2.1 How does BFR strength training work?

It is generally accepted that high loads, often above 80% of 1RM are the most effective to increase muscle mass and strength (Kraemer et al. 2002). The American College of Sports Medicine (ACSM) defines loads of 40-60% of 1RM as light to moderate and argues that such loads can only be used to increase local muscular endurance (ACSM, 2009). However, during the last decade BFRRE has gained attention in the western part of the world because of its efficacy to promote muscle growth with lower loads than what is generally recommended. Even though the amount of evidence challenging ACSM guidelines regarding training loads (e.g. Takarada et al. 2000) are increasing, mechanisms behind the effects of BFRRE are still not well known. In the next chapters, some of the current theories trying to explain this phenomenon will be discussed.

2.1.1 Motor unit recruitment

BFRRE has been shown to increase both muscular size and strength with much lower loads (20 – 30% of 1 RM) than what is traditionally used (Takarada et al. 2000a). One of the proposed explanations includes an early recruitment of type II fibres (Wernbom et al. 2008). Several researchers have reported that additional recruitment of fast-twitch fibres under the hypoxic conditions created by the partial vascular occlusion is of importance, possibly because of premature muscle fatigue (Takarada et al. 2000a, Fujita et al. 2007). Studies have shown that the integrated EMG values during BFRRE were significantly higher than with work matched free flow exercise (Moritani et al. 1992, Takarada et al. 2000b). This suggests that a greater number of muscle fibres are recruited when blood flow is restricted during exercising with that same low load (Fujita et al. 2007).

The muscle fibre recruitment follows the size principle, meaning that larger motor units containing fast twitch fibres are recruited progressively as the intensity increases (Henneman et al. 1965). However, the size principle is seemingly less significant under conditions with restricted blood flow, where fast-twitch fibres have been seen to be activated at a smaller percentage of the 1RM (Sundberg. 1994, Moritani et al. 1992). When individuals are performing traditional strength exercises without BFR at a low intensity level, the slow-twitch fibres are capable of handling the workload, however, when the blood flow is occluded, limiting the amount of oxygen to the slow-twitch muscle fibre, fast-twitch fibres are recruited early to handle the workload when slow-twich fibres are fatigued. Ingemann-Hansen et al

(1981) found that both slow and fast-twitch fibres showed glycogen depletion when examining muscle biopsies immediately after a session of BFRRE on the knee extensors (workload 14.7 Watt). This would imply that both fiber types were recruited and contributed significantly to the work produced.

2.1.2 Hormonal responses

Several studies have observed acute elevated plasma levels of hormones and local growth factors after a bout of heavy load resistance exercise (Kraemer et al. 1990). It is suggested that the acute hormonal responses occurring post-exercise is more important for hypertrophic adaptations, compared to chronic hormonal changes (Kraemer & Ratamess, 2005). Some of the hormones that increases acutely after resistance exercise includes growth hormone (GH), testosterone, cortisol, and catecholamines (Bosco et al. 2000; Kraemer et al. 1990; McCall et al. 1999).

GH concentrations increases dramatically in response to low load exercise with BFR (Abe et al. 2006; Fujita et al. 2007). Takarada et al (2000a) observed that GH increased to that of 40 µg/L, which was 290 times higher than the resting levels. These increased levels of GH reported by Takarada et al (2000a) are also higher than what Kraemer et al (1990) reported after high load resistance exercise without BFR. Other studies have also shown elevated values in GH following exercise with mild to moderate vascular occlusion (Fujita et al. 2007). However, the evidence showing a link between GH and exercise-induced hypertrophic adaptations are mixed.

Loenneke et al (2011c) argues that the current state of research suggests that the acute and chronic testosterone response to BFRRE seems to be minimal. This is consistent with other studies investigating the relationship between BFRRE and testosterone response (Fujita et al. 2007; Pierce et al. 2006; Abe et al. 2006; Reeves et al. 2006). It should however be noted that the study by Abe et al (2006) used walking as training protocol and the population in Reeves et al (2006) only performed three sets of elbow flexion and plantar flexion. Both Pierce et al (2006) and Fujita et al (2007) performed knee extension but at sub maximal intensities. This could suggest a need for higher training intensity and/or a greater training volume in order to achieve a significant response in testosterone secretion.

Rønnestad et al (2011) reported significantly ($p<0.05$) greater increase in serum testosterone and GH when resistance exercise for the elbow flexors were combined with leg exercises, compared to just arm exercises. The authors suggest that performing leg exercises prior to arm exercises, and thereby increasing the levels of serum testosterone and growth hormone, induced superior strength training adaptations compared to arm training alone. However, testosterone has previously been shown as a non-essential component for achieving muscular hypertrophy (Goldberg, 1975), but because of its importance in general anabolic processes in the muscle mass, changes in testosterone levels might affect our overall muscle mass (Raastad et al. 2010; Bhasin et al 1996/2001).

2.1.3 Signalling pathways involved in BFR induced hypertrophy

The mechanisms underlying BFRRE-induced muscle hypertrophy are not well understood. However, similar concerning traditional heavy load resistance exercise, research on BFRRE suggests that activation of different myogenic pathways stimulates translation initiation (Schoenfeld, 2010; Wernbom, 2011). Some of these pathways include different mitogen activated protein kinases (MAPK), isoforms of AKT (Protein kinase B, abbreviated as PKB) and the mammalian target of rapamycin (mTOR) (Bodine et al. 2001; O'Neil et al. 2009; Wernbom, 2011).

2.1.4 MAPK

MAPKs is a group of proteins that are composed of four signalling modules in skeletal muscle (Kramer et al. 2007). There is a direct correlation between how intense the tension a muscle is subjected to and the activation of certain MAPKs. The activation of these kinases are dependent on both the size of the tension and time under tension (Martineau & Gardiner, 2001). Six different MAPKs have been identified (Wernbom, 2011), but the three that are most associated with exercise-induced hypertrophy (Schoenfeld, 2010), and are also most studied, are ERK1/2, the p38 kinases and c-Jun NH2-terminal kinases (JNK) (Krishna & Narang, 2008).

MAPK-branches are stimulated by cytokines, growth factors, and cellular stress and regulates gene expression and metabolism in response to oxidative, energetic and mechanical stress in the muscle (Kramer et al. 2007; Force et al. 1998; Kyriakis & Avruch, 2001). The JNK seems to be most receptive to mechanical stimuli and damage to muscle fibres, especially seen with eccentric resistance training (Schoenfeld 2010). Fry et al (2010) found that ERK1/2

phosphorylation at site Thr202/Tyr204 increased fourfold at 1 h after low load exercise with BFR and was statistically significant ($P<0.05$) when compared to the work matched free flow exercise group. Recent research by Wernbom et al (2013) found elevated phosphorylation of p38MAPK (site Thr180/Tyr182) at 1-hour post-exercise in the occluded leg, but not in the free-flow leg (30% of 1-RM).

2.1.5 Akt/PKB

Activation of Akt/PKB also occurs with BFRRE (Fujita et al. 2007; Fry et al. 2010). Fujita et al. (2007) found increased phosphorylation of Akt/PKB 3 hours post-exercise. However, phosphorylation of Akt at Ser473 increased in both the BFRRE-group and the controls, with no significant difference between the groups. Nevertheless, the levels of muscle protein synthesis (MPS) differed between groups, possibly questioning the importance of Akt in BFRRE. Fry et al. (2010) found similar results with a significant ($p< 0.05$) increase in Akt phosphorylation in the BFR group 3 hour post-exercise compared with baseline levels.

2.1.6 mTOR

mTOR activation has also been observed following 1 bout of BFRRE (Fujita et al. 2007; Fry et al. 2010). Upstream signalling pathways activates the mTOR and the downstream signalling ultimately affect the protein synthesis (Hay & Sonenberg, 2004). Upstream signalling is believed to take place via several different factors involving growth factors, cytokines, amino acid availability and stretch-activated channels (Goldspink, 2002; Spangenburg, 2009; Hay & Sonenberg, 2004). The downstream signalling is thought to be regulated through the AKT/mTOR pathway, either through direct interaction or by modulating production of phosphatidic acid (Schoenfeld 2010, Hornberger et al. 2006).

Two different mTOR complexes have been identified, mTOR complex 1 and 2 (mTORC1 and mTORC2). It is suggested that mTORC 1 is the most important regulator regarding protein synthesis (Proud et al. 2007) via the downstream effectors such as p70S6K, 4E BP's and eEF2 (Baar et al. 2006; Wernbom, 2011;). Fry et al (2010) showed that the mTORC1 can be stimulated through BFRRE in older men. In their study a significant increase ($P < 0.05$) of phosphorylation of mTOR (site Ser2448) was observed. These elevated levels of phosphorylation remained significant from baseline and 3 hours post exercise in the BFR group ($P < 0.05$). These results are consistent with Wernbom et al (2013) who found an increase in the p-p70S6K (at site Thr389) at 1-h post-exercise in the BFR leg in their study.

As previously shown by Fujita et al (2007) phosphorylation of S6K1 (at site Thr389) was significantly increased at 1 and 3 h after exercise in the BFR group ($P < 0.05$). This suggests that the BFRRE enhanced mTORC1 signalling. MPS was increased with 56% 3 hours post exercise. Fujita et al (2007) also found that the eukaryotic elongation factor 2 (eEF2) was dephosphorylated, meaning less eEF2 inhibition and therefore possibly facilitating translation elongation, which is important for MPS. A recent study by Gundermann et al (2014) found that administration of rapamycin inhibited the mTORC1. The concomitant increase in MPS following BFRRE was also inhibited by rapamycin at post exercise. This suggests that the MPS-response is at least partially dependent on a rapamycin-sensitive pathway.

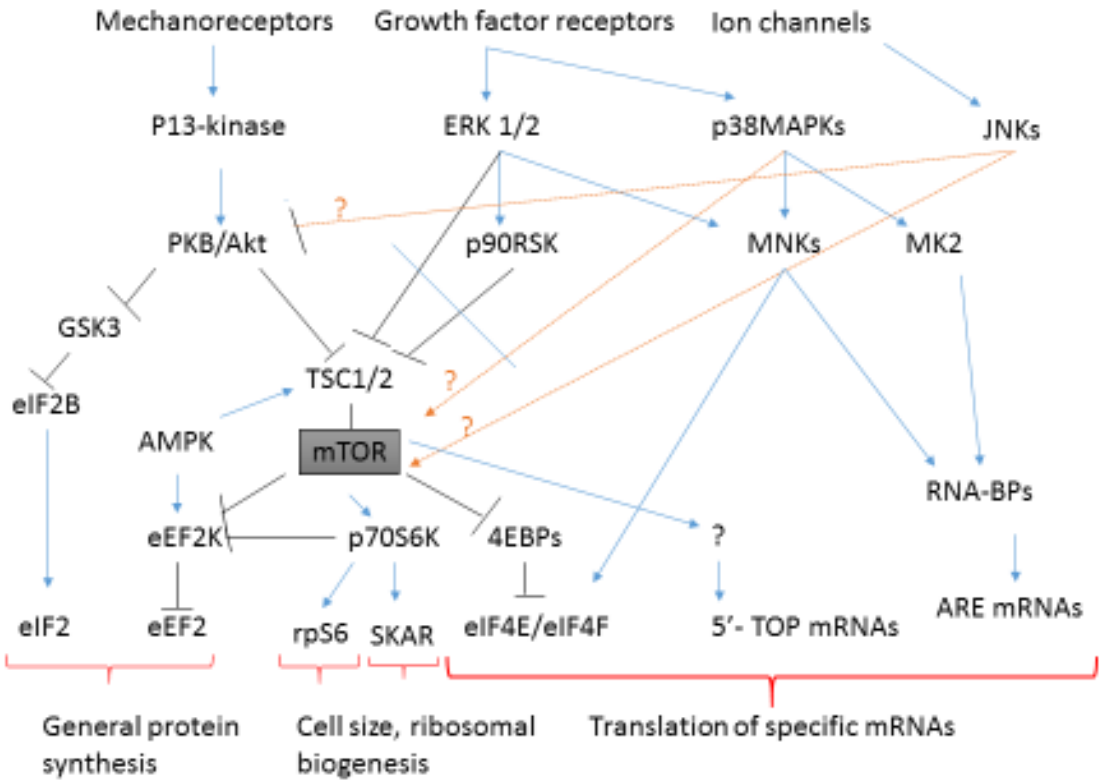

Figure 1. The figure shows some of the signaling pathways regulating translation. The figure was modified from Proud et al (2007) and later by Wernbom (2011). Blue arrows represents activation, orange arrows represents a possible connection and blunt arrows means inhibition. Question marks = uncertain significance and or not fully known mediators.

2.1.7 Other hypertrophic stimuli with BFRRE

Other possible factors explaining how BFRRE leads to muscular hypertrophy could be the reperfusion of blood to the muscle after the exercise bout, when the pressure of the tourniquets cuff is released (Drummond et al. 2008). This muscle hyperemia-reperfusion could possibly promote cell survival and cell growth adaptations within the muscle cell. Post training alterations in hypertrophy-associated genes could also be of importance (Drummond et al. 2008). A recent research report did however suggest that the reactive hyperaemic response due to BFR is not enough to stimulate MPS (Gunderman et al. 2012). However, the researchers were uncertain if they were successful in precisely reproducing the post-BFRRE response (the first 10 minutes).

There are numerous studies identifying several different transiently increases or decreases of mRNA within 24 h after one single bout of traditional heavy load resistance exercise in human skeletal muscle (Bamman et al. 2001; Kostek et al. 2007; Yang et al. 2006/2007). As previously mentioned, BFRRE seems to work in similar ways as traditional strength training. Although there is currently little data identifying changes in mRNA expression, a recent study by Laurentino et al (2012) found similar changes in mRNA expression of selected genes involved in the myostatin signalling, when traditional heavy load resistance exercise was compared to BFRRE. Drummond et al (2008) found that one single bout of BFRRE altered mRNA expression during early post-exercise recovery in human skeletal muscle for several genes associated with muscle growth and protein turnover. Namely REDD1 (↓), HIF-1> (↑), p21(↑), MyoD (↑), MuRF1 (↑), and myostatin (↓).

Reactive Oxygen Species (ROS) has shown to elicit growth in cardiac muscle and smooth muscle (Schoenfeld 2010; Rao & Berk, 1992). A similar hypertrophic effect is also thought to occur in skeletal muscle (Takarada et al. 2000a). It is well established that when performing resistance exercise the contracting muscles acutely produce ROS to an extent which is dependent on the type and intensity of the exercise (Alessio et al. 2000). Research suggests that this acute small to moderate exercise-induced increase of ROS production possibly plays an important role in the regulation of cell signalling pathways that promote gene expression leading to an increased oxidative phenotype of skeletal muscle (Droge, 2002; Jackson, 2008). Hypoxia and subsequent reperfusion, mechanisms occurring when applying BFRRE and releasing cuff pressure, are also associated with ROS production (Takarada et al. 2000a).

Similarly, Moderate production of ROS production has previously been theorized to be a very important factor in promoting tissue growth with BFRRE (Takarada et al 2000c).

Acutely, exercise-induced ROS can function as cellular signalling molecules in the response to exercise stimuli, possibly mediating hypertrophic adaptations (Ji et al. 2006; Jackson et al. 2008). Several reports are consistent with the theory that exercise-induced ROS production have the ability to alter muscle gene expression and thus contribute to adaptations of skeletal muscle mass (Powers et al. 2009).

Although the underlying mechanisms are not well known, some research has shown that ROS may affect muscle hypertrophy via enhanced MAPK signalling (Schoenfeld et al. 2010: Kefaloyianni et al. 2006). Handayaningsih et al (2011) reported that the antioxidant, N-acetylcysteine, inhibited IGF-1 induced signalling and myotube hypertrophy, suggesting that ROS is important in IGF-1 induced hypertrophy. However, Goldfarb et al (2006) reported significantly lower plasma protein carbonyl levels and blood glutathione ratio, which both are markers of ROS-mediated damage, when comparing BFRRE (3 sets at 30% of 1RM) with traditional resistance exercise (3 sets at 70% of 1RM).

Skeletal muscle satellite cells are also thought to be of importance for BFRRE-induced hypertrophy (Drummond et al. 2008; Nielsen et al 2012; Wernbom et al. 2013). Satellite cells are quiescent mononucleated myogenic cells, located between the sarcolemma and basement membrane of terminally differentiated muscle fibres (Morgan et al. 2003; Toigo & Boutellier, 2006). These are normally quiescent in adult muscle, but are able to awaken, and form new muscle fibres in cases of muscular trauma and also in response to mechanical and electrical stimuli (Seale & Rudnicki, 2000; Charge et al. 2004). Petrella et al (2008) showed that individuals who experienced large increases in mean muscle fibre CSA, also displayed a better ability to expand the satellite cell pool, suggesting there is a dose-response relationship. Proliferation of satellite cells has been found to occur within 4 days following a single bout of resistance training (Seynnes et al. 2007). This could possibly be due to what is referred to as the myonuclear domain theory which suggests that a myonucleus of a muscle cell regulates the production of mRNA and protein for a limited part of the cytoplasm in a muscle cell. Therefore, if a muscle fibre were to increase in size, it would also have to add more myonuclei to maintain the myonuclei to cytoplasmic volume ratio (Cheek, 1985; Hawke, 2005). After a bout of resistance training an increased amount of activated satellite cells can

be observed (Kadi et al. 1999, 2004; Crameri et al. 2004). Drummond et al. (2008) reported increases in the satellite cell activity markers p21 and MyoD, as well as decreased myostatin mRNA at 3 h after BFRRE. Nielsen et al (2012) observed proliferation of myogenic stem cells and myonuclei addition in human skeletal muscle in their investigations. Satellite cells per muscle fibre were 3–4 times higher after 5 days (7 exercise-bouts) of BFRRE when compared with baseline values. Wernbom et al. (2013) also found an increased number of satellite cells (33–53%) per muscle fibre one hour post-exercise with BFRRE.

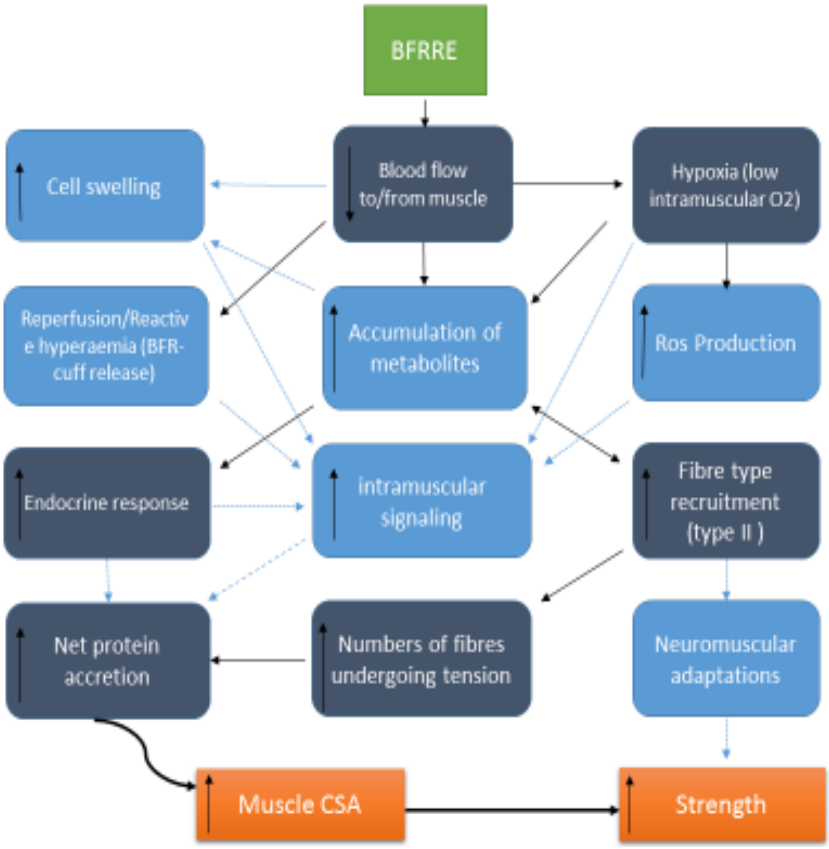

Figure 2. A simplified summary of the proposed mechanisms behind BFRRE and increase in muscular strength and size. The figure was modified by Scott et al (2014). Dark blue boxes represents likely mechanisms, whereas lighter blue boxes represents possible mechanisms in need of more research. Orange boxes represents the outcomes in which the mechanisms are trying to explain. Black arrows indicate a likely link between proposed mechanisms, and dotted blue arrows indicate a possible link requiring further investigation

2.2 Metabolic stress

When exercising with a given load, fatigue develops gradually as the number of repetitions performed increases. This fatigue can be attributed to accumulation of metabolites, or metabolic stress. Metabolic stress is thought to be an important factor for stimulating muscle growth (Raastad et al. 2010). The metabolic stress is a result of an accumulation of different metabolites. This includes lactate, inorganic phosphate (Pi) and hydrogen ions (H+) (Tesch et al. 1986; Suga et al. 2009; Goto et al. 2005), depletion of phosphocreatine (Loenneke et al. 2012a), reduction in ATP and intramuscular pH (Suga et al. 2009; Loenneke et al. 2012a). Acute muscle hypoxia also occurs. This may further increase the metabolic buildup and therefore stimulate hypertrophic adaptations (Schoenfeld, 2010).

There are numerous studies reporting that BFRRE increases blood levels of lactic acid (Takano et al. 2005; Reeves et al. 2006). Lactate level is a good indicator for metabolic demand (Robergs et al. 2004) and several studies have reported increased levels of lactate after a bout of BFRRE (Gentil et al. 2006; Reeves et al. 2006; Takarada et al. 2000a). Lactate levels have also previously been observed to be higher after moderate intensity sets of more repetitions (118 mmol/kg after 3 sets), compared to higher intensity sets of fewer repetitions (90 mmol/kg), where the energy is supplied by the phosphagen system, meaning a lower metabolic build up (MacDougall, 2003). Increased accumulation of metabolites is also associated with secretion of growth hormones (Fujita et al. 2007; Pierce et al. 2006; Reeves et al. 2006). Goto et al (2005) found that resistance exercise with rests between sets elicits a smaller amount of metabolite accumulation within the muscles compared to exercise without rest. This seems to be consistent with previous evidence suggesting that resistance exercise with BFR induce a greater growth hormone response than work match exercise with free flow (Takarada et al. 2000; Viru et al. 1998).

While exercising, the metabolic stress in the muscle cells can be enhanced in several different ways, including increasing the number of repetitions per exercise set, reducing the time of the rest between each exercise set, using slower movement (4-8 seconds) on each repetition or implement drop sets and/or forced repetitions (Raastad et al. 2010). Evidence also suggests that application of BFR during exercise might significantly elevate the metabolic accumulation in the muscle cell (Goto et al. 2005; Viru et al. 1998; Schoenfeld, 2013). Suga et al (2009) found a greater metabolic stress (i.e PCr, Pi and deprotonated phosphate) using a Magnetic Resonance Spectroscopy (MRS), during low load exercise with BFR, compared to

low load exercise without BFR. This is supported by other researchers, suggesting that exercise with vascular occlusion markedly enhances metabolite accumulation within the muscles and concomitant GH secretion (Takarada et al. 2000a; Viru et al. 1998).

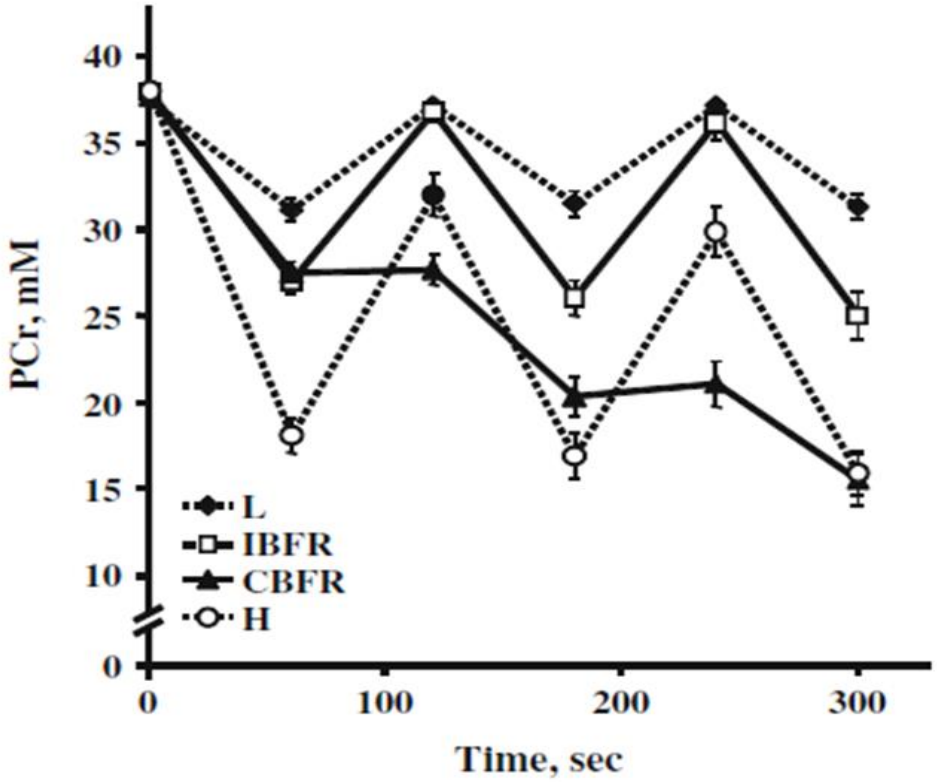

Figure 1. The figure shows the time course in seconds for phosphocreatine (PCr) concentration response during different exercise protocols. Symbols indicate means and error bars indicate standard error (SE). Total PCr change when performing exercise with Continuous BFR was significantly greater than exercise with Intermittent BFR ($P<0.001$) and similar to exercise with high intensity (H) (Suga et al. 2012).

Although not well studied, Schoenfeld (2013) argues that it is possible that hypoxia may have a direct effect on contractile protein accretion and thereby contribute to the hypertrophic response. Kon et al (2012) used a protocol where two groups of participants performed multiple sets of low intensity resistance exercise (50% of 1RM). This combined with a short rest interval in either normoxic air conditions or in conditions with only 13% oxygen, the authors found an increase in the accumulation of metabolites in the participants who exercised in the hypoxic conditions by measuring levels of blood lactate. The researcher's protocol did not involve any form of BFR, but nonetheless it shows the effect of hypoxia combined with resistance training.

Further evidence for the role of metabolic stress in relation to hypertrophy can also be observed in the method frequently used by bodybuilders where typically multiple exercise-

sets of 6 – 12 repetitions are performed with little rest between sets (Lambert & Flynn, 2002). Higher concentrations of GH have been observed in response to 10 repetitions of moderate intensity with only 1 minute rest between sets, compared to 5 repetitions with 3 min rests between sets (Kraemer et al. 1991). This type of training methods reduces the delivery of oxygen to the working muscles because of sustained compression of the arterial and venous flow over an extended period of time (Schoenfeld, 2010/2013).

2.3 Muscle cell swelling

Muscle swelling is a phenomenon seen after resistance exercise and is widely referred to as "muscle pump" (Umbell et al 2009; Yasuda et al 2011; Schoenfeld, 2013). Muscle swelling, or cellular hydration, is thought to serve as a physiological regulator of the cell function (Schoenfeld, 2010). Research has shown that cell swelling occurs in working and not inactive cells (Sjøgaard & Saltin, 1982). However, the mechanisms behind the potential anabolic effect of muscle swelling are not well understood (Ogawa et al. 2012).

Fry et al (2010) found that the acute leg circumference increased by an average of 2.5 ± 0.6 cm immediately after exercise and remained elevated for 30 min after exercise in the BFR-group ($P < 0.05$). The non BFR-control group saw an increase in the leg circumference of 1.3 ± 0.3 cm immediately after exercise. This was significantly lower than that in the BFR group ($P < 0.05$). This suggests that the increase in cell swelling is larger when BFR is applied with low load resistance training, compared to similar loads without any BFR (Fry et al. 2010). Research conducted by Umbel et al (2009) supports this claim. They found more pronounced muscle swelling in the VL of 5.5% at 24 hours post exercise in the BFR leg, compared to 2.2% swelling in the non-BFR leg.

Yasuda et al (2011) observed acute changes in muscle size in the BFR *m.tricep brachii*, following a single session of low load bench press. What was interesting is that similar acute changes were also observed in the non-occluded chest muscle, so that both the chest and tricep increased the muscle CSA post exercise. This suggests that the exercise-induced muscle swelling might be a contributing factor to the anabolic benefit that is associated with this type of training-methodology (Loenneke 2011a; Ogawa et al. 2012).

Resistance exercise alters the intra and extracellular water balance (Sjøgaard, 1986). The hypoxic environment created from the BFRRE produces at least small increases of intracellular metabolites, causing an increased flow of water into the cell to equilibrate the osmotic gradient, transiently increasing the muscle cell volume (Low et al. 1997; Loenneke et

al. 2011c). The extent of this alteration in water balance is heavily dependent on both type of exercise and the intensity. Frigeri et al (1998) argues that the muscle swelling is at a maximum if the exercise is of a type that is more reliant on glycolysis, suggesting that the accumulation of lactate is the most important contributor to osmotic changes in skeletal muscle (Frigeri et al. 1998, see also Lindinger et al 1994). Acute cell swelling, as a response to changes in extracellular osmolarity, has been shown to be an important protein regulator by stimulating protein synthesis and suppressing proteolysis (Berneis et al. 1999, Haussinger et al. 1993).

Although the anabolic mechanisms behind BFRRE are not well understood as previously stated above, there are several proposed theories. Skeletal muscle cells are in the mechanocyte family, meaning that they respond to mechanical stimuli (Goldspink & Booth, 1992). Muscle swelling can lead to increased pressure, or stretch, against the cell membrane/cytoskeleton and this might be perceived as a potential threat to cellular stability. Studies have shown that stretching of muscle fibers can elicit growth in both the CSA and the muscle length (Vandenburgh, 1987). It is possible that when the muscle cell increase in size due to the exercise-induced swelling it triggers a response in form of signaling for reinforcements in order to secure the cellular structure (Lang et al. 2007). Much like with heavy resistance training, the cell swelling could activate stretch sensitive pathways or affect the amino acid transport systems through an integrin-associated volume sensor (Schoenfeld, 2010). Low et al (1997) found that the signaling is dependent on osmossensors within the cell, these sensors then respond with activating anabolic protein-kinase transduction pathways (Lambert et al. 2008).

Other theories suggest that the venous blood pooling observed with BFRRE, could alter the balance of intra and extracellular water, even without the exercise (Loenneke et al. 2011a). Water has the ability to pass across cell membranes by diffusion in mammalian cells. Restriction of blood flow may increase the intracellular to extracellular pressure gradient, causing an increase in the water flux into the cell. This would help to drive that response, which without BFR, would not be sufficient for the sustained and rapid water fluxes needed for active regulation of water homeostasis (Frigeri et al. 2004).

Research by Low et al (1997) found that when the cell is in a hydrated state, it can initiate a process where activation of a protein kinase signaling pathway occurs in the muscle, possibly even mediate autocrine growth factors in signaling the anabolic response to membrane stretch. Muscle cell swelling could also trigger proliferation of satellite cells and facilitate their fusion to hypertrophying myofibres (Schoenfeld, 2013). Thereby enhance potential long term hypertrophic adaptations. Several research reports has also shown that inflammatory reducing administration of Non-steroidal anti-inflammatory drugs (NSAID) diminishes cell swelling, and thus impair the increase in muscle protein synthesis which would normally occur in response to resistance exercise (Palmer, 1990; Rodemann et al. 1982; Trappe et al. 2002).

2.4 Delayed-onset muscle soreness

Theodore Hough is considered the first to report on delayed onset muscle soreness (DOMS) back in in 1902 (Hough, 1902.). He reported, somewhat famously, "*when an untrained muscle makes a series of contractions against a strong spring, a soreness frequently results which cannot be regarded as a phenomenon of pure fatigue*". Still to this day, the mechanisms underlying DOMS have yet to be adequately explained (MacIntyre et al. 1995; Close et al. 2005).

However, DOMS appears to be a result of exercised-induced damage of muscle fibres (Black et al. 2007; Umbell et al. 2009) and it has often been described as a discomfort in skeletal muscle when using or palpating the muscle from 24 – 72 hours after muscular exertion (MacIntyre et al. 1996). The soreness can vary from slight muscle stiffness to severe, debilitating pain that restricts movement (Kenney et al. 2011). Other events often observed with DOMS involves decreases in muscle function, muscle injury and fatigue. Unfamiliar exercise patterns or eccentric movement is particularly associated with more muscle damage compared to concentric training, because such movement create a higher amount of force per recruited fibre (Umbell et al. 2009; Faulkner et al. 1993; Connolly et al. 2003).

One of the proposed theories behind the mechanisms of DOMS, is that an acute inflammation in the muscle after strenuous work might be responsible (MacIntyre et al. 1995/1996). This would involve disruption of collagen fibres, accumulation of neutrophils, increased cytokine release, increased levels of macrophages and monocytes, intramuscular degradation, increased vasodilatation and permeability (Jones et al. 1986; MacIntyre et al. 1995; Stauber et al. 1990). However, this is not well known and more research is needed.

Another proposed theory trying to explain the phenomenon of DOMS involves free radicals (Close et al. 2005). According to Close et al (2005), since eccentric training creates reactive oxygen species (ROS) and such movement pattern is also associated with DOMS, it is possible that ROS is an importance mechanism causing DOMS (Close et al. 2005; see also Maughan et al. 1989). However, the authors points out that there are still relatively few studies that have investigated the relationship between ROS and DOMS, compared to the more frequently studied relationship between exercise-induced muscle damage and ROS (Close et al. 2005). Research either supporting or refuting the potential link between ROS and DOMS seems to be 3 studies suggesting there is a relationship (Kaminski and Boal, 1992; Maughan et al. 1989; Thompson et al. 2001) and 4 studies suggesting there is none (Close et al. 2004/b; Thompson et al. 2001b; Lee et al. 2002).

DOMS has also been reported after BFRRE (Wernbom et al. 2006). Interestingly, in a study conducted by Umbell et al (2009), 15 subjects performed three sets of unilateral BFRRE at 35% of MVC with one limb performing only the concentric part and the contralateral leg performing only the eccentric part. DOMS was assessed with a resting soreness scale (0 - 10) and algometry (pain pressure threshold, abbreviated PPT) before exercise and 24, 48 and 96 h post-exercise. At 24 h post-exercise, the concentric BFR exercise resulted in more resting soreness than the eccentric BFR exercise (3.0±0.5 vs.1.6±0.4), and a greater decrease in MVC (9.8±2.7% decrease vs 3.4±2.5% decrease) ($p < 0.05$). These findings contradict what is traditionally stated in the literature and the reason for it is so far unclear. However, muscle soreness observed after strenuous eccentric resistance training is often attributed to mechanical stress, which may be less significant with the low training loads often used with BFRRE (Hackney et al. 2012).

Wernbom et al (2006) found that four sets of exercise with BFR induced DOMS in the region of 5.5 in a 0 – 10 point scale. These data suggest that knee extension exercise with BFR induces moderate to severe DOMS and that BFRRE elicits muscle damage under atypical conditions with low-tension concentric contractions, consistent with other research reported by the author (Wernbom et al. 2009). A recent study found significant decrements in MVC and DOMS in both the test subjects BFR and non-BFR leg (Wernbom et al. 2012). There was however some differences between the free-flow and the BFR-leg. The leg that exercised with vascular occlusion displayed higher levels of DOMS and an increased occurrence of muscle

fibres with elevated tetranectin, a plasma protein used as a marker for sarcolemmal permeability. These levels were significantly increased from 9% pre exercise to 27 – 38% at 1, 24 and 48 h post-exercise in the occluded leg. The changes in the free-flow leg was significant only at 24 h (19%).

2.5 Time course for increases in muscle strength and mass with BFRRE

With traditional strength training, we can often observe an early increase in muscle strength shortly after the onset of a training regime. These early improvements in strength are often attributed to neural factors (Hakkinen et al. 1996; Sale, 1998). According to Moritani et al (1979) the initial increase in muscle strength might be due to enhanced ability to activate already existing muscle mass. Neurological adaptations are typically seen as enhanced EMG activity in the working muscle during a maximal contraction (Hakkinen et al. 1998; Narici et al. 1989). Initially in this period the increase in muscle strength, when measured with a 1 RM-test, appears to be 1% per bout, compared to increases in cross sectional area of the working muscle which has been observed to be 0.1 – 0.5% per bout (Raastad et al. 2010).

There are individual differences in the time course for increases in muscle strength and mass. Generally, individuals with little to no previous exercise history will experience larger and more rapid increases, when compared to individuals who are already fit and are exercising regularly. There are also differences between body parts. A review by Wernbom et al (2007) suggests that the strength training induced rate of gain of the CSA in *m.bicep brachii* were 0.20% per day, while in comparison the rate of gain in the quadriceps were 0.11% per day. Interestingly, increases in quadriceps CSA have been observed to be 0.5 – 0.55 % per day with intensive BFRRE (Fujita et al. 2008; Abe et al. 2005a). Research on BFRRE with less frequent bouts (2/week) have reported gains in the quadriceps CSA of 0.18 – 0.22% per day (Takarada et al 2002/2004) Resistance training in the upper body is considered to elicit larger hypertrophic gains compared to the lower body (Abe et al. 2000), as suggested by Wernbom et al (2007). This could possibly be attributed to a more frequent use of the lower body extremities in daily living activities (Raastad et al. 2010).

Researchers suggests that it takes in the region of 8 – 12 weeks of resistance exercise before muscle hypertrophy occurs (Hakkinen et al. 1998; Takarada et al. 2002; Hubal et al. 2005). However, since the cellular responses to the strength training have been observed to occur acutely after exercise, this may be heavily dependent on the accuracy and sensitivity of the method used to measure the increases (MacDougall 2003; Seynnes et al. 2007). This could

suggest that the instruments used to measure hypertrophic adaptations are better to use when assessing accumulated muscle mass after the time period mentioned, rather than measure progression through a shorter period with a more frequent time interval.

BFRRE have been shown to increase muscle cross sectional area (Abe et al. 2005b; Madarame et al. 2008; Takarada et al. 2000), maximal strength assesd as one repetition maximum (Abe 2005; Madarame 2008), isometric strength (Moore, 2004; Takarada et al. 2004), and isokinetic strength (Burgomaster et al. 2003; Sumide et al. 2009). However, the time course for these adaptations varies a lot between different studies. As already discussed, one of the benefits with BFRRE is the low mechanical tension, which causes little no to muscle damage (Wernbom et al. 2007). Therefore, it is a training method that can be used with high frequency. This option for training frequencies is something that is less feasible with traditional strength training and it is something that separates the studies on exercise with BFR and traditional resistance exercise.

Seemingly, there are few long-term studies on BFRRE. The two longest running for 16 weeks (Ohta et al. 2003; Takarada et al. 2000b). Studies running for 7 weeks and more typically tend to use fewer bouts per week (2 – 4) (Abe et a. 2010; Ozaki et al. 2011; Madarame et al. 2008), than studies running for shorter periods, i.e <4 weeks (4 – 12 bouts per week) (Nielsen et al. 2012; Abe et al. 2005/b). This suggests that the time course for BFRRE-induced hypertrophic adaptations could depend on the frequencies of the bouts.

In a meta-analysis on BFRRE, the authors point out that muscular hypertrophy can be observed in less than 4 weeks (Loenneke et al 2011a). The meta-analysis however suggest that muscular strength seems to increases at a slower rate when compared to muscular size. Interestingly, this is the opposite with traditional resistance exercise where individuals seemingly can increase in strength from bout to bout in the initial onset of a strength training regime (Wernbom et al. 2007). Interestingly, this might suggest that the majority of the initial increase in muscle size after the onset of a BFRRE-regime is not functional hypertrophy, but muscle cell swelling.

Madarame et al (2008) randomly assigned 15 untrained men into an BFR-group (N = 8) and a control group (N = 7). The BFR-group performed leg exercise with BFR (3 sets, 15–30 repetitions with 30% of 1RM), whereas the control group performed the same leg exercise

without BFR. Bouts were performed 2 times per week for 10 weeks. The researchers found that the cross sectional area and isometric torque of thigh muscles increased significantly in the occlusion group (pre 82.8±0.6, post 86.4±9.2), whereas no significant changes were found in the control group (pre 89.4±11.4, post 88.2±11.9).

Takarada et al (2002) investigated the effect of bilateral BFRRE twice a week for eight weeks on the knee extensors in (n= 17) elite rugby players. Subjects performed exercise with 50% of 1RM and a pressure of 200 mmHg. Both groups trained in an isotonic leg extension machine and the group with BFR (n = 6) showed a significantly increased CSA ($p<0.01$) of the knee extensors. Pre and post-test MR-analysis done only in the BFR-group showed an increase of about 15% of the CSA in the knee extensors after 8 weeks.

Abe et al (2005) conducted a study running for 2 weeks where the participants performed 2 BFR training bouts per day (20% of 1RM), six days a week with 3 sets of squat and leg curl and 30 seconds rest between sets (Figure 4). The researchers found increases in 1RM strength (17% for squat, 23% for leg curl), and increases in muscle volume in the quadriceps (7.7%), bicep femoris (10.1%), and gluteus maximus (9.1%). This study used a lower load than the previous one by Takarada et al (2002), but with use of much more frequent bouts.

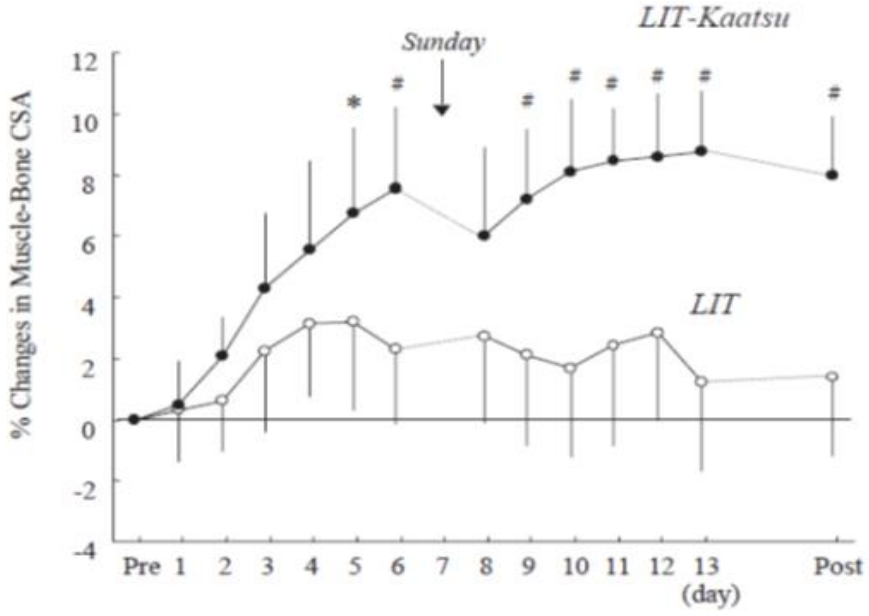

Figure 2. The figure shows the daily % of change in mid-thigh muscle-bone CSA between low intensity resistance exercise with (black symbols, LIT-Kaatsu) and without (white symbols, LIT) BFR. Values are means ± SD. * Indicate $p<0.05$ and # indicate $p<0.01$ between LIT- kaatsu and LIT (Abe et al. 2005a).

Nielsen et al (2012) conducted a study running over 19 days consisting of 23 BFRRE-bouts for the knee extensors in 20 healthy male subjects. The training group (n= 10) used 20% of 1RM for four unilateral sets, with a 100 mmHg pressure cuff (15 cm width) to concentric failure. Significant increases were observed in maximal isometric knee extensor strength increased by 7.1% (post 5) and 10.6% (post 12), also increases in type I and II myofibre area (MFA) were seen of 38% (mid 8), 35-37% (post 3) and 31-32% post 10. The number of satellite cells per myofibre increased with BFRRE, the same did myonuclei per myofibre.

One particular interesting study conducted by Abe et al (2005b) used a design where college track and field athletes trained 14 bouts in 8 days with 20% of 1RM. 15 athletes was divided into one BFR-group (n = 9) and one control group (n = 6). The muscle-bone CSA increased 4.5% ($p<0.01$) in the BFR-group and was unchanged in the control group. Quadriceps and hamstrings mid-thigh muscle thickness significantly increased by 5.9% and 4.5%, respectively, in the BFR-group but did not change in the control group. Leg press strength increased (9.6%, $p<0.01$) in the BFR-group but not (4.8%, $p>0.05$) in the control group.

These studies support the theory that increases in muscle size and strength with BFRRE are dependent on the frequency of the bouts, rather than training background, gender or other variables. However, because hypertrophic responses with BFRRE occurs rapidly, it can be difficult to differentiate between functional hypertrophy and muscle cell swelling due to the exercise itself. Looking at the results from Abe et al (2005) (Figure 4), the figure shows how the percentage of muscle CSA increased with 7% from baseline to the end of the first of two training weeks. After resting on sunday in the first week, the percentage of CSA was down to 6%. Similar reduction was observed from the last test day to the post testing.

2.7 Is blood flow restricted training safe?

By now several studies have demonstrated the potential benefit of a BFRRE-regime (Takarada et al. 2000a; Abe et al. 2005; Fujita et al. 2007). Fewer studies have assessed the safety of such training methodology (Clark et al. 2011). This is important for several different reasons. One obvious reason is the potential harm human tissue is susceptible to if oxygen supply is totally cut off (Margovsky et al. 1997), making BFRRE a somewhat controversial training method. Secondly, safety needs to be assessed if this training methodology is going to be adopted and accepted in clinical settings throughout western countries, as it is in the eastern part of the world.

In 2006 a questionnaire survey was conducted throughout Japan by a research team (Nakajima et al. 2006). Out of 195 facilities, 105 then currently using BFRRE replied. Based on the survey 12642 people between the age of 20 - 80 had used BFRRE. The incidents of side or adverse effects were venous thrombosis (0.055%), pulmonary embolism (0.008%) and rhabdomyolysis (0.008 %) and deterioration of ischemic heart disease (0.016%). The main side effects however included petechial hemorrhage beneath the skin (which disappeared as training progressed) (13.1%), numbness (1.297%) dizziness (0.277%). and chills (0.127%). Nakajima et al reported to have conducted exercise with BFR for around 700 persons yearly since 2007, mainly on patients with a cardiovascular problem, and they reported that they had never experienced any side effects or problems (Nakajima et al. 2011). Both reports argue that BFRRE is safe (Nakajima et al. 2006/2011)

Few studies have however investigated the safety of BFRRE specifically (Takano et al. 2005). If safety is mentioned it is usually mentioned as a sentence in the conclusion stating that BFR was an effective method for the study population, but also that more studies are needed to evaluate the general safety (Takarada et al. 2000/2000b).

Clark et al investigated the relative safety of 4 weeks of BFRRE (Clark et al. 2011). The study population consisted of young healthy men. The authors concluded that no changes were observed in blood levels of fibrinogen, D-dimer or hsCRP ($P>0.05$). These findings suggest that the BFRRE-protocol increased the strength without altering nerve or vascular function, and that a single bout of BFRRE increases fibrinolytic activity, which may restrict thrombus formation (Nakajima et al. 2007), without altering selected markers of coagulation or inflammation in healthy individuals. However, Iversen & Røstad (2010) report a case on a 31 year old ice hockey player who performed BFRRE to fight persisting quadriceps atrophy and weakness, 11 months after a knee articular cartilage resection and microfracture. The authors remained unsure of why the subject responded the way he did. It should however be noted that upon recovery the subject performed one exercise-set more than what was initially planned, possibly enough to induce rhabdomyolysis.

Another concern which might arise in regard to this type of training is its effect on blood pressure. Renzi et al (2010) found that heart rate increased more when BFR was applied during walking, compared to walking without BFR. Consequently, an increase in double product, which is an index of myocardial oxygen demand, was observed to be more than

threefold higher in the BFR group. The study concluded that BFR of limbs during walking exercise may have to be cautiously used in those with cardiovascular conditions. Nakajima et al (2006/2007) points out that increase in catecholamines, which increases the blood pressure and heart beats, has been observed to be slightly more elevated during BFRRE, compared to traditional strength training without BFR (Takano et al. 2005; Lida et al. 2007; Nakajima et al. 2011).

Restricting blood flow to the working muscles is arguably not without any risk. However, one can make the case that no domains or types of physical activity are without any risk of harm. Based on the current amount of research done during the last 10 – 15 years, and its results in a wide range of different populations with different training backgrounds, BFRRE is a safe training methodology who is suitable for all.

3.0 Methods

This study was based on and part of a larger study called *Occlusion 3*, which took place in the Norwegian School of Sports Science from September to November of 2013.

3.0.1 Subjects

Thirteen young men (n = 9) and women (n = 4) volunteered to participate in the study. During the study course 3 subjects dropped out due pain from biopsy, severe DOMS and difficulty combining study requirements and school lectures. One subject was excluded due to only exercising one leg.

A total of 9 healthy subjects (table 1) completed the intervention and all tests. All subjects were physically active (e.g., cross country skiing, handball, football), however none of them participated in any systematic strength training (≤ 1 bout per 2 week) within the last year. Participants was recruited from the Norwegian School of Sports Science and other local universities by use of social media (student mail, facebook groups), oral information prior to lectures, information stands and placing posters in strategic places (super market, school cafeteria). All subjects were screened for the following exclusion criteria's; allergy to local anaesthesia, any injury preventing the exercises or medication and/or supplementation that could affect the results. All participants received oral and written information of the procedures, risks, and benefits, and signed a written informed consent document before participation. The local ethics committee approved the study.

Table 1. Characteristics of study participants.

	Men	Woman
n	6	3
Age	25 (2.0)	23 (1.7)
Height (cm)	182 (7.4)	173 (4.7)
Weight (kg)	87 (10.3)	67 (2.0)

Values are means (SD); n, no. of study participants.

3.1 Study design

3.1.1 Pre testing

A pilot study was conducted one month prior to baseline. This was carried out in order to train test-personnel, detect and correct potential methodological flaws and ensure reliability and validity of the test-protocols.

Subjects performed familiarization and pre-tests for the strength tests and pre-biopsies one week prior to the start of the intervention in order to determine and adjust individual settings for the test-protocols (later described in detail), however they did not try the BFRRE-protocol. Mean values between the familiarization and pre-tests was set as baseline values for all tests.

3.1.2 General study description

The subjects underwent a one month supervised BFRRE-intervention, consisting of two training weeks of a total of 14 unilateral training sessions per leg, 7 sessions per training week. Training was conducted once per day (mon–wed) and twice per day (thu–fri) during both weeks. A 10-day rest period separated the two training weeks. Successive training sessions (thu-fri) was separated by at least 4 h (Abe et al. 2006).

The timeline for study procedures is shown in Figure 5. The first day of each training week (day 1 and 15) was called acute days. On these days, subjects performed the BFRRE-bout and additional testing consisting of 2 ultrasound measurements, 2 muscle function tests, 3 blood samples and 1 biopsy. Participants started this day with a 30-minute time interval, after eating a standardized breakfast. On the ordinary training days (day 2 - 5 and 16 - 19), DOMS-analysis and ultrasound measurements was obtained, before subjects performed the training-protocol. On day 9, in addition to the tests previously mentioned, we measured muscle function and maximal strength. All tests performed on day 9 were assessed again on day 22 and 29 (3 and 10 days after the last training session).

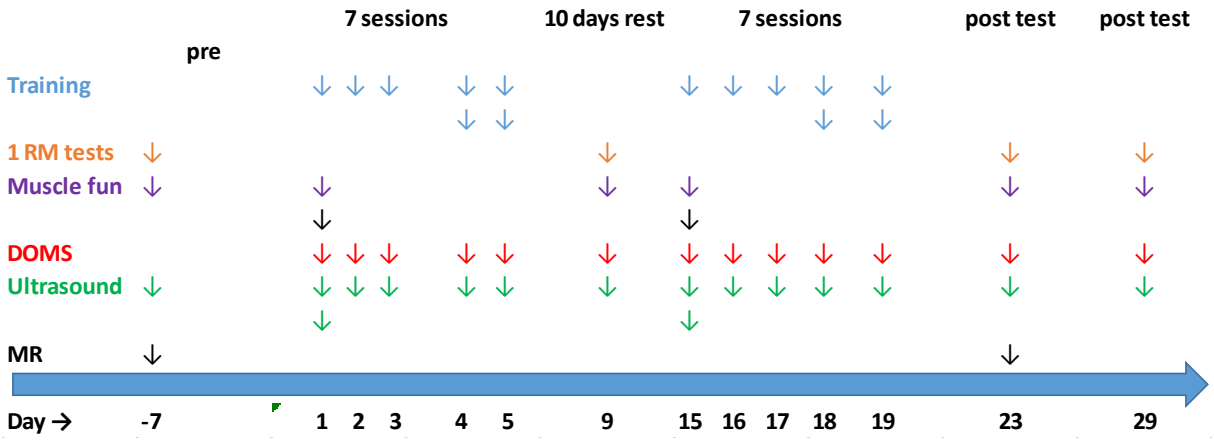

Figure 5. Complete timeline for the tests-procedures during the intervention.

3.2 Test protocols

3.2.1 Training protocol

A pneumatic cuff (150 mm width, 9-7350-003, Delfi Medical, Vancouver BC, Canada) was placed around the proximal part of the thigh (as seen in Figure 6) before exercise start. Subjects were instructed to pull the cuff up towards the groin area. The right leg was exercised before the left, with a 5-minute break in between legs. Repetitions were set at 1 sec concentric and 1 sec eccentric, controlled by a metronome (Korg Metronome MA-30. China). The pneumatic cuff was plugged into a computerized tornique system (Zimmer A.T.S. 2000, USA) ensuring stable pressure. Manuel hand pumps (VMB Medizintechik Gmbh. Germany) was on standby in case of system failure. Warm up consisted of 15 repetitions of 5 kg resistance performed with the pneumatic cuff remaining non-inflated. Following warm up the cuff was inflated (100 mmHg for men and 90 mmHg for women) and the subjects performed four sets of unilateral dynamic knee extensions (in a Gym 2000 Gym Equipment) at 20% of 1RM to concentric failure. Each set was separated by 30 seconds of rest. Blood flow restriction was maintained during the entire training session, including rest periods, and was released immediately upon completion of the fourth set. Strong verbal encouragement was given for all subjects during every set.

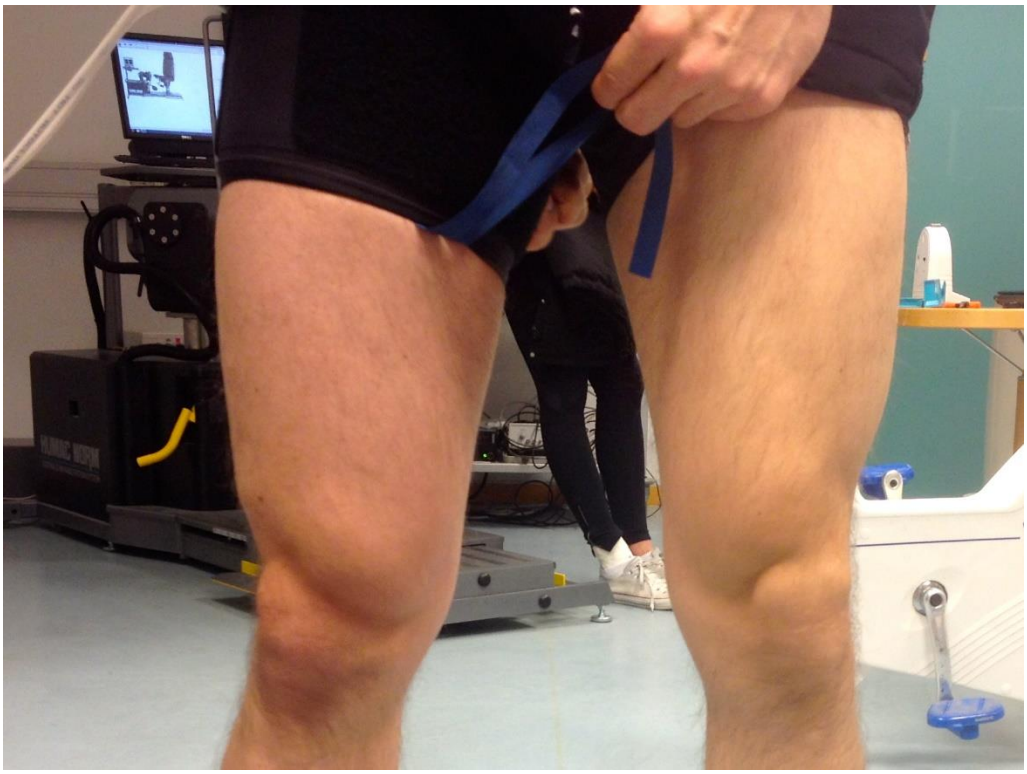

Figure 6. The pneumatic cuff placed on the proximal part of the thigh. The pictures was taken immediately after completion of a training bout as indicated by the redness and swelling seen on the right leg

3.2.2 Muscle function

Muscle function was examined by testing maximal isokinetic torque and maximal isometric (MVC) strength in a strength dynamometer (Humac 2009nomr csmi. Testing and Rehabilitation System. USA). The isokinetic testing of the knee extensors was assessed by subjects performing 3 isokinetic repetitions at 60 degrees/second with maximal force in the concentric phase and no force in the eccentric phase. The highest torque obtained from the three trials was selected for further analyses (peak torque). The MVC-tests was conducted at 90, 80 and 70 degrees (0 degrees = full extension), with two repetitions performed at each angle and 60 seconds rest in between repetitions. The trial with the highest torque was selected for further analysis. Test subjects were strapped to the dynamometer chair with two belts crossing over their chest. Hands were placed on these belts. Subjects were instructed to sit in an upright position with their back straight and their legs firmly on the lever of the machine. The thigh was secured to the chair with a strap. The lever arm was placed right above the ankle joint and fastened with a foot-strap. The lateral epicondyle of the knee was aligned with the rotational axis of the dynamometer. This position was maintained throughout all the tests. General warm up consisted of 5 minute cycling with a standardised watt load for each test-subject. Specific warm up for the isokinetic testing consisted of 4 repetitions at 60 degrees, where the resistance increased gradually. No specific warm up was done before the

isometric tests. Subjects were instructed to contract as hard as possible and strong verbal encouragement was given during all tests. The same test instructor performed all tests

3.2.3 1RM-test

1 RM-tests were conducted in a knee extension machine (Gym 2000 Gym Equipment). Subjects were secured to the chair by a seat belt around the waist and their hands placed on handles on the side of the chair. The lateral epicondyle of the knee was aligned with the rotational axis of the machine. The foot pedal was placed just above the ankle joint. After warm up, single max repetitions with increasingly heavy load were performed until the load that induced concentric failure was found. The lift was counted if the knee joint reached an angle of 10 degrees or lower (0 degrees = full extension). In order to control this, marks were drawn at the machine, so to indicate where the weight-plates needed to reach for the rep to count. Specific warm up consisted of sets with 10 repetitions (50% of 1RM), 6 repetitions (70% of 1RM) and 3 repetitions (80% of 1RM). The right leg performed the exercise before the left leg at all tests. Strong verbal encouragement was given for all trials. 2 – 3 minutes was given before each max try in order to ensure sufficient recovery (Abe et al. 2000).

3.2.4 Ultrasound

Ultrasound-measurements was conducted with a Philips HD11XE Ultrasound system. The images obtained was analysed blindly in a random order with the free software imageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The muscle thickness of the VL and the CSA of the RF was measured.

Test subjects were instructed to lie supine with their heels up on the bench relaxing their legs. The ultrasound machine was used to locate the greater trochanter and the distal end of the VL, where the muscle met the knee tendon. The middle point between these positions was located with a 150 cm measuring band and marked with a waterproof marker. When good measuring sites were identified, eyeliner was used to draw around the probe to ensure visible marks in the gel. A transparent sheet was put over the leg to copy the marks and thus ensuring reliable measuring positions.

The muscle thickness of the VL was measured at mid-distance between the greater trochanter and the distal end of the VL by placing the ultrasound transducer perpendicular to the axis of the VL. A small grid consisting of three metallic wires was apposed onto the skin to generate echo-absorptive markers at the scanning location of the RF muscle (Figure 7). Axial-plane images of RF CSA were obtained on the medial and lateral sides of the muscle. The ultrasound transducer was coated with Aquasonic transmission gel. Additional gel was

applied to the measuring sites to prevent contact with the skin. Therefore, little or no pressure was applied to the muscle, causing no obstruction of images. An average of three trials per picture, for three pictures was used each time ultrasound was measured. Test-retest measurements from the familiarization to the pre tests revealed a coefficient of variation (CV) of 0.3% (VL muscle thickness) and 1.3% (RF CSA). Previously taken pictures was always used as reference when measuring VL muscle thickness and RF CSA with ultrasound.

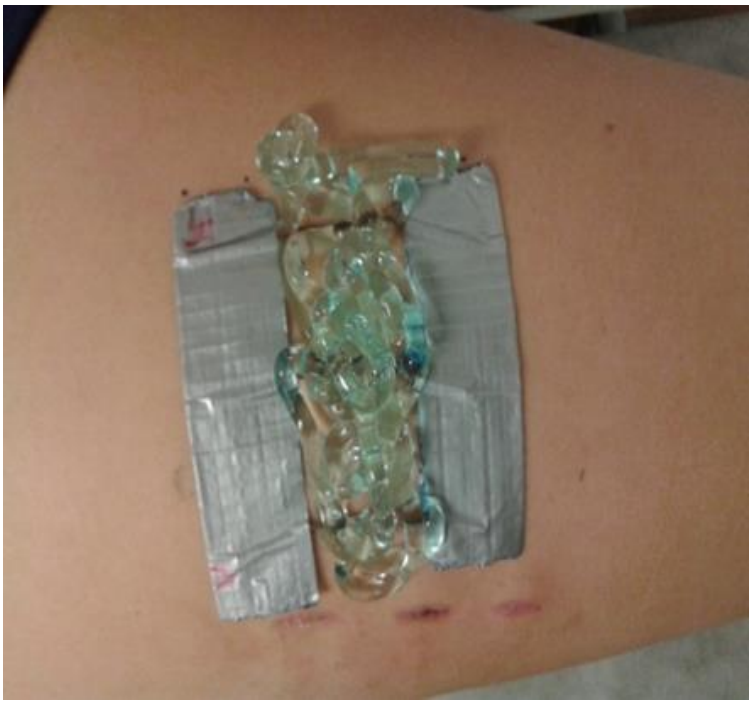

Figure 7. A close-up of the small grid of metallic wires used to measure the CSA of the RF

RF CSA and VL thickness, were measured offline, using ImageJ software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The two portions of the RF CSA (part 1 + 2 and part 2 + 3) were stitched together by superimposing the central marker and aligning aponeuroses of both images (Figure 8).

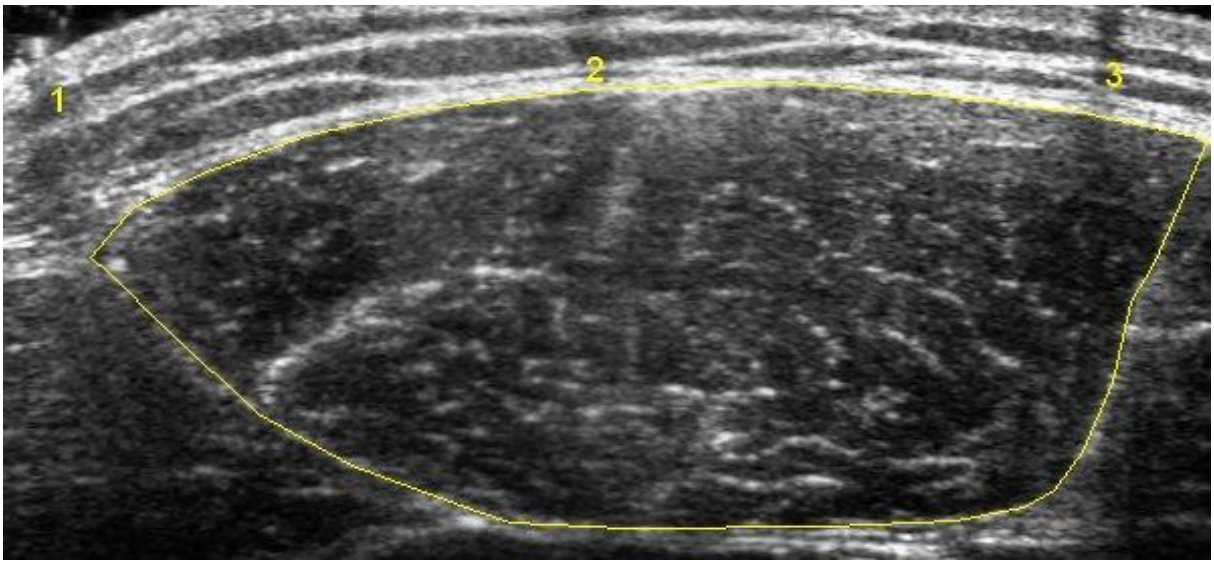

Figure 8. Ultrasound image showing the stitched version of the left (part 1 and 2) and the right (part 2 and 3) part of the CSA of RF. The numbers 1, 2 and 3 shows the small metallic grids used as reference points.

The muscle thickness was calculated as the mean of the three vertical distances (left, middle and right) measured between the superficial and deeper aponeurosis (Figure 9).

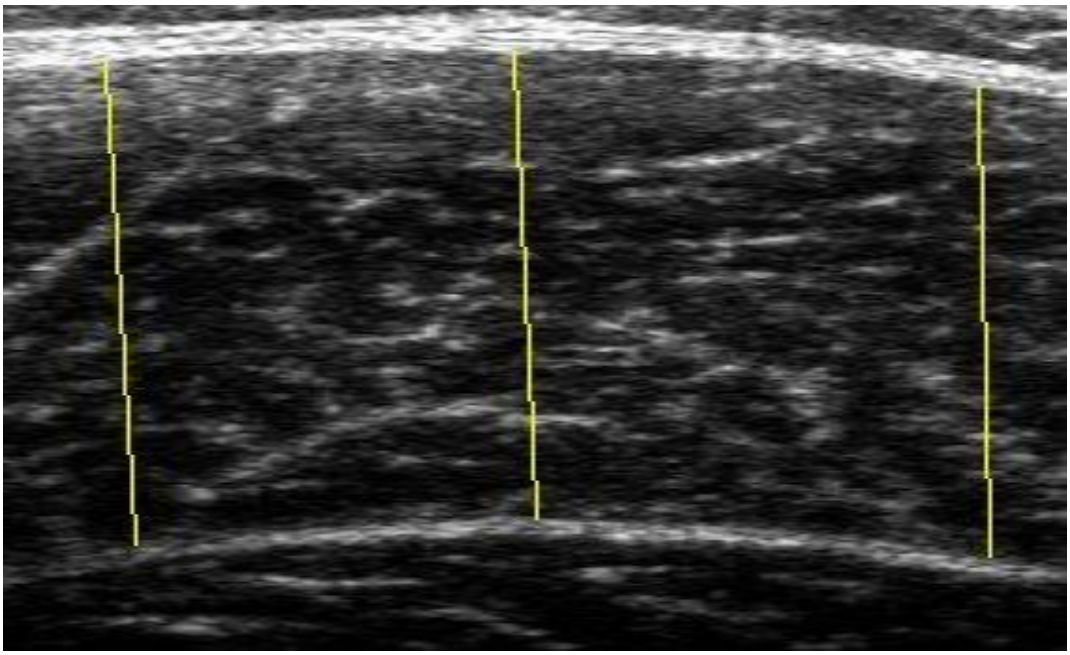

Figure 9. Ultrasound image from the thickest part of the VL. Yellow vertical lines represents the measurements from the superficial to the deeper aponeurosis.

3.2.5 1.5 Magnetic resonance imaging (MR)

MR scans was conducted one week prior and one week after the training intervention (table 2). The scans was carried out using a MR-machine (Siemens MAGNETOM Avanto, A Tim+Dot System 1,5-T) on supine lying subject. Every muscle in the quadriceps (m. vastus lateralis (VL), m. vastus medialis (VM), m. vastus intermedius (VI) and m. rectus femoris (RF) was analysed from the proximal to the distal part, divided in 9 slices. The muscles was analysed with 5 mm thick sections and with 39,7 to 40,0 mm between each section. The computer program OsiriX (OsiriX medical imaging software, OsiriX, Atlanta, USA) was used to analyse the CSA of the muscles. The most distal incision was done 40 mm above the knee joint (with the joint gap used as a reference point) and did not show visual muscle tissue in all cases. From the most distal slice, pictures were taken with 40 mm intervals in proximal direction up the thigh. In cases where the boundaries between muscles were hard to distinguish, a MR-atlas was used as reference (Möller & Reif, 2007). Mid-thigh CSA was found by first estimating femur length as ¼ of the subjects height (cm) and 50% of this length was used as mid-thigh. The MR slice located closest to the mid-thigh area was used.

3.2.6 DOMS-analysis

Subjects were asked to quantify their level of quadriceps muscle soreness by self-palpating on five different positions on both legs. Palpations were done in a standing position. First distal, middle and proximal on the VL, then distal and middle on *m.vastus medialis* (VM). Soreness for each site was marked on a visual analog 0–10 scale prior to ultrasound measurements. This scale has previously been found to be reliable and valid (Summers et al. 2001). The value of 10 was defined as extreme soreness and the value of 0 was defined as no soreness at all. Subjects were instructed to not include soreness or pain from biopsies in the assessment.

3.2.7 Statistical analysis

All statistical analyses were calculated in excel (Microsoft excel, 2013, USA). Two way paired t-test was used to detect any significant changes from baseline to post 29. Pearsons's correlation was used to investigate all correlations. Significance level was set at $\alpha<0.05$. All values are expressed as means ± standard deviation.

4.0 Results

No significant differences was found in VL muscle thickness or RF CSA between the right and left leg at baseline.

4.1.1 Time course for changes in VL muscle thickness

The muscle thickness of the VL increased significantly in both legs from baseline to day 29, subsequently with 5.7±2.6% ($p < 0.05$) in the right leg and 6.4±3.8% ($p < 0.05$) in the left leg (Figure 10).

The muscle thickness of the right leg increased gradually from baseline to the end of week 1 (day 5) with 5.2±2.8% ($p<0.05$). After 4 days of rest (day 9) the muscle thickness was reduced to 3.2±2.2% ($p<0.05$), which was still significant compared to baseline. The muscle thickness had increased to 3.7±3.1% ($p < 0.05$) at the beginning of the second training week (day 15). Throughout the second training week (day 15 - 19) the muscle thickness gradually increased to 6.6±6.6% ($p<0.05$) on day 19, compared to baseline. The left leg showed an increase of 2.0±0.7% ($p<0.11$) from baseline to day 9. The measures stabilized for both legs from day 22 to 29.

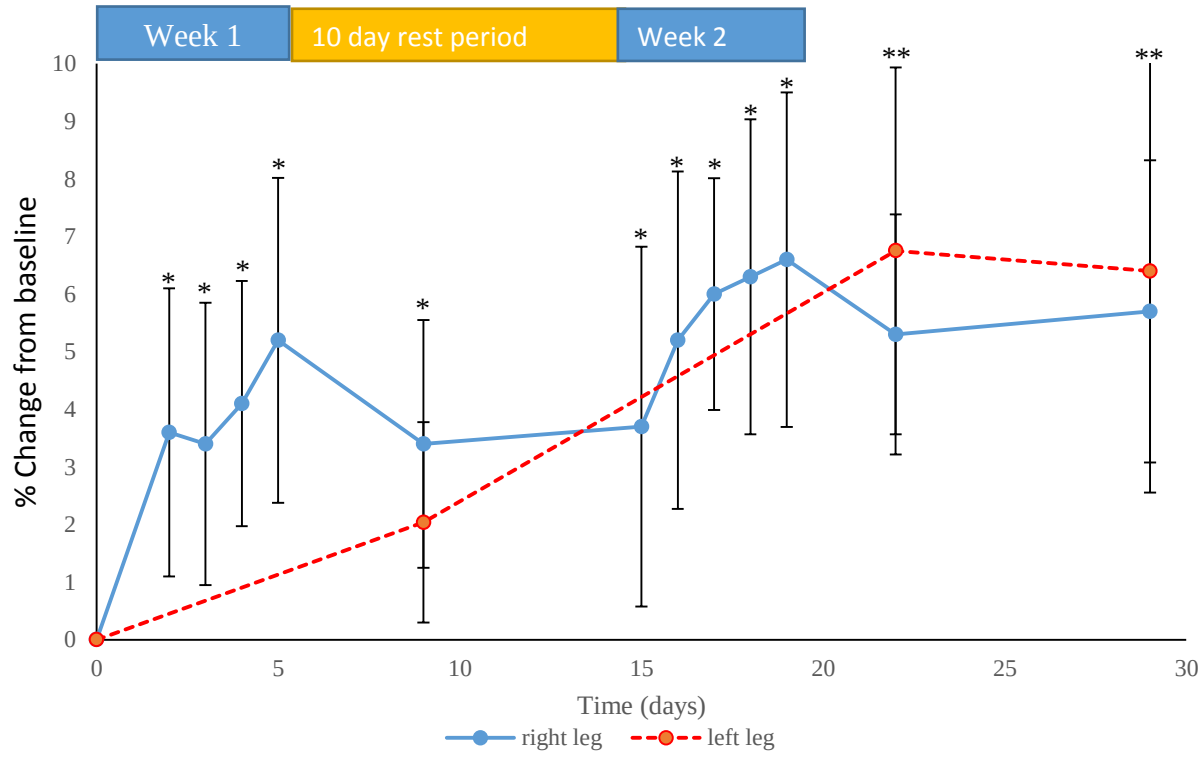

Figure 10. The time course for mean % changes in muscle thickness of the VL from day to day over the whole intervention period for the right (blue line) and left leg (red stapled line). Values are means±SD (error bars). * indicates significant ($p<0.05$) difference from baseline. ** indicates significant difference for both legs.

4.1.2 Time course for changes of CSA in RF

The CSA of the RF increased significantly in both legs from baseline to day 29. The right leg showed an increase of 7.0±4.1% ($p < 0.05$) compared to baseline and the left leg showed an increase of 8.8±5.2% ($p < 0.05$) compared to baseline (Figure 11).

The right leg increased gradually from baseline to day 5 with 6.5±4.4% ($p<0.05$). A marked increase of 2.1±3.8% ($p<0.59$) was found when CSA of RF was measured during the rest week (day 9). At the start of the second training week, the CSA was at a non-significant 1.7±4.27% ($p<0.65$) increase compared to baseline. Throughout the second training week (day 15 - 19) the CSA increased significantly to 7.5±2.9% ($p<0.05$) at day 19, compared to baseline. The left leg displayed a significant increase of 5.4±4.0% ($p < 0.05$) from baseline to day 9. The measures stabilized for both legs from day 22 to 29.

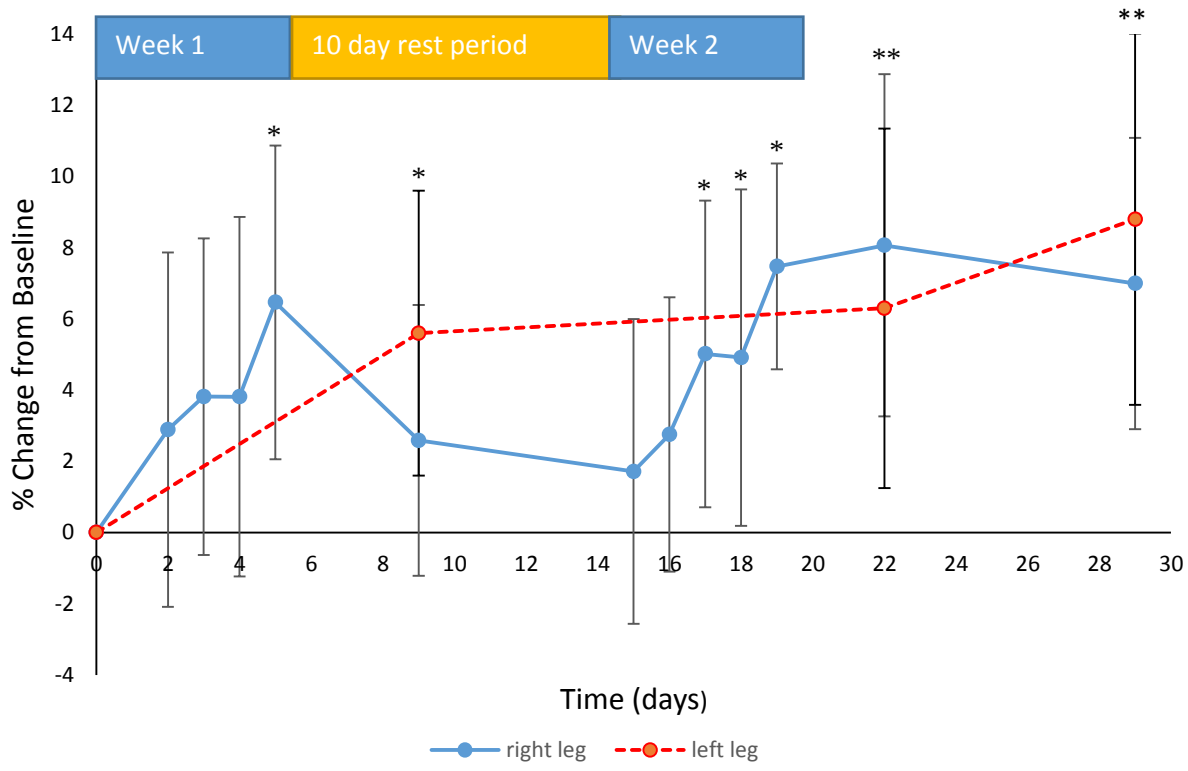

Figure 11. Time course for mean % changes in the CSA of the RF from day to day over the whole intervention period in the right (blue line) and left (red line) leg. Values are means±SD (error bars). * indicates significant ($p<0.05$) difference from baseline. ** indicates significant difference in both legs.

4.1.3 Changes in CSA measured with MR

No significant differences were detected between the left and right legs in CSA for VL, VM, VI or total quadriceps CSA (Figure 12). A significant difference ($p<0.05$) was detected in the RF CSA between the right and left leg, where the RF of the left leg was bigger. A significant increase was found in the RF (5±6.8%), but not in any of the other muscles in the quadriceps (VM = 1.23±3.1%, VI = -1.15±2.2%, VL = 1.59±3.9% and total quadriceps 1.17±2.7%).

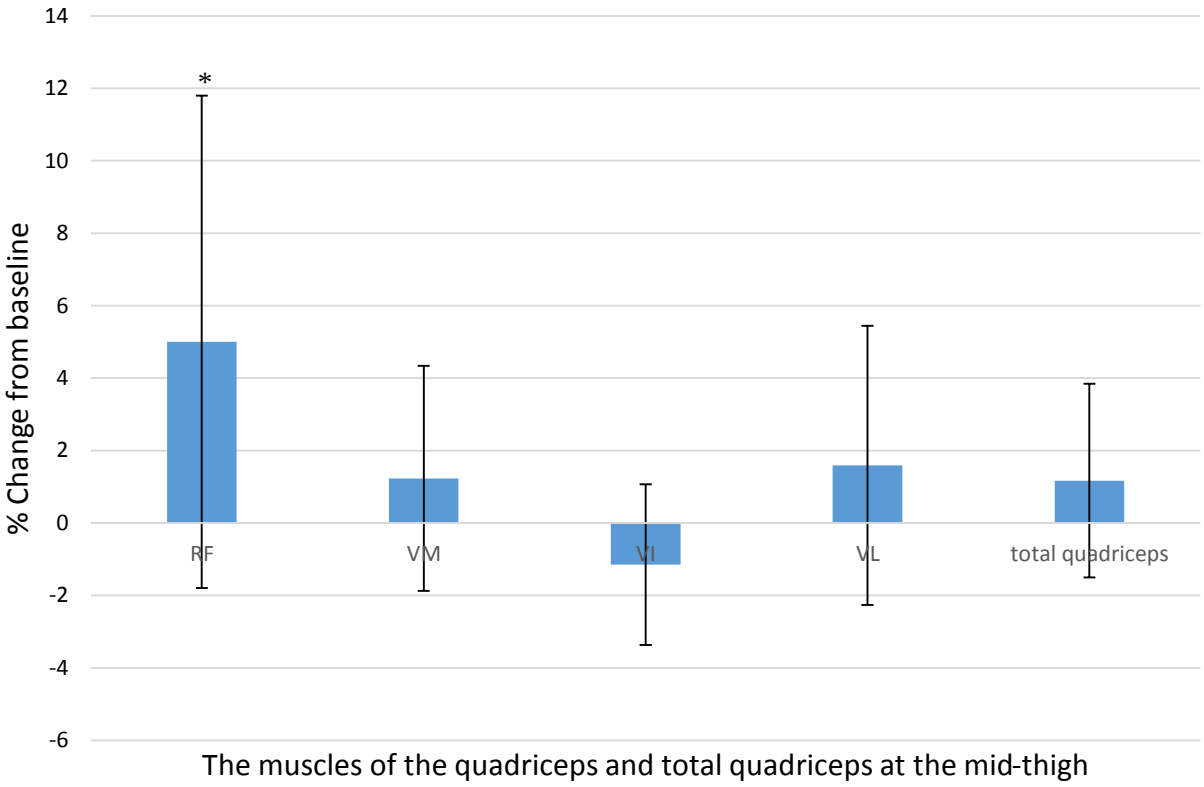

Figure 12. The figure shows the % change in CSA of the individual muscles of the quadriceps and the total quadriceps as measured on the mid-thigh site. Values are means±SD (error bars). * indicates significant ($p<0.05$) difference from baseline.

4.2 Muscular Strength

4.2.1 Strength tests

No significant differences were observed between the three different strength tests on the familiarization testing and pre-testing, or between the right and left leg on any of the tests. From baseline to day 29 the maximal isometric strength increased significantly with 17.1±7.3% ($p<0.05$), whereas no significant changes in maximal isokinetic strength (2.6±18.5%, $p<0.79$) or 1RM in dynamic knee extension-test was observed (1.4±7.1%, $p<0.75$) (Figure 13).

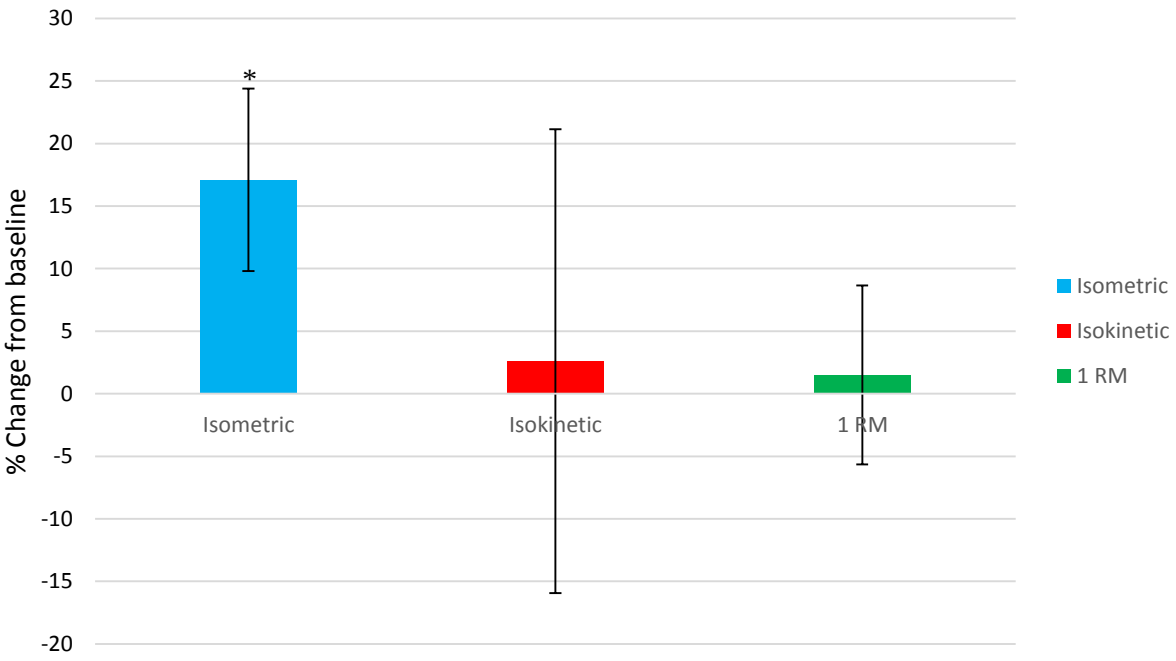

Figure 13. Relative (%) changes in muscle strength measured in the different strength-tests from baseline to day 29. Values are means (±SD, as shown with error bars) between the right and left leg as measured on day 29. * indicated $p<0.05$.

4.2.2 Acute muscle swelling

No significant differences was observed in VL muscle thickness or RF CSA from the pre-tests (1 week prior to acute 1) to acute 1 (day 1).

The acute muscle swelling was measured immediately upon release of cuff pressure, less than one minute after completion of the last exercise set. An average increase of 22.9±6.0% was found in acute muscle cell in the RF (Figure 14).

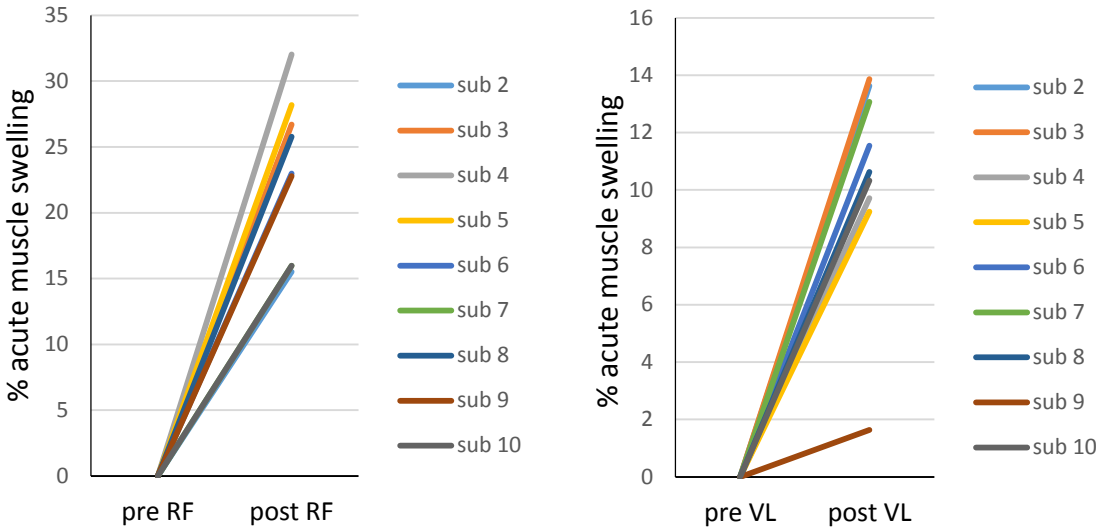

Figure 14. The figure shows the % increase acute muscle cell swelling in the RF for each test participant. Values are mean between right and left leg from acute 1 and 2 (day 1 and 15). Note; Subject 7 and 10 both displayed an increase of 16 %. Because of this, the green line is somewhat obscured

4.3 Correlations

All values for VL muscle thickness & RF CSA are means of the percentage change between the right and left leg for each test-subject as measured on day 29. This applies for all correlations.

4.3.1 Increase in acute swelling vs muscle size

No correlation (r = 0.02) was found between the acute muscle swelling measured after the first session in each training week increase in VL muscle thickness and the increase in VL muscle thickness during the whole period (Figure 15). A moderate negative correlation (r = -0.46) was found between the mean % of acute muscle swelling and the increase in RF CSA.

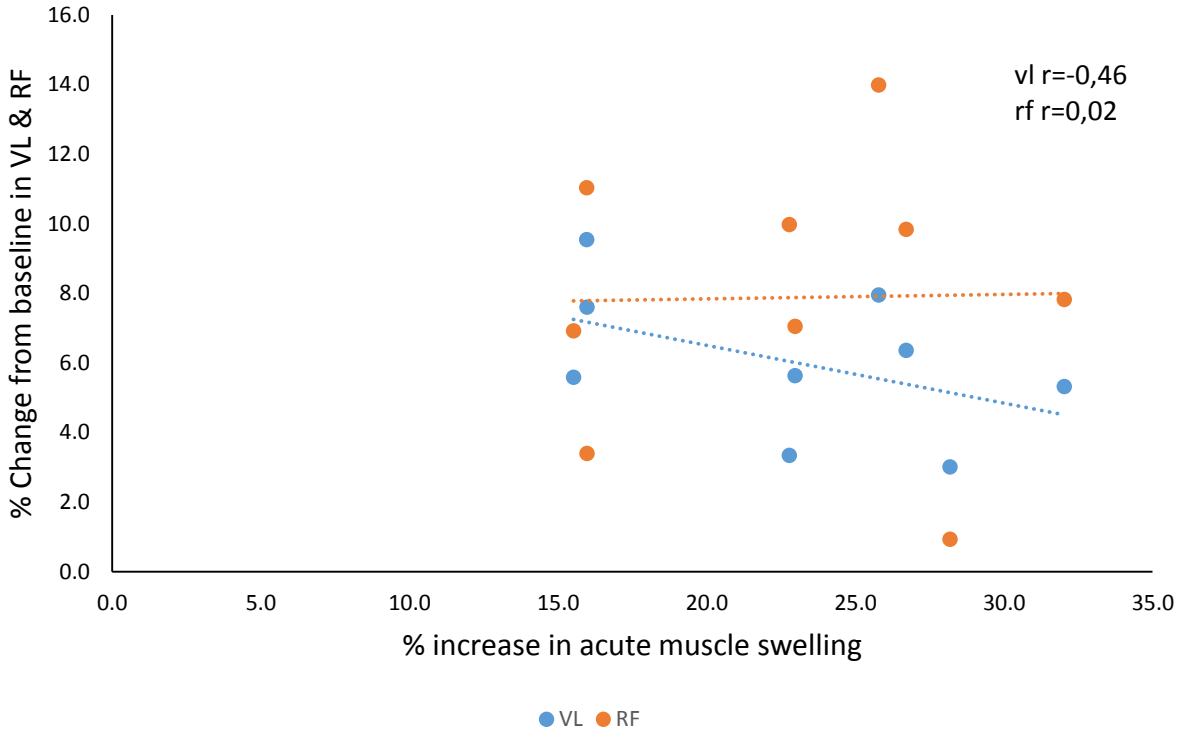

Figure 15. Correlation between the % increases in muscle size for VL and RF and the acute % increase in muscle swelling measured immediately after BFRRE. Values for muscle cell swelling are expressed as means between acute days for left (day 1) and right leg (day 15).

4.3.2 Maximal isometric strength (MVC) vs hypertrophy

No correlation (0.08) was found between the increases in VL muscle thickness and increases in maximal isometric strength (Figure 16). A moderate negative correlation (r = -0.50) was found between increase in the CSA of RF and increase in isometric strength.

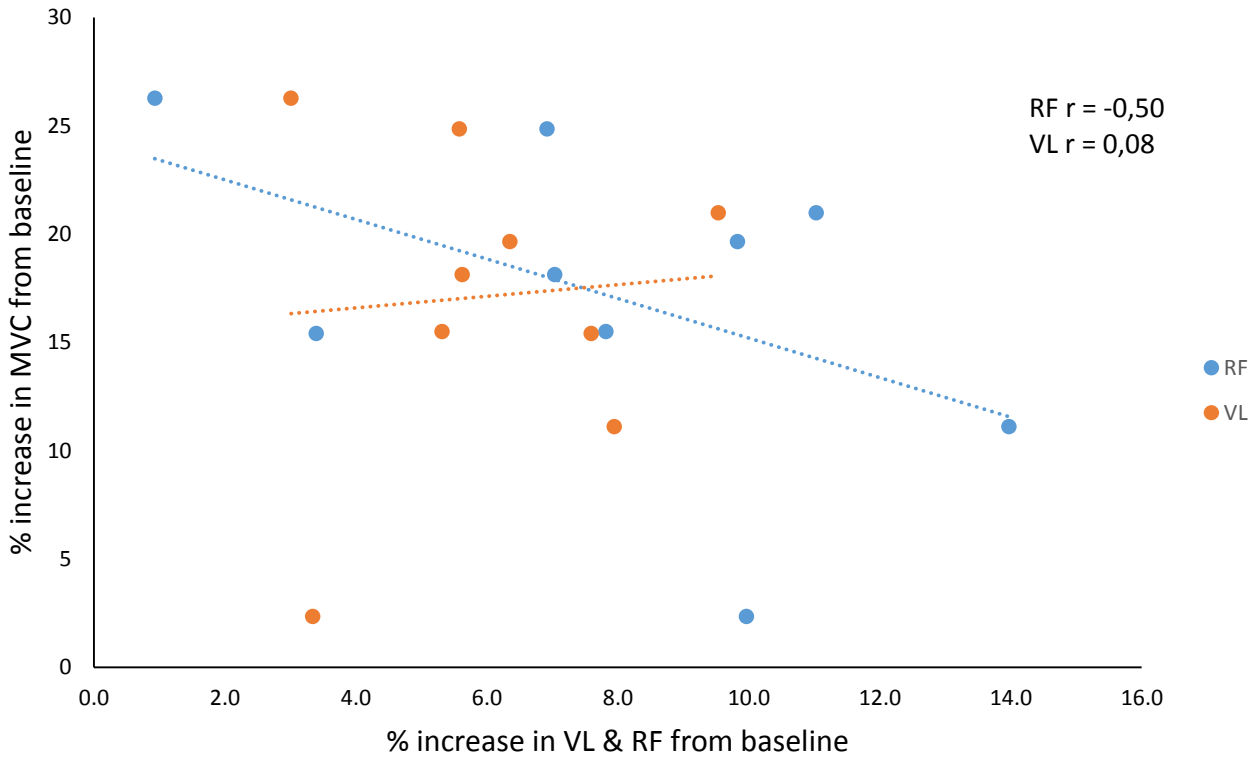

Figure 16. Correlation between increases in VL and RF and increases in MVC. Values for isometric strength increases are means between tests performed at 70, 80 and 90 degrees knee joint angle.

4.3.3 Maximal isokinetic torque vs hypertrophy

A moderate negative correlation of -0.34 was found between increases in maximal isokinetic torque at 60 degrees and increases in CSA of the RF (Figure 17). A moderate positive correlation of 0.56 was found between increases in muscle thickness of the VL and increases in isokinetic strength.

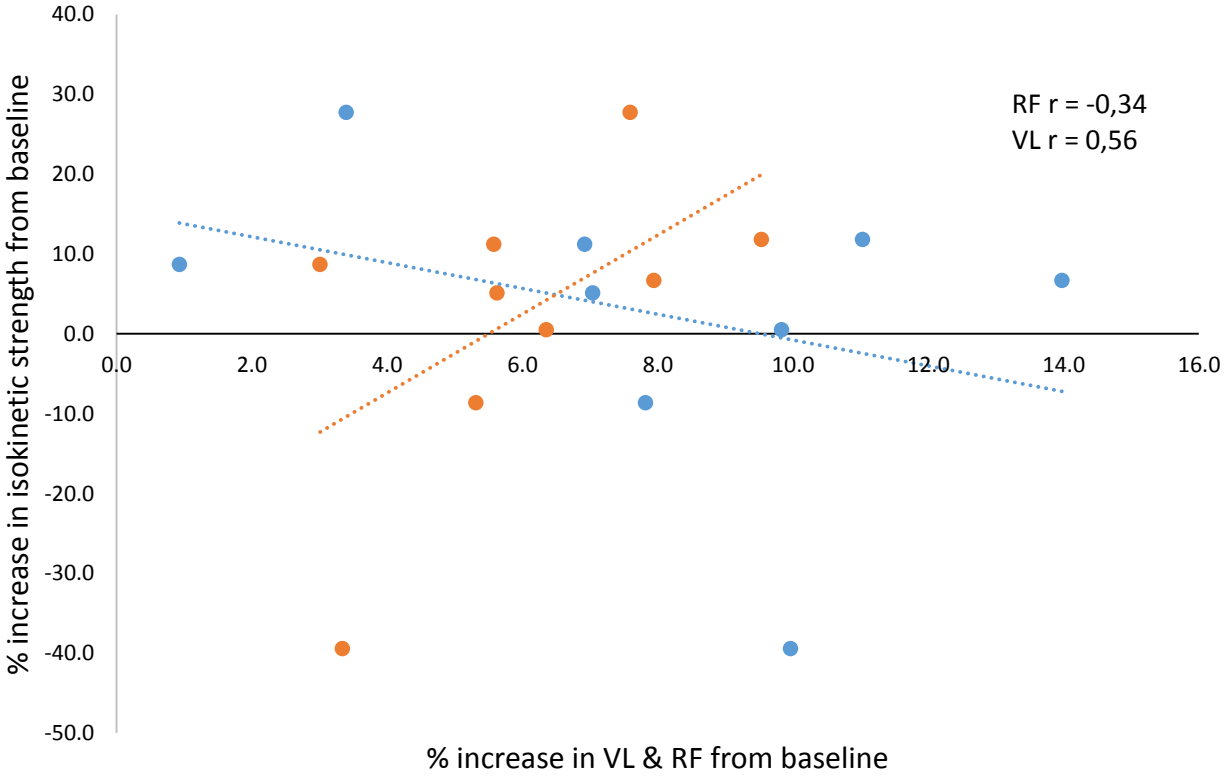

Figure 17. The figure shows the correlation between the increases in VL and RF and the increase in maximal isokinetic torque at 60 degrees.

5.0 Discussion

The purpose of this study was to investigate the time course for hypertrophic adaptations with intensive BFRRE in healthy young subjects, following two training weeks consisting of 7 training bouts each. Training weeks were separated by a 10-day rest period. Previous studies have shown BFRRE to induce rapid muscular hypertrophic increases, however very few studies have established the exact time course for when these adaptations occur.

Other research questions examined was the potential correlation between hypertrophic adaptations and muscle strength. Furthermore, it was hypothesized that the acute muscle cell swelling observed immediately after one session of BFRRE, would be correlated to the long term training-induced change in muscle size.

In summary, significant increases was found in VL muscle thickness and RF CSA in both legs with ultrasound. VL muscle thickness and RF CSA increased gradually throughout the first training week, but decreased during the rest period, suggesting that there was a considerable amount of muscle swelling. A similar gradual increase was observed during the second training week, before a more stable and permanent increase seemed to manifest from after the second training week and to the post-tests on day 22 and 29. Only the RF displayed any significant increase in CSA measured with MR. Additionally, significant increases in isometric MVC was found, but surprisingly, no significant increase in maximal isokinetic torque at 60 degrees or 1RM knee extensions was found.

5.1 Time course for increase in muscular size

Both VL muscle thickness and RF CSA increased gradually from baseline to the end of week 1 (day 5) in the right leg. This gradual, bout-to-bout increase has been reported in previous research (Abe et al. 2005/b/c). Consecutive day-to-day ultrasound measurements on the same location on the thighs before exercise, suggests that the results obtained are both reliable and valid. CV values of 0.3% (VL muscle thickness) and 1.3% (RF CSA) between test – retest pre baseline supports this. Previously taken pictures was always used as reference when measuring VL muscle thickness and RF CSA with ultrasound.

However, because of daily training sessions, it is difficult to distinguish the muscle cell swelling from any functional muscle hypertrophy occurring within the training weeks (day 1 - 5 and day 15 - 19). Acute muscle cell swelling was measured on two separate occasions (day 1 and 15), immediately after finishing the exercise bout. As expected, muscle swelling was observed at both time points. These findings are consistent with other investigators (Fry et al. 2010; Yasuda et al. 2011). When combining the values of acute swelling obtained from both legs, increases of 11% and 23% was found in VL muscle thickness and RF CSA. Other research on BFR has also found RF to increase more than VL both with (Kacin & Strazar, 2011) and in the absence of exercise (Loenneke et al. 2012b).

This large acute swelling observed after the very first training bout is likely to also affect the measurements on the next following days. The decrease in muscle size for both VL and RF observed in both legs from day 5 to day 9 suggest that the muscles were still in a swelled state when measured consecutively throughout the training week. It might also suggest that the muscles were in a swelled state during most of the week, which could perhaps be a strong anabolic signal?

The first reliable time point for assessing any plausible increase in muscle size was on day 9, during the 10-day rest period. VL muscle thickness of the right leg and RF CSA of the left leg displayed significant changes at this time point, their contralateral legs did not. This was found after 4 days of rest, possibly enough time for the legs to recuperate and any residual muscle swelling to disappear. Briefly looking at the other studies shows that the number of days of rest from the last training bout to the post testing was either fewer or consistent with ours. Abe et al (2005) did their post testing 3 days after the final bout, training twice every day. Abe et al. (2005b) only had post-tests on day 9 and 10 after two bouts performed on day

8. Consequently their percentage increase from day 8 to 9 and 10 lies within 0.5% of each other. Fujita et al (2008) used two days of rest from the last bout.

However, several subjects stated feeling unrested during the testing on day 9, which also revealed no increases in muscular strength (2.4%, 0.15% and -2.5% for isometric, isokinetic and 1 RM knee extension, respectively) (described in detail later). This could be due to the entire volume of exercise sets to failure during the prior training week, onset of a new unfamiliar exercise-regime, or possibly because of the two consecutive days with double bouts at the end of the training week. An interesting observation in favour of this last factor could be the considerable jump in size, consistent for both VL muscle thickness and RF CSA from day 4 to day 5 in the right leg. This indicates a considerable amount of muscle swelling.

For the VL muscle thickness, after the rapid initial increase observed from day 1 to day 2 (3.6%), the percentage increase seemed to stabilize, seeing only an increase of 0.5% from day 2 to day 4, then from day 4 to day 5, after the first double bout, an additional increase of 1.1% was found. The numbers found for the CSA of RF was even greater, where an additional increase of 2.7% was observed from day 4 to day 5. Almost exactly the same as the initial increase of 2.9% from day 1 to day 2.

This rapid increase at the end of the week was found after the first day of double bouts (day 4), the size increase after the second double bouts on day 5 was not measured before day 9. Even though a training intensity of 20% of 1RM is thought to produce minimal muscle damage (Takarada et al. 2000a), and less recovery time is thought to be needed (Abe et al. 2004), analysed data from day 9 should perhaps be interpreted with caution due to the possibility of residual swelling and fatigue from the high training volume. However, it should also be pointed out that the muscle thickness of the VL increased from day 9 to day 15 with 0.3%, strongly suggesting that hypertrophic adaptations had manifested. Simultaneously, somewhat conflicting, the RF CSA decreased in the same time period with 0.9%. A good portion of the exercise-induced changes found on the last day in training week 1 was reduced at day 9 and at the beginning of week 2 (day 15). This suggests that muscle swelling contributed substantially to the changes observed throughout week 1.

The exercise-induced changes in muscle size were also measured on the start of the second training week (day 15). At this time point, all subjects had 10 days of rest, which would have to be considered enough time for the swelling to go down, suggesting that any increase found at this point likely would be attributed to hypertrophic adaptations. Increases was found in

both VL and RF on day 15 in the right leg, but only significant for the VL. Throughout the second training week (day 15 - 19) the muscle thickness of the VL gradually increased from 3.7% to 6.6%, but the large leap observed after the first double bout in week 1 was only seen in the RF CSA (+ 2.6% from day 18 to day 19). The exercise-induced changes then stabilized between the post-tests on day 22 to 29, suggesting a more permanent increase in muscle size occured.

5.2 Comparison with other studies

The exercise-induced changes in VL muscle thickness and RF CSA was measured with ultrasound pre – post and before every training bout. MR was used to measure the hypertrophic response in quadriceps CSA pre – post. Increases in muscular strength will be mentioned but further discussed in the next chapter.

Few studies are comparable to the current one in terms of consecutive day-to-day assessment of the time course for increases in muscle size. Three studies have been identified as the most comparable ones (Abe et al. 2005/b; Fujita et al 2008) (Table 2). All of these studies assessed the change in muscle size from bout to bout. Two of these studies also conducted MR measurements pre and post intervention (Abe et al. 2005; Fujita et al 2008). The last study used ultrasound to measure quadriceps muscle thickness (pre-post) (Abe et al 2005b).

Table 2. The table gives an overview over the three studies most comparable to the current study

Study	n/age	Sex/training status	Method	Load (% of 1RM)	Set/reps/rest between sets	Training frequency	Changes in muscle strength	Changes in muscle size
Abe et al. 2005	9 23(±6)	M/ active	Squat & leg curl with continous BFR. 160 mmHg at baseline, daily 10 mmHg increases up to 240 mmHg. Used 33 mm Kaatsu cuff. An equation was used to measure daily changes in mid-thigh CSA	20 %	6 sets/15 reps (90 reps/day) 30 sec rest	2/day for 6 days/ week for 2 weeks	↑ 16.8 % squat, ↑22.6 % leg curl	↑ 8.5 % CSA (MR), ↑9% mid thigh (equation)
Abe et al. 2005b	9 nr	m/college athletes	Squat & leg curl with continous BFR. 160 mmHg pressure at baseline, daily 20 mmHg increases up to 240 mmHg. Used 33 mm kaatsu cuff. An equation was used to measure daily changes in mid-thigh CSA	20 %	6 sets/15 reps (90 reps/day) 30 sec rest	2/day for 8 days	↑ 9% leg press	↑ 4.5% CSA mid-thigh (equation) ↑ 5.9% quadricep MT ↑4.5% hamstring MT (ultrasound)
Fujita et al. 2008	8 22(±3)	m/ active	Bilateral knee extension with continous BFR. 160 mmHg at baseline. Increased 10 mmHg a day up to 220 mmHg. An equation was used to measure daily changes in mid-thigh CSA. Cuff size nr	20 %	4 sets/30,15, 15,15 (150 reps) 30 sec rest	2/day for 6 days	↑ 6.7% knee-extension	↑ 3.5% mid-thigh quadriceps CSA (MR) ↑3.4% mid-thigh muscle-bone CSA (equation)

nr = Not reported, MT = Muscle thickness

When comparing our results with the results of other studies a couple of problems arise. The first one being that the current study only investigated the time course on specific muscles in the quadriceps area, namely the muscle thickness of the VL and the CSA of the RF. The other research teams have tended to refer to mid-thigh CSA, without distinguishing between any of the quadriceps muscles. The two studies by Abe et al (2005/b) also trained the hamstring muscles, thus induced a hypertrophic response in the hamstrings as well. This would arguably increase the mid-thigh CSA, without any further distinction from the muscles in the quadriceps.

Furthermore, while the current study used ultrasound as previously mentioned, the other investigations used an estimation-equation to measure the daily training-induced changes in mid-thigh CSA (Gurney & Jelliffe, 1973). This equation gives an estimated number of mid-thigh CSA of the quadriceps muscles. The researchers carried this out every morning prior to the training session (Fujita et al. 2008; Abe et al. 2005/b).

There is a rather large discrepancy between mid-thigh quadriceps CSA found in the different studies. Given that the studies length differed and the study by Fujita et al (2008) only ran for 6 days, results from day 6 for all studies will be used as reference in the next paragraph. This will be comparable knowing that all three studies all used a submaximal exercised-protocol with twice daily exercise-bouts with 20% of 1RM. Day 5 from the current study will be used in this context.

At day 6, the increase in mid-thigh CSA was found to be 3.4% in Fujita et al (2008) and 3% in Abe et al (2005b) compared to baseline. Interestingly, Abe et al (2005) found an increase of almost 8% at day 6. The mid-thigh CSA increased with over 1% over the next two days in Abe et al (2005b), suggesting that the 6 days in the study by Fujita might not be enough time to maximize the dividends of this training method. Consistent between all studies is the daily gradual increase in muscle size throughout the training week, before a small decrease was found when measured the next day (Abe et al 2005b), after 3 days of rest (Abe et al. 2005) and after 2 days of rest (Fujita et al. 2008). These decreases suggest that some of the exercise-induced changes is muscle swelling. The current study found increases of 6.5% in RF CSA of the right leg on day 5. Although no mid-thigh CSA-estimation was performed, the increase in RF CSA follow a similar gradual day-by-day pattern as the mid-thigh CSA reported from the other studies.

Post-tests revealed increases of 9% (Abe et al 2005), 4.5% (Abe et al 2005b) and 2.4% (Fujita et al. 2008) in mid-thigh CSA. The current study found RF CSA increases of 8.8% in left leg and 7.0% in the right leg. Interestingly, consistent between the current study and the study by Abe et al (2005) is that the difference in increased muscle size between the first and second week is very small, indicating that this training form induce an initial rapid response as previously shown by Takarada et al (2000a).

The MR results of the current study revealed no changes (+1.2%) in the mid-thigh CSA. Abe et al (2005) and Fujita et al (2008) found increase of 8.5% and 3.5% in the mid-thigh CSA with MR. The MR results suggested an increase of 4.8% and 5.1% in the left and right RF CSA. This was slightly smaller than what was found with ultrasound (7.0% in the right and 8.8% in left leg). However, the increase in CSA of the other muscles of the quadriceps measured at mid-thigh were smaller (VI = -1.15%, VM = mean 1.23%, VL = 1.17%). This was found 7 days after the last training bout. Previous studies on knee extension have reported no differences in contribution of RF and VL during knee extension exercises (Alkner et al. 2000; Vaz et al. 1996). This seems to apply with VM as well (Jakobsen et al. 2012). Interestingly, Kacin & Strazar (2011) experienced a significant reduction in CSA and a tendency towards reduced hypertrophy in VI due to high cuff pressure (230 mmHg). Given that the VI is the muscle adjacent to the bone, it appears to be the most vulnerable part of the quadriceps when BFR is applied.

There are several possible explanation for the different increases in muscle size between the studies. One of them could perhaps be due to the usage of different training-protocols. The two studies by Abe et al (2005/b) performed leg curls in combination with squats, thus also exercising the test participant's hamstring muscles. Muscle volumes of *m,bicep femoris* (Abe et al. 2005) and hamstring muscle thickness (Abe et al. 2005b) was reported to increase with 10% and 4.5% respectively, thus give the mid-thigh CSA a considerable increase, compared to mid-thigh CSA without exercise for the hamstrings.

While the current study performed repetitions to concentric failure, the other studies used sub-maximal training protocols. It has been reported that submaximal exercise-protocols can produce increases in anabolic signalling and protein synthesis similar or even greater than protocols with higher intensity in conventional resistance exercise (Burd et al. 2010; Hulmi et al. 2010). However, more so than intensity, continuous rather than intermittent blood flow

restriction during BFRRE seems to be of greater importance for increasing the metabolic demands and motor unit recruitment (Fahs et al 2012). All studies used a continuous approach with BFR during the training.

The current study performed 4 sets of knee extensions with 30 seconds rest between sets. Both of the studies by Abe et al (2005/b) did 3 sets of 15 repetitions of squat and leg curl (20% of 1RM) with 30 seconds rest between sets, twice per day (6 sets per day, for a total of 90 repetitions). Fujita et al (2008) did 4 sets (30, 15, 15, 15 reps) of knee extension (20% of 1RM) with 30 seconds rest, twice per day (8 sets per day, for a total of 150 repetitions) (Fujita et al. 2008). Fujita et al (2008) did two more exercise-sets and a total of 60 repetitions more per day than both studies by Abe et al (2005/b) and 4 sets and about 80 repetitions more than our study. However, given that the subjects in all studies were previously untrained, the additional volume is unlikely to cause any hypertrophic difference (Krieger, 2010). However, the studies by Abe et al (2005/b) performed different exercises than our study and Fujita's study. Squats are known to recruit more muscles than leg extensions. The squat has previously been dubbed "the king of all lower-body exercises" (Poliquin, 2006) and this movement would arguably activate additional muscles such as *m.gluteus maximus*, compared to knee-extensions which generally are thought to only involve the muscles of the quadriceps (Raastad et al. 2010). This might be of significance because exercises that involve larger muscle mass produce larger hormonal responses compared with exercise involving smaller amounts of muscle mass (Kraemer et al. 1998). However, it is uncertain whether this is of significance when it comes to the local response in the quadriceps.

If training program was the most important component for explaining the differences between the studies, the two studies by Abe et al could possibly be attributed to the subjects training backgrounds, given that they performed the same exercises. Abe et al (2005) used untrained individuals, whereas Abe et al (2005b) used track and field athletes. It is likely that the latter group had previous experience with some kind of resistance exercise in order to perform better in their respective sports. Resistance exercise would be especially relevant for the sprinters in the study. This could also explain the initial low increase (<1% increase from day 1 - 3) in the beginning of the intervention, compared to the much larger initial increase in the non-athlete population in Abe et al (2005). There were only slight differences in the training protocols. An interesting notion, albeit somewhat speculative could be that an initial discrepancy in total amount of muscle mass between study participants at baseline was

determining. The subjects in the study of Abe et al (2005) were reported to exclusively participate in aerobic activities. The mean BMI for this group was 22.2 with an average of 14.9% body fat. Subjects in the study by Fujita et al (2008) and Abe et al (2005b) had an BMI of 21.8 and 21,9, respectively. These numbers are considered normal, however, the subjects of the current study had an average BMI of 24.7. Also considered normal, but close to the upper limit of the category. Given that all study participants of the current were physically active, this high mean BMI value could possibly be attributed to a large amount of muscle mass at baseline. Of course, none of the study participants from any study performed regular strength training, but subjects from our study reported performing sports where muscle mass arguably would be beneficial (Football, handball).

The current study started the training intervention with standardized cuff pressure (100 mmHg for men and 90 mmHg for women). Simultaneously, the two studies by Abe et al (2005/b) started the cuff pressure at 160 mmHG and increased pressure daily 10 mmHg daily in Abe et al (2005) and 20 mmHg daily in Abe et al (2005b) until 240 mmHg was reached. Fujita et al (2008) started their pressure at 160 and increased 20 mmHg daily until they reached 220 mmHg. This gradual increase in pressure could perhaps build up a tolerance for the pressure and prevent excessive level of DOMS, and thus allow the subjects to perform with max effort every training session. This approach could also be a way of achieving a progressive overload to further enhance the training adaptations (Fahs et al. 2012). It should be noted that the pressure cuff used in the current study was wider than the ones used in the other studies. This is of significance because wider pressure cuffs are generally more effective in restricting arterial blood flow to the working muscles at lower pressures, compared to narrower pressure cuffs (Fahs et al. 2012). We found a 60% reduction in arterial blood flow with the pressure cuff that we used. The other studies did not report any numbers for reduction in arterial blood flow with their pressure cuffs.

Furthermore, differences in muscular adaptations could also be attributed to individual variability. According to Hubal et al (2005) muscular adaptations induced by resistance training are highly individual. This variability can be attributed to genetics, differences in training programs, how people comply with the training, dietary habits and/or training status (Raastad et al, 2010; Wernbom et al 2007).

It should also be noted that we included 3 female subjects in our study. Few studies have been conducted on young untrained females and BFRRE. According to a meta-analysis by Loenneke et al (2012), BFRRE studies including both sexes have yielded less muscular hypertrophy when compared to male only studies. However, when assessing the average between both legs we observed a 7.6% and 5.2% increase in VL muscle thickness and 10.9% and 6.3% increase in RF CSA, for females and males subsequently. These numbers could have been different with a larger study sample, but nonetheless they indicate that sex was not a determining factor in this study.

A significant amount of other research reports has also found increased muscle size after a period of BFRRE. Kacin and Strazar (2010) found an increase of 9.3% in RF CSA and 4.5% increase in VL CSA. These results are larger than our MR-results, but the 9.3% in RF CSA was found with an MR-machine and is similar to our 7.0% and 8.8% increase in the right and left RF CSA found with ultrasound. Also consistent was the fact that RF seemed to respond more than VL. Their study consisted of a similar amount of training bouts as the current study, but differently distributed over time (4 session/per week for 4 weeks). Notably, MVC did not increase in their study. Madarame et al (2008) found an increase of a 4.3% increase in the quadriceps CSA and 5.7% increase in the hamstring CSA. Subjects performed two bilateral BFRRE-bouts per week, for 10 weeks. A 19.6% increase was found in MVC. Interestingly, this increase in MVC is similar to that of the current study, but found after a much longer time period. Kubo et al (2006) found a significant increase of 5.9% in quadriceps volume and a 7.8% increases in MVC after 3 bouts of knee extension per week for 12 weeks. Takarada et al (2004) found an increases of 10.3% in the quadriceps CSA after 8 weeks of knee extensions performed twice a week. When averaged between isokinetic and MVC strength, an increase of 9.2±2.2% was found.

5.3 Muscle strength

All strength-parameters tested has previously shown to adapt with BFRRE (Takarada et al. 2004; Abe et al. 2005b; Burgomaster et al. 2003). As far as this author knows, no other study on resistance exercise with BFR has investigated increases in MVC, Isokinetic strength and 1-RM strength all at once.

According to a meta-analysis by Loenneke et al (2011) the effect size for low intensity BFRRE is greater in untrained individuals (ES = 1.38), compared to already active individuals

(ES = 0.37). These findings are consistent with conventional strength training (Wernbom et al. 2007).

In the current study, MVC increased to a similar extent as what was found by Madarame et al (2008) (19.6%). Their study however ran over a much larger time course. Nielsen et al (2012) found an increase of 10.6% in MVC within a similar timeframe, but with 9 training bouts more performed only at 70 degrees. Individual data from the increases in MVC at only 70 degrees from the current study suggests an almost two fold increase (+ 20.42%) as what was found in Nielsen's study. There was found large discrepancies between results in MVC, isokinetic and 1RM strength, which are difficult to explain. Isometric strength was found to increase with 17.1 % (Range 2.35% - 26.27%) and isokinetic strength was not changed (2.6 %, (range -39.4% – 27.7%). A review by Wernbom et al (2007) investigated the hypertrophic effect of MVC and isokinetic strength training without BFR. The authors found an average daily increase of 0.11% (MVC) and 0.13% (isokinetic strength training) in muscular CSA with similar training frequency over similar time periods. The difference in individual ranges seems to be a determining factor.

Increased muscle strength did in fact occur after the increased muscular size. We did find significant increases in VL muscle thickness and RF CSA, with ultrasound, However, the MR results suggested no increase in total quadriceps CSA. This can perhaps explain any lack of increased strength.

Clark et al (2011) found a 8% increase in MVC at 60 degrees after 4 weeks of bilateral BFR knee extensions. This increase was lower compared to the current study, which used a mean of 90, 80 and 70 degrees to determine the increase in strength. These angles could possibly be more favourable in terms of developing force due to a larger range of motion. Individual data from the current study however, suggested a larger increase at 70 degrees, compared to 80 and 90 degrees. Kubo et al (2006) found an increase of 7.8% of MVC after performing knee extensions at 90 degrees. The large increase in MVC of the current study, compared to other research reports could be a result of more frequent testing. Shinohara et al (1998) conducted a study supporting this theory. They found an increase of 26% in MVC. The rather large increase in MVC could arguably be attributed to the specific MVC training protocol. Interestingly, after 2 weeks of training a 9% increase was found. After the next two weeks, the increase had risen to 26%, compared to baseline. Interestingly, this also means that the strength gain was bigger within the last two weeks. This time course is consistent with our findings.

However, if the theory of frequent testing was accurate we would likely see a larger increase in isokinetic torque and 1RM knee extension as well. This frequent testing could also put subjects at a disadvantage. Testing 1RM is likely to take a toll on the muscles. Frequent max testing (pre, day 1, 9, 15, 22 and 29) for several tests, combined with an already high training volume could suggest that more restitution would have been beneficial for the strength tests on day 29. Laurentino et al (2012) found an increase of 40.1% in 1RM knee extension. This is amongst the highest reported increases in 1RM and significantly bigger than what we found (+1.4%). The current study assessed both MVC and Isokinetic strength before the 1RM-test. This could possibly induce a certain level of fatigue. Furthermore, Laurentino et al (2012) warmed up with 5 minutes jogging on a treadmill, whereas our subjects warmed up on an ergometer cycle for 5 minutes. Ergometer cycle is likely to induce a more local fatigue in the quadriceps, compared to the jogging protocol. The specific warm up was also different where Laurentino et al (2012) performed fewer warm up sets at with lighter loads.

Maybe more importantly the current study lasted only 1 month, including 10 days of rest, whereas the study by Laurentino et al (2012) lasted 8 weeks, with a lower training frequency. This would likely allow their test participants to recuperate better and optimizing strength adaptations. The authors used an additional second of the concentric and eccentric phase, compared to the current study. An observation from the current study was that when fatigue set in, usually around 20 – 30 repetitions in the first set, the first noticeable sign was an increase in speed in the eccentric phase. This could probably be attributed to a compensation, trying to preserve energy, thus possibly making the repetitions less effective.

Laurentino et al (2008) found an increase of 35.3% increase in 1RM knee extension after having subjects perform either a moderate load (n=12, 60% of 1RM) or a high load (n= 6, 80% of 1RM) with BFR. Interestingly, the subjects performed 3-4 sets of intermittent submaximal unilateral knee-extension twice per week, for 8 weeks. Continuous BFR is thought to give better results, but these results might indicate that this is of more relevance with lower loads. The study used 120 seconds rest between exercise-sets, Also noteworthy, loads was adjusted higher if subjects were able to finish all sets before failure and lowered if subjects failed to perform the determined repetitions minus 2, optimizing load to correspond with daily form. The authors used a higher load than what is common with BFRRE and had substantially longer rest intervals than the 30 seconds of the current study. This could suggest that a higher load is sufficient to induce a metabolic build up even with cuff deflation between

exercise sets. In this regard, 120 seconds of rest might be too long for experienced lifters, but it might be adequate in untrained.

No increase was found in Isokinetic torque at 60 degrees (+2.6%). The mean increase in isokinetic strength was largely affected by one subject who displayed a reduction of -39.4% at day 29. One of the possible explanation for this could be that this subject was the only study participant who did not attend the Norwegian school of sports science. It is therefore a possibility that the other subjects were able to recuperate faster between bouts due to better training statuses. Decreases in muscle strength on day 9 and 22 was also observed for this subject. This could supports the possibility of a slower recuperation. Interestingly, on day 22, a 35% decrease in maximal isokinetic torque was found. This means that the subject experienced additional decrease in strength from day 22 to day 29. The CPK-levels remained similar (77 U/L) between these to time points, indicating no difference in muscle damage. The subject also experienced a decreases in maximal knee extension (-0.8% in 1RM) on day 29.

5.4 Correlations

A number of correlations were investigated. These will be justified and explained further in detail in the next few paragraphs

5.4.1 Increase in muscle size vs acute swelling

Given that muscle cell swelling is thought to be important for hypertrophic adaptations, we investigated the correlation between the acute muscle cell swelling and the long term increase in muscle size, as measured on day 29. Correlation analysis revealed no correlation for acute muscle cell swelling and long term increases in RF ($r = 0.02$) and moderate correlation in acute muscle cell swelling and long term increase in VL (r.=.0.46).

The lack of any apparent relationship could be due to the difference in magnitude between the two variables. The fact that we measured the acute muscle cell swelling after the very first BFRRE-bout (day 1) and then on day 15, after a 10 day rest period could mean that the time points for when these measurements was done was extra favourable in terms of the percentage of increase. Therefore, the numbers could look different if they were measured sometime later in the training week.

5.4.2 Correlation between increased muscle strength and increase in muscle size

It is generally accepted that muscle CSA determines muscle strength (Tonson et al. 2008; Raastad et al. 2010). Consequently, an increase in muscle size after a period of resistance exercise will typically be followed by an increase in maximal strength. Therefore, it was investigated whether the increased muscle size observed some days after the last training

session (day 29) would be correlated with an increase in maximal muscle strength. Specifically how the increase muscle size affected isokinetic strength and MVC.

However, no correlation (r = 0,08) was found between the increases in VL muscle thickness and increases in maximal isometric strength. A moderate negative correlation (r= -0.50) was found between increase in the CSA of RF and increase in isometric strength. A moderate positive correlation (r = 0.56) was found between increases in VL muscle thickness and increases in maximal isokinetic strength. A moderate negative correlation (r = -0.34) was found between increases in maximal isokinetic strength and increases in CSA of the RF. Previous studies on knee extension exercise have reported no differences in contribution of RF and VL during knee extension exercises (Alkner et al. 2000; Vaz et al. 1996).

BFRRE has previously shown to increase both isometric (Abe et al. 2006; Moore et al. 2004; Shinohara et al. 1998) and isokinetic strength (Burgomaster et al. 2003; Takarada et al. 2002; Sumide et al. 2009). This is consistent in the literature without BFRRE for both isometric (Narici et al. 1996; Ahtiainen et al. 2005) and isokinetic strength (Houch et al. 1996; Ruther et al. 1995; Nickols-Richardson et al. 2007). The lack of any strong correlation can be explained by differences in the magnitude of change in muscle strength and size, and also partly from individual differences where some deviant values affected the mean.

However, Individual studies reveals a varying degree of correlation between muscular strength and size. Several studies have found no correlation between strength and size both with (Takarada et al. 2004; Abe et al 2005b; Madarame et al. 2008) and without BFR (Jones et al. 1987; Garfinkel et al. 1992; Schott et al. 1995). Individual differences seems to decide what increases the most.

5.5 Delimitations

Some delimitations were made in order to make the results as precise as possible. We conducted post-tests 3 and 10 days after the last training bout. This is consistent with other similar research reports (Nielsen et al.2012). However, based on our experience with the strength tests on day 9, where several of the subjects reported feeling fatigued it could be that more rest than 3 days was needed. Furthermore, when considering either a decrease or increase in VL muscle thickness and RF CSA observed from day 5 to day 9 and so forth to day 15, it was decided to only use the post-test results from day 29. Consequently, this allowed for ultrasound measurements on legs without any swelling and strength tests with fresh leg. However, even though students were told to abstain from vigorous exercise the last

48 hours before the post-test, it is not possible to rule out participation in any activities that could conflict with the tests. Especially considering the students attended a sports school where some classes might be mandatory.

The mid-thigh portion of the quadriceps was needed in order to compare results from ultrasound with MR and the MR from the current study with other studies. Given that the femur bone is roughly 25% of height (Feldesman et al. 1990), mid-thigh was located by using the following estimation; Height (cm)/4. This answer represented the length of the femur and 50% of it was accepted as the mid-thigh. This estimation does not take into account individual differences in the height to femur length ratio, but it was used because no actual measurement of femur length was obtained. Consequently, the estimated mid-thigh location might have some errors, but the change in CSA at this location was rather stable over some cm in the proximal and distal direction.

5.6 Limitations

All ultrasound measurements were conducted on the same locations on the thigh. This would be considered a reliable and valid way of measuring pre and post effects after an intervention. Simultaneously, it is a weakness in the sense that ultrasound only measures one dimension at one location, in this case on the middle part of the thigh. Because of this we cannot exclude the possibility that we missed any other increases at a more distal or proximal location on the thigh during the intervention. The MR data suggests that the largest increases happened at a more distal location to the mid-thigh area used. This was consistent for both VL and RF.

Furthermore, ultrasound measurements were conducted only in the VL and RF. These muscles were chosen because muscle biopsies were obtained from the VL, and RF was considered most suitable for CSA measurements. VI and VM were disregarded because of time and resources. However, data from additional consecutive examination of VI and VM would be useful for comparison with MR data. Given that increases found on day 5 was either reduced or increased at day 9 and day 15, it could also be of interest to do more frequent measurements between day 9 and 15. This data could perhaps be helpful for determining the exact time course for the hypertrophic responses occurred.

The use of echo intensity could have made it easier to differentiate between muscle cell swelling and functional hypertrophy. However this test was not considered an option before the intervention period had started and the setting was not an option on the ultrasound machine.

BFRRE is a very intense type of training form and it has been known to cause severe DOMS (Wernbom et al. 2006). Four sets to volitional failure would suggests that a certain amount of motivation is required for someone to endure BFRRE over time. Students at NIH would have to be considered a very homogenous group. Largely because of the extensive training backgrounds and similar interest in sports. This could potentially mean that they would be better suited to handle such strenuous work out over time. Therefore, our findings can not be generalized to a broader or even another population. Furthermore, in all likelihood, the group in the current study would probably have a better understanding for such a training methodology and their expectations could have been affected by this, which again would make our participants less comparable to other populations.

In the current study we used the same size pressure cuff with a standardized cuff pressure for all test-participants. This is normal procedure in studies on BFRRE argues Fahs et al (2012). However, it is suggested that the pressure applied should be decided based on individuals anthropometrics and the width of the cuff (Shaw & Murray, 1982; McEwen et al. 2002; Younger et al. 2004). According to Loenneke et al (2011b), limb circumference seems to be the most important factor in determining the pressure occluding the arterial blood flow. Laurentino et al (2012) demonstrated that the wider the cuff, the less amount of pressure is needed to restrict blood flow, which is consistent with previous research (Crenshaw et al. 1988). Neglecting to consider these factors may not only decrease the general safety of BFRRE, it may also reduce the effectiveness (Loenneke et al. 2013b).

Another possible limitation could be the fact that several different studies were being conducted at the same time as the current one. This meant that a lot of people had access to and needed to be in the strength laboratory. It was not uncommon that other people, unrelated to our study, walked in or out of the lab during testing. It would be impossible to rule out a positive or a negative effect on the subject's performance on the tests under these conditions. Some subjects might thrive when they have an audience, increasing their effort and some

might give in faster when the strain and discomfort sets in. Also sometimes when performing tests subjects would get additional encouragement from highly regarded scientists who were in the lab at the time.

The BFRRE-protocol and the 1RM-tests had several different test leaders and internal validity was only addressed during the training of the researchers. While the 1RM-tests were easy to control, the BFRRE-protocol required more of the leader because of numerous additional variables. As a result, one subject was able to perform 52 and 90 repetitions on the first exercise-set on two different test days, with different test-leaders. No difference in CPK-levels was observed between the two days or the rest of the week. We attributed this to one test-leader allowing more movement in the hip, while the other test leader argued that additional hip movement was a sign of compensation due to concentric failure in the knee extensors and thus stopped the set.

5.7 Future perspectives

5.7.1 Tracking time course for muscle swelling

Tracking the increase and decrease of the exercise-induced muscle swelling over time could possibly be of interest. The acute muscle cell swelling might not increase to a similar extent later in the training week, compared to what was found during the first training days (day 1 and 15). If indeed muscle swelling is the potential anabolic stimuli it is thought to be, it should be measured often to see how the swelling behave after several bouts. Identifying it's time course from immediately after a bout to total recovery could give the opportunity to manipulate the swelling with exercise so that the muscle cells are in a constant stretched state, thus optimizing the anabolic environment in the body. This could also perhaps give new insight for optimal training frequencies with BFRRE.

Furthermore it should be investigated how intensity affects muscle swelling. Specifically, if there is a difference in the size of the acute muscle swelling depending on whether the BFRRE is performed to failure or not. If there is a difference in the size of the swelling, it could be important to know if twice daily bouts of sub maximal intensity can induce muscle swelling to a similar extent as bouts performed to failure. This could have important implications for how long a time subjects should rest before performing post-tests.

5.7.2 Long term effects of training with BFR

Resistance exercise is associated with increases in muscle strength and numerous other health beneficial adaptations (ACSM 2009). Recently, new developed norwegian exercise guidelines were outlined and published (Helsedirektoratet, 2014). These recommendations includes at least 150 min of exercise/per week with moderate intensity. Exercise that improves muscle strength and the skeletal system should be performed at least three times per week. These recommendations are meant to be followed on a regular basis throughout the life span for lasting effect.

The efficacy of BFRRE for increased muscle growth and size has been well described earlier in this paper (Fujita et al 2008; Wernbom et al. 2008; Loenneke et al. 2012), however few studies have run over a long time course. This proposes the question whether BFRRE is a sustainable or even effective training methodology over a longer time-period.

Takarada et al. (2000b) and Ohta et al (2003) conducted the two longest studies on BFRRE to date. The subjects in the latter studies borrowed manual air pumps for an occlusion-set and trained at home for 16 weeks after ACL-surgery, making the results questionable. In the study by Takarada et al (2000b) 16 weeks of BFRRE was found to be well tolerated in 24 post-menopausal women (58.2±6.6 years old). Another important point is that, while the current results are promising in regards to safety, the most important factor in establishing clear evidence of safety would have to be more long-term studies.

5.7.3 Assessing safety

A lot of research has been conducted on resistance exercise with blood flow restriction, but the safety aspect of the training still is brought up as something that needs further examination (Wernbom et al. 2006; Clark et al. 2011).

Earlier in this report, the author made a reference to a survey by Nakajima et al (2006). The survey included 105 facilities that offered BFRRE at the time (2006) and attempted to discover any adverse effects observed because of BFRRE. The authors findings suggested that the adverse effect of BFRRE were minimal. It should however be noted that the survey by Nakajima and his research team is not without any weaknesses. It can be argued that it was in all of the different establishment's best interests that BFRRE looked safe, so the numbers reported back may be biased and even incorrect.

One of the subject in the current study dropped out due to severe DOMS. Blood samples revealed CPK- levels of 4188 U/L. Resistance exercise has been observed to elevate CPK-levels from 2000 - 10000 U/L depending on the intensity and movement pattern of the exercise (Clarkson et al. 1992). Increased serum CPK is also considered the most reliable indicator for rhabdomyolysis and levels elevated to > 10 000 U/L is diagnosed as rhabdomyolysis (Iversen & Røstad 2010).

Clarkson et al (2008) argues that different individuals responds different to exercise and that some produces higher CPK-levels than others. Our subject might be a high-responder. Even though CPK-levels suggested a good amount of muscle damage, but no severe health problem, the subject still had to use crutches for one week after the incident. The subject also reported discomfort while sleeping and pain whenever he used his leg. Arguably, all exercise comes with a certain risk. The current body of evidence suggest that BFRRE is a safe and well-tolerated exercise methodology in all populations. Still, for wider acceptance and usage of BFRRE a couple of guidelines needs to be outlined. Particularly guidelines for cuff width seems to be of importance.

Given that the prospect of BFRRE seems to grow globally, a solution could be to design an universal standardized pressure cuff in different sizes. The lack of a standardized cuff type and pressure causes several methodological concerns in terms of determining best study design and comparability between research reports. For instance, studies have shown that cuff width is important for pressure. Previous research has used pressure cuff with widths of 4 (Madarame et al. 2008) 5 (Fujita et al. 2008) and 9 cm (Takarada et al. 2004) on the lower limbs. In these cases, the cuff pressures were 160-240 mmHg, which was found to be both effective and well tolerated for increasing muscle strength in the knee extensors (Fahs et al. 2012). On the other hand, a 13 cm wide pressure cuff inflated to greater or similar to 230 mmHg, was observed to cause complete arterial occlusion (Kacin & Strazar, 2011). In this study the authors found significantly reduced CSA in VI and a tendency for reduced hypertrophy of the other three quadriceps muscles at the proximal part of the thigh where the cuff was applied. This study illustrates how cuff width is important for choice of cuff pressure. Soft tissue surrounding the artery could also be of importance in terms of arterial blood flow restriction. Higher cuff pressure is also associated with higher increase in blood pressure and heart rate (Sakamaki et al. 2011).

6.0 Conclusion

As expected, the onset of BFRRE induced a rapid increase in VL muscle thickness and RF CSA during the first training week. Importantly, the decrease in VL muscle thickness and RF CSA observed during the rest period suggests that a large portion of the increase found in the first training week likely was a result of cell swelling. Slight differences in increases in muscle size after the end of the second training week and the first post test (day 22), as well stable measurements of muscle size from day 22 to 29, suggest that a more permanent increase had manifested after the second training week. The increase in RF CSA was verified by MR analysis at the mid-thigh region, but no changes in VL CSA was observed in the MRI analysis.

It was also hypothesized that any increase in muscle size would be followed by increase in muscle strength**.** This gave some conflicting results where a significant increase in MVC was observed, but no significant increases in 1RM in knee extensions or in peak isokinetic torque at 60 degrees/second was observed. Even though increases was observed in VL muscle thickness and RF CSA, the lack in muscle strength for isokinetic strength and 1RM can likely be explained by no change in total quadriceps CSA and/or not enough rest from the last training bout to day 29 in order to recover from any overreaching effect.

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