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Tissue oxygen saturation during different blood flow restriction exercise protocols

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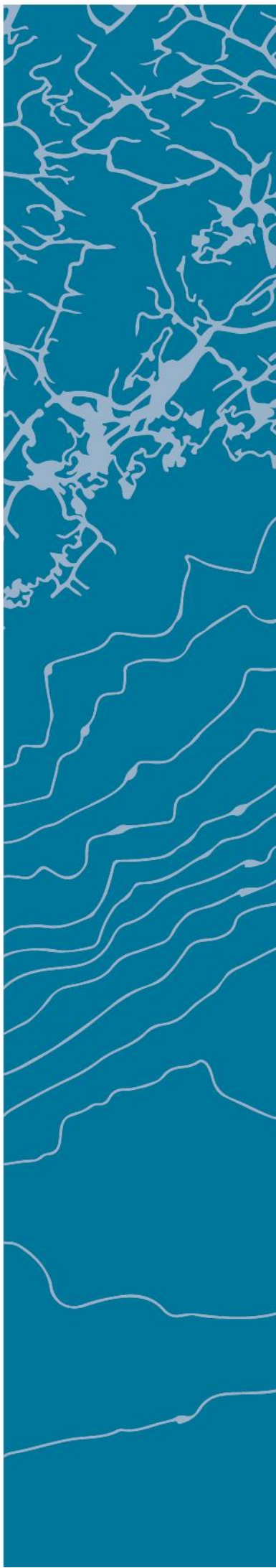

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ABBREVIATIONS

BFRRE – Blood flow restricted resistance exercise

BFR-Blood flow restriction

NIRS- Near- Infrared Spectroscopy

MVC- Maximal voluntary contraction

O_2 – oxygen

StO_2 – Tissue oxygen saturation

1 RM – 1 repetition maximum

mRNA – Messenger RNA

mTOR – Mammaligan target of rapamyosin

mTORC1- mammaligan target of rapamycin complex 1

MAPK – Mitogen activated protein kinases

RCT – Randomized controlled trial

CV – Coefficient of variation

MRI – Magnetic Resonance Imaging

CT - Computed tomography

DOMS – Delayed onset of muscle soreness

RPE – Rate of perceived exertion

CSA- Cross sectional area

LOP- Limb occlusion pressure

MRI- Magnetic resonance imaging

EMG- electromyography

IGF-1- Insulin growth factor-1

ABSTRACT

Introduction: Research is limited in regard to how hypoxia affect the muscular adaptions seen in blood flow restricted resistance exercise (BFRRE). The primary objective of the present study was to investigate the level of tissue oxygen saturation (StO_2) during a failure and a submaximal BFRRE protocol in three muscle areas; vastus lateralis distal (VLd), vastus lateralis proximal (VLp) and vastus medialis (VM). Secondarily the association between StO2 during one session of BFRRE and muscle thickness 17 days post intervention. In addition, the correlation between StO_2 and the amount of subcutaneous fat.

Methods: Twenty untrained men and women performed high intensity, low load, unilateral leg extensions with BFRRE. Each subject had one leg randomized to follow a failure protocol with four sets to concentric failure, the other leg to follow a submaximal protocol, with training load set to 20% of 1 RM. Near-Infrared Spectroscopy was used to quantify StO_2 in VLd, VLp and VM during training. Ultrasonography was used to measure muscle thickness and subcutaneous fat.

Results: No significant difference was observed between the submaximal and failure groups in StO_2 level for VLd, VLp or VM. StO_2 during BFRRE was not correlated to muscle thickness post intervention. There was a significant correlation between amount of subcutaneous fat and StO_2 in VLd ($r=0.52$; $p<0.001$), and VLp ($r=0.58$; $p=0.001$).

Conclusion: The submaximal and failure protocols induce similar StO_2 levels. Subcutaneous fat should be taken into account in NIRS-measurements. More research is needed regarding how different BFRRE protocols may affect the level of hypoxia.

Keywords: kaatsu; occlusion; hypoxia; near infrared spectroscopy.

SAMMENDRAG

Introduksjon: Det er manglende forskning angående hvordan hypoksi påvirker de muskulære tilpassingene sett i trening med redusert blodtilførsel (BFRRE). Målet med studien var å undersøke nivå av oksygenmetning i muskelvev (StO_2) under en utmattelse-protokoll og en submaximal protokoll av BFRRE i tre muskel-områder; vastus lateralis distal (VLd), vastus lateralis proximal (VLp) and vastus medialis (VM). Sekundært assosiasjonen mellom StO2 under økt nummer tre av BFRRE og muskeltykkelse dag 17 post intervensjon. I tillegg, korrelasjonen mellom StO_2 og mengden subkutant fett.

Metode: Tjue utrente menn og kvinner utførte høy intensitet, lav belastning, ett-bens kneekstensjon med BFRRE. Hver deltager fikk ett ben randomisert til å gjennomføre fire sett til utmattelse, det andre benet fulgte en submaximal protokoll, med treningsbelastning på 20% av 1 RM. Near-Infrared Spectoscopy ble brukt til å kvantifisere StO_2 i m. vastus lateralis proximal (VLp), m. Vastus lateralis distal (VLd) og m. vastus medialis (VM) under trening. Ultralyd ble brukt til å måle muskeltykkelse og underhudsfett.

Resultat: Ingen signifikant forskjell ble observert mellom den submaximale og utmattende-protokollen i StO_2-nivå for noen av muskel områdene. StO_2 under BFRRE var ikke korrelert med muskeltykkelse 17 dager post intervensjon. Det var en signifikant korrelasjon mellom mengden subkutant fett og StO_2 i VLd ($r=0.52$; $p<0.001$), og VLp ($r=0.58$; $p=0.001$).

Konklusjon: Den submaximale og utmattende protokollen førte til tilsvarende nivåer av StO2. Subkutant fett bør tas i betraktning under NIRS-målinger. Det er behov for mer forskning om hvordan nivået av StO_2 påvirker hypertrofi.

Nøkkelord: kaatsu; okklusjon; hypoxi; near-infrared spectroscopy.

PART 1:

THEORETICAL FRAMEWORK & METHODS

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1 Introduction

Increases in muscle growth and strength is most commonly known to be promoted by high intensity resistance exercise of >60% of 1 repetition maximum (1RM) (Evetovich, 2009). However, more recent studies have investigated resistance exercise with intensities as low as 20% of 1RM in combination with vascular blood flow restriction (Karabulut et al., 2012). With similar muscular benefits as high intensity resistance exercise, blood flow restricted resistance exercise (BFRRE), also called Kaatsu has become a popular exercise modality in a variety of specific populations (B. J. Schoenfeld, 2013). Reports have shown sufficient muscle hypertrophy and strength adaptions following blood flow restricted exercise with very little or even no resistance (Abe, Kearns, & Sato, 2006), resulting in minimal joint and ligament strains. This make BFRRE convenient for rehabilitation for (Wernbom, Järrebring, Andreasson, & Augustsson, 2009), trained individuals and athletes (Manimmanakorn et al., 2013) and elderly (Cook, LaRoche, Villa, Barile, & Manini, 2017).

The present master thesis is a part of a larger project. The overall purpose of this project was form a submaximal protocol and compare it to the existing failure protocol prescribed by Nielsen et al., (2012). The submaximal protocol has been added and adjusted through the previous research within our group. After one week of BFRRE, Nielsen and co-workers observed an increase in muscle growth (Nielsen et al., 2012). However, this plateaued in the second and third week. A 10-day recovery period was therefor included in the intervention executed by Bjornsen et al., (2018). It was hypothesized that this would lead to further gain in the second block of BFRRE (Bjornsen et al., 2018). A higher training volume was also seen performed by the participants in this study, which was believed to be a result of the difference in contraction velocity (1.5-seconds cadence in (Nielsen et al., 2012), versus 1-second cadence used in previous work completed by our research group). There have been speculations with respect to whether the exercise volume and level of stress in previous studies (Nielsen et al., 2012) might have been considerably lower than what was received in the previous work conducted by our research group. This has led to the implication of a comparison of a submaximal and a failure BFFRE protocol in previous in previous work by our group, however, the submaximal protocol was concluded to have been too strenuous. Based on this, we hypothesize that a submaximal protocol adjusted to be less strenuous than the one previously introduced in previous research within our group, and we will further compare it to a failure protocol in the present project.

The detailed mechanisms of the muscle growth seen after BFRRE are not yet fully understood and still under investigation (Abe et al., 2005). However, it is suggested that local hypoxia induced by the restriction of local blood flow plays a greater part in the anabolic muscular responses observed after a period of BFRRE. Muscle contractions combined with high loads will itself restrict blood flow and is considered an important mechanical stimulus for muscle growth (de Ruiter, de Boer, Spanjaard & de Haan, 2007). The restriction of blood flow during low load resistance exercise promoted by the application of a tourniquet (restriction band) and air pressure induces an intermuscular hypoxic environment, thought to increase the metabolic stress (Loenneke, Fahs, Rossow, Abe & Bemben, 2012), another stimulus for muscle growth.

There is a consensus amongst researchers that mechanical tension and metabolic stress are both contributing factors to the muscular adaptions seen in BFRRE. However, metabolic stress including the hypoxic muscular environment and the accumulation of metabolites are thought to be responsible for a greater proportion of the stimulus (Ganesan et al., 2015). Hypoxia seem to also be closely linked to cell swelling, fast twitch muscle fiber recruitment, production of reactive oxygen species, elevated systemic hormone production, muscle damage, all considered important factors in the process leading to muscle growth (Nishimura et al., 2010). Nishimura and colleagues have shed light on the need of more investigation around how the level of O_2 affects muscle hypertrophy, suggesting the use of several levels of hypoxia to determine a dose-response effect (Nishimura et al., 2010). Investigations of muscle tissue oxygenation (StO_2) currently most commonly performed through the none-invasive method of Near- Infrared Spectroscopy (NIRS). This method is used to gain insight into the local muscle oxygen saturation. Additionally, it will give indications of the type of metabolism in a specific area of a working muscle under a particular activity and/or intensity (Perrey & Ferrari, 2017). Ultimately, the investigation of hypoxia in relation to muscular adaption seen in BFRRE is important to develop proper prescriptions of the training method in the future.

2 Objectives

The main objective of the present study was to investigate the level of StO_2 during and a submaximal protocol (20-10-10-10 repetitions) and a failure protocol (four sets to voluntary concentric failure) of unilateral knee extensions with low load BFRRE.
Secondary objectives are listed below:

1) To investigate the association between the level of StO_2 during one session of BFRRE and the change of muscle thickness after a period of BFRRE.
2) To investigate the association between StO_2 during a session of BFRRE and the amount of subcutaneous fat.

3 Theoretical framework

3.1 Muscle growth

Skeletal muscle is a dynamic and very adaptable to change. An increase in muscle size could occur as a result of increase in fiber size through increase in the number of muscle fibers (hyperplasia) and/or an increase in of connective tissue within the muscle (hypertrophy) (Komi, 2003. S. 252). Muscle growth is a result of greater regeneration of muscle proteins than breakdown of proteins over a longer period of time (Ratamess, Alvar, & Evetoch, 2009). Muscle protein synthesis and breakdown is regulated by a series of complex interactions of anabolic and catabolic signalling pathways, increased satellite cell activation, proliferation and fusion of satellite cells (Pearson & Hussain, 2015). Many of these signalling pathways are yet to be investigated (Schoenfeld, 2010), whilst some have been demonstrated to link directly to cellular processes that influence transcription and translation (Mayhew, Hornberger, Lincoln & Bamma, 2011). Amongst the key signalling pathways are mammaligan target of rapamycin (mTOR); known to increase muscle protein synthesis and prevent protein breakdown (Sue et al., 2001) and mitogen-activated protein kinase-family (MAPK); known to mediate swelling-induced anabolism (Loenneke, Fahs, Rossow, Abe & Bemben, 2012a). The two primary stimuli to trigger muscle growth are mechanical tension and metabolic stress, most likely combined.

3.1.1 Metabolic stress

The accumulation of metabolites such as inorganic phosphate, lactate and H^+ have been shown to result in metabolic stress, an important mechanism for muscle growth (Schoenfeld, 2013; Takarada et al., 2012; Loenneke & Pujol, 2009; Suga et al., 2009). Metabolic stress is thought to work alongside mechanical tension as a mechanism to promote muscle growth (Schoenfeld, 2010). When comparing resistance training protocols with and without blood flow restriction metabolic stress is thought to play an even greater role in BFRRE than traditional high load resistance exercise (Loenneke, Wilson & Wilson, 2010; Pearson & Hussain, 2015). Elevation of systemic hormone production (Reeves et al., 2006), increased

production of ROS (Kaijser et al., 1990; Kawada & Ishii, 2005), muscle cell swelling (Loenneke et al., 2012a), increased recruitment of fast twitch muscle fibers (Yasuda et al., 2009) and muscle damage are all considered to be a result of metabolic stress and will eventually affect muscle growth (Pearson & Hussain, 2015). However, this underscores that some of these mechanisms potentially are more strongly correlated with mechanical tension than metabolic stress (Pearson & Hussain, 2015).

3.1.2 Mechanical tension

Mechanical tension is achieved through the generation of force and muscle stretch (Schoenfeld, 2010), and is considered to act as a primary mechanism along with metabolic stress for muscle growth during BFRRE (Pearson & Hussain, 2015). Several underlying mechanisms such as mechanotransduction (Schoenfeld, 2013) increased localised hormone production (Tatsumi et al., 2006), muscle damage (Tatsumi et al., 2006), reactive oxygen species (ROS) production (Uchiyama, Tsukamoto, Yoshimura, & Tamaki, 2006) and increased fast-twitch fibre recruitment (Suga et al., 2009) are initiated by mechanical tension. This results in increased protein synthesis through activation of signalling pathways (Sue et al., 2001) and/or satellite cell activation and proliferation (Tatsumi et al., 2006). The low mechanical tension linked with BFRRE is the reason for questioning if the above-mentioned mechanisms are induced to any greater extent.

The amount of muscle mass seems to be highly associated with strength (Folland & Williams, 2007), and therefore, play an important role in the increase of muscle strength. Muscle strength is beneficial for people of all ages. It has been demonstrated to affect and improve physical performance, prevent injuries, improve the everyday life, in particular for elderly or improve psychological factors, such as self-esteem or self-imaging (Raastad, Paulsen, Refsnes, Rønnestad & Wisnes, 2010; Suchomel, Nimphius & Stone, 2016). When considering the importance of muscular growth and its significance for muscular strength, it is necessary to point out that other components also contributes to increases in maximal strength. Along with sex and age, there are peripheral factors such as pennation angle, fibre type, muscle fibre length and the specific muscle activation, and central factors such as neural factors, the interactions between antagonists and synergists and the voluntary activation (Folland & Williams, 2007).

3.2 Blood flow restrictive resistance exercise

It is commonly known that high intensity resistance exercise using loads of >60% 1 repetition maximum (1RM) promotes an increase in muscle growth (hypertrophy) and strength However, more recent studies have investigated resistance exercise with intensities as low as 20% of 1RM in combination with vascular blood flow restriction (Scott, Loenneke, Slattery, & Dascombe, 2015). These studies demonstrate that it is as beneficial for muscle hypertrophy as conventional strength training >60% 1RM (Takarada et al., 2002). Although it should be noted that BFRRE have no further increases in hypertrophy, strength or fibre recruitment beyond that of traditional high load resistance training (Laurentino et al., 2008). Exercise with blood flow restriction (also called KAATSU training) was first introduced for experimentation in 1966 by Yoshiaki Sato, and further patented in 1997 (Sato, 2005). Since then, it has been shown to be an effective method for maintaining or increasing hypertrophy and muscular strength in different populations such as; elderly or injured (Wernbom et al., 2009), astronauts (Loenneke & Pujol, 2009) and athletes (Manimmanakorn et al., 2013).

Blood flow restriction (BFR) is typically achieved by restricting blood flow to the muscle with the application of external pressure such as a pressurized cuff, a tourniquet or an elastic banding applied over the proximal portion of the upper or lower extremities (Pearson & Hussain, 2015). Pressure is suggested to be sufficient when the venous outflow is reduced but the arterial inflow is maintained in the blood flow restricted extremities. However, the vasoconstriction induced by the reduced venous outflow may also result in partial reduction of arterial inflow (Pearson & Hussain, 2015).

The five acute training variables introduced by Fleck and Kraemer (Fleck & Kraemer, 1988) choice of exercise; order of exercise; load or intensity; volume of exercise; and rest directly affect the great variability of resistance exercise. Scott et al. (2014) includes factors that affect the dose of hypoxia during BFRRE, such as the type and dimensions of the cuff, pressure and duration of BFR and exercise to failure or a predetermined number of repetitions. Wernbom, Augustsson, & Thomee, (2007) suggests that the rate of muscle hypertrophy is greatly affected by training frequency when conducting training over shorter periods. They also suspect a potential stagnation or overtraining using high frequency over longer periods, there is however a lack of data/studies/evidence proving this. Wernbom et al. (2007) further conclude that moderately heavy loads seem to be the most beneficial for the greatest gains of hypertrophy.

Evidence suggests greater cuff pressure appears more effective than less, >150mmHg, although it is highlighted that cuff pressure should be individualized based on the perimeter of the thigh (Slysz et al., 2016). The absolute cuff pressures have been used to study effects of BFRRE interventions (Abe et al., 2005). These may be highly unreliable as individuals with different thigh perimeter will have received different levels of BFRRE under the same pressure and further experience different training responses (Fahs, Loenneke, Rossow, Tiebaud, & Bemben, 2012). An increasingly used method to achieve similar pressure despite differences in anthropological measurements is the limb occlusion pressure (LOP), found through individual measurements of the pressure needed to completely occlude the blood flow in the particular limb. The relative limb occlusion pressure is calculated based on the individual's respective limb occlusion pressure (LOP) and thought to be as important for BFRRE as relative intensity is to traditional strength training (Buckley et al., 2015; Scott et al., 2015).

3.2.1 Frequency and load

Wernbom et al. (2007) conclude that training preformed with maximum effort or to failure, achieving the greatest recruitment of muscle fibers possible might be as important as training load. However, the authors also stated that the program designs that produce the largest increases in strength does not necessarily result in the largest increases in muscle mass.

Martìn-Hernàndez et al. (2013) investigated muscle strength and thickness after two different volumes of BFR and compared it with high-intensity training. The study included four groups, two using BFRRE; one low volume, low intensity using 20% 1RM (performing a single bout of 30-15-15-15 repetitions with 1-minute rest in between sets); one high volume, low intensity, using 20% 1RM (using two bouts, 5 minutes between, each including a bout of 30-15-15-15 repetitions with 1-minute inter-set rest). There was no evidence of a relationship between the dose of low-load BFRRE and the adaption of muscle strength or muscle thickness. Martín-Hernández et al. (2013) suggest a maximum of 75 reps per session per muscle due to the high effort required when performing BFRRE.

Slysz et al. (2016) suggests in their meta-analysis that greater strength and hypertrophic gains comes with loads of ≥20% 1 RM than aerobic exercise alone. They speculate that the efficacy will improve proportionally with greater loads. Interventions lasting ≥ 8 weeks seem to work

better for increases in muscle size than < 8 weeks. Slysz et al. (2016) also conclude that loads 20-50%1RM is necessary to induce similar adaptions in muscle strength and hypertrophy as conventional training using 45-60% 1RM in untrained individuals and 80-85% in trained individuals.

High frequency of training is suggested to greatly affect gains in muscle hypertrophy for shorter periods of exercise. High frequent resistance exercise over longer periods of time are yet to be investigated, although it is suggested that this might induce stagnation or overtraining. Most types of exercise seem to achieve the greatest gains through the use of moderate to heavy loads. However, performance of sets with maximum effort to near failure or failure seem to and is suggested to be the important factor for muscle gains (Loenneke, Wilson, Marín, Zourdos & Bemben, 2012b). Wernbom et al. (2007) investigated the dose-response relationships for the development of hypertrophy using three different models. The meta-analysis by Loenneke et al., (2012b) reported greater hypertrophy after an exercise frequency of 2-3 days per week compared to 4-5 days per week. Schoenfeld, Ogborn & Krieger (2016) observed that two sessions per week induced greater increases in hypertrophy than one session per week. It is agreement around the importance of progression and individualisation for exercise prescriptions (Kraemer et al., 2002). Overall volume and/or intensity may also need a gradual increase to further achieve physiological adaptions, and sometimes also periodization (Wernbom et al., 2007).

3.2.2 Summary

Low load BFRRE induce more strength and hypertrophy than work-matched resistance exercise alone. Although the potential for hypertrophic adaptions seem to be comparable between high load conventional resistance exercise and low load BFRRE. Adaptions in hypertrophy following BFRRE seem to be greater with loads of 30% than 20%. There does not seem to be any relationship between the dose of low load BFRRE and the muscular adaption hypertrophy. There is lack of research of lower volumes, in terms of what is necessary for muscular adaptions. Hypertrophy is speculated to be more affected by frequency when exercising over shorter periods. Evidence clearly indicates that BFRRE induce an intermuscular hypoxic condition (Takarada et al., 2000). No one has to our knowledge compared a submaximal and a failure protocol in regard to the level of StO_2 during BFRRE. This gives us the opportunity to get a better insight to the presence and level of

hypoxia and its potential effect on the muscular gains seen in BFRRE, and also if and to what extent there are differences in a low load BFRRE completed to submaximal or failure.

3.3 Hypoxia

Hypoxia can be defined as the "reduction in oxygen level below that normally experienced by an organism or cell" (Nikinmaa, 2013). In altitude, this is often referred to as oxygen availability (West, 2006) and is an example of hypobaric hypoxia, a result of a decrease in total air pressure (Nikinmaa, 2013). The oxygen availability of which the organism or cell normally experience can be referred to as normoxia (Nikinmaa, 2013).

Oxygen within the body is transported in the blood component called plasma, and more importantly bound to haemoglobin (within red blood cells). The O_2 delivery system is flexible and provide rapid regulations to adjust for changes in oxygen requirements within tissue, or to survive low level of nutrition or nutritional deficiencies. This haemoglobin affinity for oxygen is a system with a sigmoid shape curve, also called the "oxygen equilibrium curve" (Jensen, 2004). The haemoglobin molecule is in equilibrium between two states; the relaxed structure with a high affinity for oxygen (oxyheamoglobin) and the tensed structure with a low affinity for oxygen (deoxygenated haemoglobin) (Jensen, 2004). These structures determine the binding and delivery properties of the red blood cells. With a high oxygen tension, such as in the lungs, blood normally become fully saturated and haemoglobin assume the relaxed structure. The decrease in the partial pressure of oxygen seen in the microcirculation promote oxygen offloading and haemoglobin will shift to the tensed structure (Jensen, 2004). Only 25% of oxygen is extracted from the blood during rest, and the venous point will be on the shoulder off the curve. During exercise, a further increase in oxygen unloading will happen at the steep end of the curve. A decrease in pH will further facilitate oxygen unloading, also called "the Bohr effect" and is seen as a right shift of the oxygen equilibrium curve (Jensen, 2004). Oxygen delivery depends to a large extent on blood flow. A hypoxic vasodilation is a mechanism which task is to reinsure a sufficient blood supply to the tissues when the O_2 demand increases (Galdwin et al., 2005; Jensen, 2009).

3.3.1 Systemic hypoxia

When hypoxia depend upon the respiratory system, the haemoglobin concentration will determine which direction the shift of the equilibrium curve will take: An increase in haemoglobin will lead to a right shift, whilst a normal amount of haemoglobin will lead to a left shift (Lenfant, Ways, Aucutt, & Cruz, 1969). The exposure of hypoxia through a reduced barometric pressure in high altitudes and decrease in total air pressure will therefore (in people with normal amount of haemoglobin) negatively affect the equilibrium curve by shifting it to the left and initiate a lower saturation of oxygen between inspired air and haemoglobin within the body (Lenfant et al., 1969). The arterial oxygen content and blood flow is thought to determine the muscular oxygen content (Jensen, 2004). Thus, a decrease in oxygen pressure within inspired air will result in a decrease in oxygen available for extraction within the muscles both at rest and during exercise (Raynaud et al., 1986). A decrease in muscle oxygenation can also be a result of an increase in oxygen consumption, such as during exercise (Kawaguchi, Tabusadani, Sekikawa, Hayashi, & Onari, 2001). The consequence will be a hypoxic environment, and when it affects the entire body it will be referred to as 'systemic hypoxia' (Jensen, 2004; Winslow, 2007).

3.3.2 Local Hypoxia

Local oxygen exchange is dependent upon the peripheral arterio-venous O_2 diffusion-capacity and arterial blood flow, more commonly known as "Fick´s principle". As the oxygen demands by the tissue increases, such as from rest to exercise, a dilation of arterioles occur. Arteriolar networks are the mechanism controlling the distribution and magnitude of capillary perfusion within the tissues. More capillaries are recruited to extract O_2 from the incoming blood. This allows for more surface area for O_2 exchange. Under physical conditions, peripheral O_2 exchange is thought to be limited by the blood flow (Pittman, 2011).

During hypoxic exercise the overall muscle oxygen content is decreased by reduced arterial oxygen content and metabolic demands. During normoxic exercise however, only the venous blood is deoxygenated by metabolic demands (Costes et al., 1996). Restriction of blood flow is a method for inducing localized hypoxia in normoxic environments, aiming to induce hypoxia in a part of the body. This is done by wrapping a band or placing a cuff around the proximal part of any of the limbs and inflating it to create blood flow restriction (Takarada et al., 2000) (often using a pressure between mean systolic and diastolic blood pressure, which is considered a moderate occlusion thought to compress underlying veins (Wolthuis, Bergman,

& Nicogossian, 1974). This will limit the oxygen delivery to the working muscles and induce a hypoxic environment distal to the restricted area (Sato, 2005). According to (Takarada et al., 2000), an external pressure between the mean systolic and diastolic blood pressure is thought to suppress both the venous outflow and arterial inflow to the muscle. Hamaoka et al. (2000) found that during arterial occlusion, partial oxygen pressure within the muscles and the venous partial oxygen pressure were decreased to a similar level, indicating no oxygen gradient between the muscle tissue and the vessels. This results in a decrease in mitochondrial oxygen availability and respiration, leading to a more anaerobic metabolism. The level of pH within the muscle is thought to be a factor reflecting this and has been used as an indication of present hypoxia within muscles (Hamaoka et al., 2000). Hypoxia created by occlusion pressure is further facilitated by muscle contractions during exercise (Winslow, 2007). Despite findings of hypertrophic responses after resistance exercise in environments intended to induce a systemic hypoxia (e.g. hyperbaric chamber or hypoxic environment), a greater variability seem present due to physiologic adaptions linked to the cardiovascular systems and not related to a local stimulus (Slysz, Stultz, & Burr, 2016).

3.3.3 Hypoxia during exercise

Skeletal muscles undergo different changes in their mechanical and metabolic properties as a result of exercise stimuli. These changes are specific to the type of stimuli (Takarada et al., 2000). Resistance training with lower loads or endurance exercise results in an increase in the oxidative capacity (Holloszy & Booth, 1976), and also atrophy (Sundberg, Eiken, Nygren, & Kaijser, 1993), which is consistent with findings after a chronic exposure of high altitude. Notably, factors such as malnutrition, reduction in activity level and hypoxia have been shown as potential triggers for this decrease in muscle size observed (Narici & Kayser, 1995). In contradiction, several studies including Nishimura et al. (2010) and Kurobe et al. (2015), has conducted experiments of “train high, live low” with results of muscular hypertrophy. A combination of vascular occlusion and resistance training is thought to lead to muscle hypertrophy because of the hypoxia (Abe et al., 2006; Nishimura et al., 2010). Although, Nishimura et al. (2010) further states that hypoxia alone is insufficient to increase strength and hypertrophy.

Hypoxia is hypothesized to enhance the effect of strength training by its attribution to increase level of metabolic stress (Takarada, Sato, & Ishii, 2002), enhance muscle fibre recruitment (Moritani, Sherman, Shibata, Matsumoto, & Shinohara, 1992; Yasuda et al., 2009), increase

hormone activity (Reeves et al., 2006), initiate intercellular swelling (Loenneke, Fahs, Rossow, Abe & Bemben, 2012), increase muscle damage (Schoenfeld, 2013), and intercellular signalling (Schoenfeld, 2013). Some of the above-mentioned factors are theorized to thought to mediate muscle protein signalling and/or satellite cell proliferation for the induction of muscle growth (Pearson & Hussain, 2015). More detailed, hypoxia (independently or in combination with external loading) may lead to hypertrophy through the following mechanisms: Microfocal damage followed by subsequent regeneration of the muscle cell membrane (Grembowicz, Sprague, & McNeil, 1999) eventually resulting in changes in myogenic stem cell proliferation (Nielsen et al., 2012). Hypoxia is also speculated to, independently and/or in combination with stretch and/or contraction-induced nitric oxide, stimulate different myogenic stem cell mediators resulting in an addition of myonuclei to the myofibers resulting in hyperplasia (Nielsen et al., 2012). Scott, Loenneke, Slattery, & Dascombe, (2014) have illustrated the potential mechanisms of muscular strength and growth observed in BFRRE and their likely relationships (figure 1).

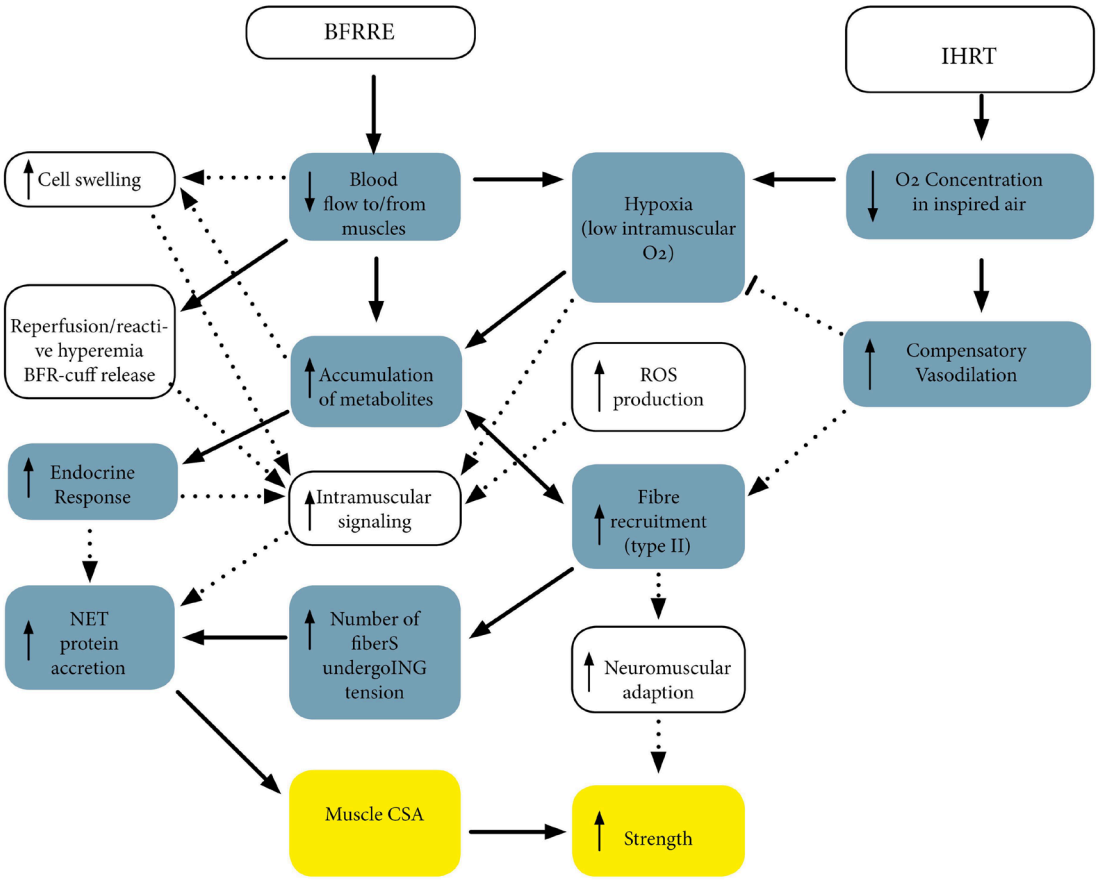

Figure 1: Overview of the potential mechanisms of BFRRE = blood flow restricted resistance exercise, obtained and modified after Scott et al. (2014). ROS = Reactive oxygen species, CSA = cross-sectional area, BFR = blood flow restriction, IHRT = intermittent hypoxic resistance training. Blue boxes present the likely mechanisms, white boxes present possible mechanisms. Yellow boxes present the possible hypertrophic and strength adaptions. Black arrows = likely links between mechanisms. Dotted arrows = possible links between mechanisms.

3.3.4 Systemic hypoxia and resistance exercise

Scott, Slattery, Sculley, Lockhart & Dascombe (2017) investigated the acute physiological responses to resistance training with moderate load in hypoxia. Three sets of 10 repetitions of squats and deadlifts with a load of 60% 1 RM was used, while the inter-set rest period was 60 seconds. The findings revealed no difference in tissue oxygen saturation (StO_2) between training in hypoxia vs training in normoxia. It is, however, discussed that the dose of hypoxia measured at the muscle were non- significant as a likely result of the moderate level of

hypoxic air used to exercise in. Scott et al. (2015) suggests that hypoxia during moderate load resistance exercise induce metabolite accumulation and muscle activation.

Katamaya et al. (2010) investigated acute deoxygenation in vastus lateralis during intermittent and sustained isometric, unilatereal knee extensions (at 60 % of maximal voluntary contraction) whilst breathing normoxic or hypoxic air. StO_2 was measured using NIRS. The exercise conducted in hypoxia showed a greater increase in deoxyhemoglobin/myoglobin and larger reduction in tissue oxygenation than the exercise conducted in normoxia. During sustained isometric exercise however, there were no significant differences in muscle oxygenation variables when exercising in hypoxia compared to normoxia. The differences in muscle oxygenation between normoxia and hypoxia during intermittent exercise are suggested to result in variations of accumulated metabolites and thus affect the rate of muscle fatigue, which are shown to occur significantly earlier in the hypoxic group (Katamaya et al., 2010).

A comparison of StO_2 during non-loaded submaximal squat exercise in altitude (1800 m) with exercise near sea level has shown that both altitudes resulted in dramatic decreases in oxy-haemoglobin after the start of exercise. A significantly lower decrease in StO_2 from resting levels in 1800 m than near sea level was observed after exercise in the 1800 m (Oguri et al., 2004). A similar size of StO_2 was also observed during submaximal sprint exercise when performed in hypoxic environments (Oguri et al., 2008). Based on this, it is suggested that acute exposure to moderate altitudes cause a more dramatic decline in peripheral StO_2 which is thought to potentially lead to a more hypoxic stress level within the muscles. It should also be mentioned that sprint athletes also have reached a higher degree of deoxygenation in hypoxia compared to untrained subjects suggesting they are able to approach closer to the physiological limit of oxygen extraction (Oguri et al., 2008).

StO_2 pattern during high load resistance exercise have previously been investigated during (10 RM) arm curls (Tamaki, Uchiyama, Tamura, & Nakano, 1994) through three sets with short rests. It was observed that the restriction of venous blood and the hypoxic state occurring in the dynamic muscle were accelerated throughout the three sets. This pattern also corresponded to the pattern registered during an additional blood flow restricted experiment also conducted on the arm (Tamaki et al., 1994) and thus suggesting similar muscular

responses. Several researchers have investigated the degree and pattern of StO_2 during different protocols of BFRRE (Ganesan et al., 2015; Karabulut et al., 2012).

3.4 Measuring tissue oxygen saturation

Oxygen saturation measurements of working muscle tissue may be a useful tool to get insight in the specific strength and endurance characteristics in knee extension training conducted with blood flow restriction (Hamaoka, McCully, Niwayama & Chance, 2011). A Near-Infrared Spectroscopy (NIRS) can be used to monitor local changes in oxy- and deoxyhemoglobin (and myoglobin) superficially in the muscles *in vivo.* Oxy- and deoxy-haemoglobin have different light absorption spectra, which make it possible to quantify them using NIRS sensors rending and receiving infrared light through the tissue (Benni, MacLeod, Ikeda, & Lin, 2018). The two components together provide an estimate of the total StO_2. The complex light propagation within tissue, individual variations in subcutaneous fat thickness and the maximal depth of NIRS (approximately half the distance between the light source and detector) are all factors known to potentially influence the measurements (Ruiter, Boer, Spanjaard, & Haan, 2005). It is also found that the light path and metabolism of subcutaneous fat is different from that in muscle tissue. Therefore, as the subcutaneous fat increases, measurements by NIRS will lead to an underestimation of StO2 (Van Beekvelt et al., 2001; Nasseri et al., 2016). Also, regional differences in muscle oxygenation and metabolism in different areas within or between agonists has been registered. Individual anatomical differences can result in underestimations or overestimations of StO_2 and potentially provide a different training response for the subjects when not using individualised occlusion pressure (stimuli). Thus, are there consistent suggestions of applying a relative occlusion pressure based on each individuals' limb occlusion pressure, and thigh circumference (Fahs et al., 2012; Lixandrão et al., 2018). Despite this, both non-individualised and individualised prescriptions have been shown to provide sufficient blood flow restriction to the working muscle (Lixandrão et al., 2018).

Muscle oxygenation is dynamic and can be measured invasively, yet perhaps more easily in vitro. NIRS is an increasingly used method for modelling oxygenation in muscle tissue and venous blood during and after exercise. This can be beneficial in investigating the mechanisms controlling local, internal (and external) respiration (Lai et al., 2009). Muscle oxygenation is dynamic and measurements using NIRS are assumed to mirror the invasively

measured venous oxygen saturation, although, the patterns are not always linked (Lai et al., 2009).

Exercise in normoxia seem to initiate a rapid decrease in oxygenated haemoglobin and myoglobin concentrations before monotonically increase again. Exercise in hypoxia the initial decrease is exponential and seem to reach a steady state within ~2 min. The dynamic concentrations of oxy- and deoxygenated haemoglobin and myoglobin has been shown to contribute comparably to the NIRS-signal through model simulations. The different oxygen saturation patterns of muscle and venous oxygen saturation dynamics observed under normoxia and hypoxia is thought to be caused by changes in oxygenated haemoglobin and myoglobin (Lai et al., 2009).

Near- Infrared Light (700-1000 mm) penetrates skin, subcutaneous fat or skull and underlying muscle/brain, and is either absorbed or scattered within the tissue. A number of factors is responsible for the relatively high attenuation of near- infrared light in tissue: Oxygen-dependent absorption from chromophores of variable concentration such as haemoglobin and myoglobin, absorption from chromophores of fixed concentration such as skin melanine, or light scattering. The detected signal is to a greater part from haemoglobin in small vessels (<1 mm diameter) or from myoglobin (Ferrari, Mottola, & Quaresima, 2004). The optimal pathlength of the light is affected by the scattering effects of the different types of tissue and is therefore longer than the distance between the source and the detector. The amount of subcutaneous fat is of great implication to the penetration depth of the near- infrared light; with a deeper penetration with low amounts of subcutaneous fat and a superficial penetration of the muscle tissue with high amounts of subcutaneous fat. The type of information required determines what type if NIRS device needed (Strangman, Boas, & Sutton, 2002). According to Kohri et al., (2002) it is generally accepted that the maximum muscle sensitivity is in the area between the source and the detector fiber tip and roughly half the distance of that between the source and detector down, below the skin surface, creating a banana-shaped region of sensitivity which extends both above and below this depth.

The main, and most known limitations of measuring muscle oxygen saturation using NIRS includes: The interference of subcutaneous fat. The uncertainty around the contribution of myoglobin to the NIRS signal. The effect of changes in blood volume. Also, there is difficulties predicting the effect of the changes in flow and volume on the observed NIRS

signal changes (Ferrari et al., 2004). Matsushita, Homma & Okada (1998) concluded that a subcutaneous fat and skin layer of 1.5 cm allows the near- infrared light to reach the shallow areas of muscles in the legs. How the subcutaneous fat affects the near-infrared light propagation has been investigated by many researchers (Hamaoka et al., 2000).

3.5 Hypoxia as stimuli and mechanism

3.5.1 Cell swelling

Cell swelling is thought to be the foundational mechanism where inhibition of catabolism and a switch of protein balance towards anabolism occurs through intracellular signalling (Loenneke, Fahs, Rossow, Abe & Bemben, 2012a; Schoenfeld, 2013). At least small increases in intracellular accumulation of metabolites is thought to be a result of hypoxia during BFRRE (Schoenfeld, 2013). The increased accumulation of metabolites creates a pressure gradient leading the blood flow into the extracellular matrix capsuling the muscle fiber and water into the intracellular space in the muscle fibres. The result is muscle swelling, a reaction that works as a threat on the structural integrity of the cell membrane (Loenneke et al., 2012a), causing the initiation of a signalling response that leads to reinforcement of its ultrastructure (Schoenfeld, 2010. Intrinsic volume sensors detect cell swelling which may further lead to activation of the mammaligan target of rapamycin (mTOR) and mitogen-activated proteinkinase (MAPK) pathways (Loenneke et al., 2012a). The mTOR pathway is believed to act to increase muscle protein synthesis through downstream targets (Sue et al., 2001). MAPK has been shown to link cellular stress with adaptive response in myocytes, regulating both growth and differentiation (Roux & Blenis, 2004). Both pathways are suggested to be needed for a maximal muscle protein synthetic response following resistance exercise (Fry et al., 2010; Loenneke et al., 2012). It should be mentioned that the complete mechanisms of cell swelling and its role in promoting potential anabolic effect is not well understood. Also, whether the cell swelling contribution to hypertrophy is mediated by metabolic stress alone or if mechanical tension also has an impact is still a standing question.

3.5.2 Satellite cells and myonuclei

In adult, uninjured skeletal muscle there has been found satellite cells in a resting state (Tatsumi et al., 2006). When myofibers are exposed to a myotrauma, the resident satellite cells become activated, proliferate and express myogenic markers (Hawke & Garry, 2001).

Thus, the regeneration of skeletal muscle is to a greater extent dependent upon satellite cells. Satellite cells expressing myogenic markers, also termed myoblasts, eventually fuse together or into existing muscle fibers or together and form new myofibers during regeneration of damaged muscle (Schultz & McCormick, 1994). This activation is initiated by hepatocyte growth factor and Nitric oxide (Tatsumi et al., 2006).

BFRRE has been suggested to initiate a microfocal damage of the muscle cell, followed by a consecutive regeneration (Grembowicz et al., 1999), a consequence of external loading, hypoxia or a combination of the two. Another promoted reason for the large amount of muscle satellite cell proliferation and activation following BFRRE is the stretch and/or contraction on different myogenic stem cell mediators, is thought to be mediated by hypoxia. Nielsen et al. (2012) conducted a study consisting of 3 weeks of high-frequent, low load BFRRE. The result from this study demonstrates highly marked gains in muscle fiber size, which was thought to be a result of considerable upregulation in myogenic satellite cell number. This process is suggested to potentially be a consequence of the hypoxic stimulation.

A recent investigation of the anabolic response after a single session of 1-leg knee extension in moderate hypoxic or in a normoxic environment has been conducted by Gnimassou et al. (2018). Their results showed blunted activation of protein synthesis after resistance exercise and downregulated the transcriptional program of autophagy. Hypoxia did regulate the expression of genes involved in a glucose metabolism, a myoblast differentiation and fusion and in muscle contraction machinery post exercise. This suggest that hypoxia did not potentiate the anabolic response on a short-term after resistance exercise. Exercise in hypoxia did however initiate transcriptional regulations which may possibly to translate into satellite cell incorporation and higher force production observed on a long- term (Gnimassou et al., 2018).

3.5.3 Reactive Oxygen Species

ROS signalling has been shown to contribute to muscle fiber adaption after muscular exercise and also after a period of prolonged inactivity. These contradictive effects of ROS signalling leading to either promotion of proteolysis and perhaps cell death (Jackson, 2008) or cellular adaption and protection against future stress is likely based upon the temporal pattern and the magnitude of the ROS generation (Powers, Talbert, & Adhihetty, 2011). Nitric oxide is one of the primary radicals generated in cells. BFR- induced stretch-, hypoxia-, and or contraction-

induced nitric oxide is suggested to activate signalling pathways within the cell such as the activation and proliferation of muscle satellite cells (Gutteridge & Halliwell, 2010).

3.5.4 Fast twitch muscle fiber recruitment

A muscle unit include the motor neuron and the specific fibers it innervates and are recruited after Henneman's size principle (Henneman, Somjen, & Carpenter, 1965). A motor unit consists of only a single type of muscle fibers, either the slow-twitch (type I) recruited first, at lower intensities, or the fast-twitch (type II). Force and speed of contraction are known to affect the recruitment of high threshold motor units (Moritani et al., 1992). It is also hypothesized that the availability of oxygen can affect the high threshold motor unit recruitment. During muscle contractions using a handgrip exercise with occlusion, there has been significant decreases in the availability of oxygen and blood borne substrates such as glucose and free fatty acids. This, in addition to the findings of significant increases in muscle unit firing rate and muscle unit spike amplitude in association with occlusion by Moritani et al. (1992) has led to the hypothesis that muscle units are recruited progressively to compensate for the deficit in force development (Bigland-Ritchie, Cafarelli & Vollestad, 1986; Moritani, Muro & Nagata, 1986).The additional support to this theory is the oxygen delivery dependent recovery rate of phosphocreatine, demonstrated by Bylund-Fellenius et al. (1981).

It is stated that the recruitment of type II muscle fibres is essential for reaching hypertrophic effects (Loenneke, Fahs, Wilson & Bemben, 2011), and despite that they are recruited first at higher intensities, it has been demonstrated possible to achieve at very low intensities when using blood flow restriction (Yasuda et al., 2010; Yasuda et al., 2009). This is thought to happen as a consequence of the hypoxic environment preventing oxygen for slow-twitch fibres and the high metabolite accumulation (Moritani et al., 1992). The recruitment and activation of fast-twitch fibres following BFRRE may stimulate muscle protein synthesis, although it is not always the case to be observed. Also, traditional high intensity resistance exercise still seems to recruit a greater amount of fast-twitch fibres (Suga et al., 2009), indicating that mechanical tension has a greater association with fast-twitch fibre recruitment than metabolic stress (Pearson & Hussain, 2015).

Takarada and others observed that increased integrated electromyography (Takarada et al., 2000) and inorganic phosphate splitting (Suga et al., 2010) is a result of reduction in oxygen

and subsequent metabolic accumulation during BFRRE. Reduced oxygen and metabolic accumulation are factors considered to be important for increased fibre recruitment in order to maintain force and protect against conduction failure (Yasuda et al., 2010). Performing an exercise protocol with similar low load without blood flow restriction would require significantly more repetitions to be completed in order to achieve the activation of fast twitch fibres (Wernbom et al., 2009). The highest level of muscle activity has been shown to occur during the last few repetitions of each set of low-load BFRRE, when performing unilateral dynamic knee extensions, both when exercising with blood flood flow restriction, and without (Wernbom et al., 2009). It should be noted that the recruitment of fast twitch fibres is not believed to be the only factor important for hypertrophic response to an exercise stimulus (Loenneke et al., 2011).

3.5.5 Elevated systemic hormone production

The increase in metabolic stress triggers a strong anabolic response post exercise (Takarada et al., 2000). Low-intensity BFRRE facilitates the expression of many systemic hormones including growth hormone (Loenneke et al., 2012a; Reeves et al., 2006) and insulin growth factor-1 (IGF-1) (Takano et al., 2005). Growth hormone appear to not be associated with increased muscle protein synthesis or long term hypertrophic adaptions (West & Phillips, 2012). There has been observations of increased cross-sectional area, strength and in myofibrillar protein synthesis as a response to resistance exercise protocol independently of changes in growth hormone, IGF-1 and testosterone levels (West & Phillips, 2012). This is in opposition to the likely small importance of systemic, mechanical-tension-induced localised hormones that may contribute to BFRRE induced hypertrophic adaptions. The localised IGF-1 isoform, IGF-1Ec, known as mechano-growth factor, is the only of the isoforms (IGF-1Ea, IGF-1Eb and IGF-1Ec) that appears to be locally activated by mechanical stimuli and cellular damage (Hameed et al., 2004). Mechano-growth factor is claimed to act as the generator for post-exercise hypertrophy response and facilitate local repair of damaged tissue (Goldspink, 2005), it is thought to have a role in carrying out anabolic signalling such as mTOR, mitogen-activated protein kinase, calcium-dependent pathways and possibly activate satellite cells, proliferation, and differentiation (Pearson & Hussain, 2015). To which extent these factors exist within BFRRE is still under investigation.

3.5.6 Muscle damage

Exercise-induced muscle damage is claimed to be essential for satellite cell-mediated compensatory muscle growth (Hill & Goldspink, 2003). The support for this stems from studies showing absence of hypertrophy responses after excluding the eccentric phase of the exercise, which is the phase where the greatest muscle damage has been observed (Hather, Tesch, Bunchanan & Dudley, 1991). In addition, the amount of strain on individual muscle fibers, initial muscle length and force per active area influence muscle damage. Increases in intensity and number of contractions mediate the degree of muscle damage. This will eventually overstretch and disrupt the sarcomere and in the end disruption of the cytoskeletal matrix (Proske & Morgan, 2001). The downfall in StO_2 and the depletion of energy stores when exposed to hypoxia is thought to result in a build-up of lactic acid and a decrease in pH (Wang, Baynosa, & Zamboni, 2011). The following reperfusion of BFRRE enlarge this damage due to an increase in reactive oxygen species in the mitochondria (Wang et al., 2011). Numerous studies show beneficial increase in muscle damage. It would not be beneficial for high-frequent training (which often is done with BFRRE) to have a large increase in muscle damage (Nielsen et al., 2012). The role of muscle damage in the hypertrophic adaptions of BFRRE show conflicting results, perhaps as a result of the wide variety in the degree of muscle damage that is observed between studies using different exercise prescriptions.

3.5.1 Reactive hyperemia – another stimulus for muscle hypertrophy?

This amplitude of changes from hypoxia to hyperoxia (reactive hyperemia) in BFRRE is thought to play a part in muscle hypertrophy (Takarada et al., 2000). Fry et al. (2010) and Fujita et al. (2007) have reported increased rates of muscle protein synthesis and stimulation of mammaligan target of rapamycin complex 1 (mTORC1) and MAPK-mediated anabolic signalling after low- load BFRRE. The reactive hyperaemia post BFRRE enhances the delivery of nutrients to the working muscles and is hypothesized to be the mechanism stimulating these processes. (Gundermann et al., 2012) tested this hypothesis by a randomized crossover study with two trials: Low-intensity resistance exercise with blood flow restriction and low-intensity resistance exercise with a pharmacological vasodilator infusion into the femoral artery immediately after exercise to simulate the reactive hyperaemia after blood flow restriction exercise. It is concluded by Gudermann et al. (2012) that reactive hyperaemia is not the primary mechanism stimulating mTORC1 signalling and muscle protein synthesis

following BFRRE. However, they do highlight the possibility for it to be a supplementary variable (Gundermann et al., 2012). To further establish to what extent reactive hyperemia affect mTORC1 signalling and muscle protein synthesis more investigation is required.

3.6 Safety regarding blood flow restriction resistance exercise

BFRRE is considered a safe training method for athletes and healthy people. Although, minor side effects have been reported, the most frequent are; subcutaneous haemorrhage and numbness (Nakajima et al., 2006). Other observed side effects were venous thrombus, dizziness and fainting. Muscle damage with prolonged loss of muscle strength and muscle soreness and cellular stress been observed to be highly present. Recently, some cases of rhabdomyolysis and excessive muscle damage has been noted, especially after high intensity BFRRE, close to failure (Clark & Manini, 2017; Sieljacks et al., 2016; Wernbom, Paulsen, Nilsen, Hisdal, & Raastad, 2012). Responses to BFRRE seem to act in a similar fashion to regular physical activity (Loenneke et al., 2011), and the method is considered highly safe in controlled environments where pressure and intensity can be monitored (Nakajima et al., 2006).

4 Methods

4.1 Study Design and participants

The present study was conducted as a randomized controlled experiment trial (quasi experimental trial) with a within subject design. The data for this study was collected as a part of a larger study. During September 2017, a recruitment process was conducted at the University of Agder (UOA) and nearby locations, Kristiansand, Southern Norway. Recruitment was done through social media, stands in the UOA's canteen, information-presentations during seminars at UOA and posters (appendix 3 & 4). Thirty-two untrained, healthy men and women volunteered to participate, two subjects declined the option to participate before the start of the intervention due to time commitment. One subject was excluded due to medical problems. Twenty-nine subjects, 14 females and 15 males were randomly assigned to a blood flow restricted resistance exercise (BFRRE) or a non-exercising control group using block randomization; 2/3 BFRRE and 1/3 control. The control group is not included in this acute study, leaving the BFRRE group for participation (N=20), 10 females and 10 males (figure 2). The subjects were included based on the following criteria's: Subjects could not have conducted systematic strength training on the legs (>1 sessions per week) during the last six months before initiating the study and had to be new to BFRRE

training. Endurance exercise had to be kept to a minimum (≤1 session per week) during the intervention. Obtaining or sustaining injuries that could prevent the subjects from conducting BFRRE would have been excluded. Any use of medication, drugs or supplements such as vitamins, creatin, amino acids etc. during the intervention would also lead to exclusion. Subjects had to be able to attend all BFRRE sessions.

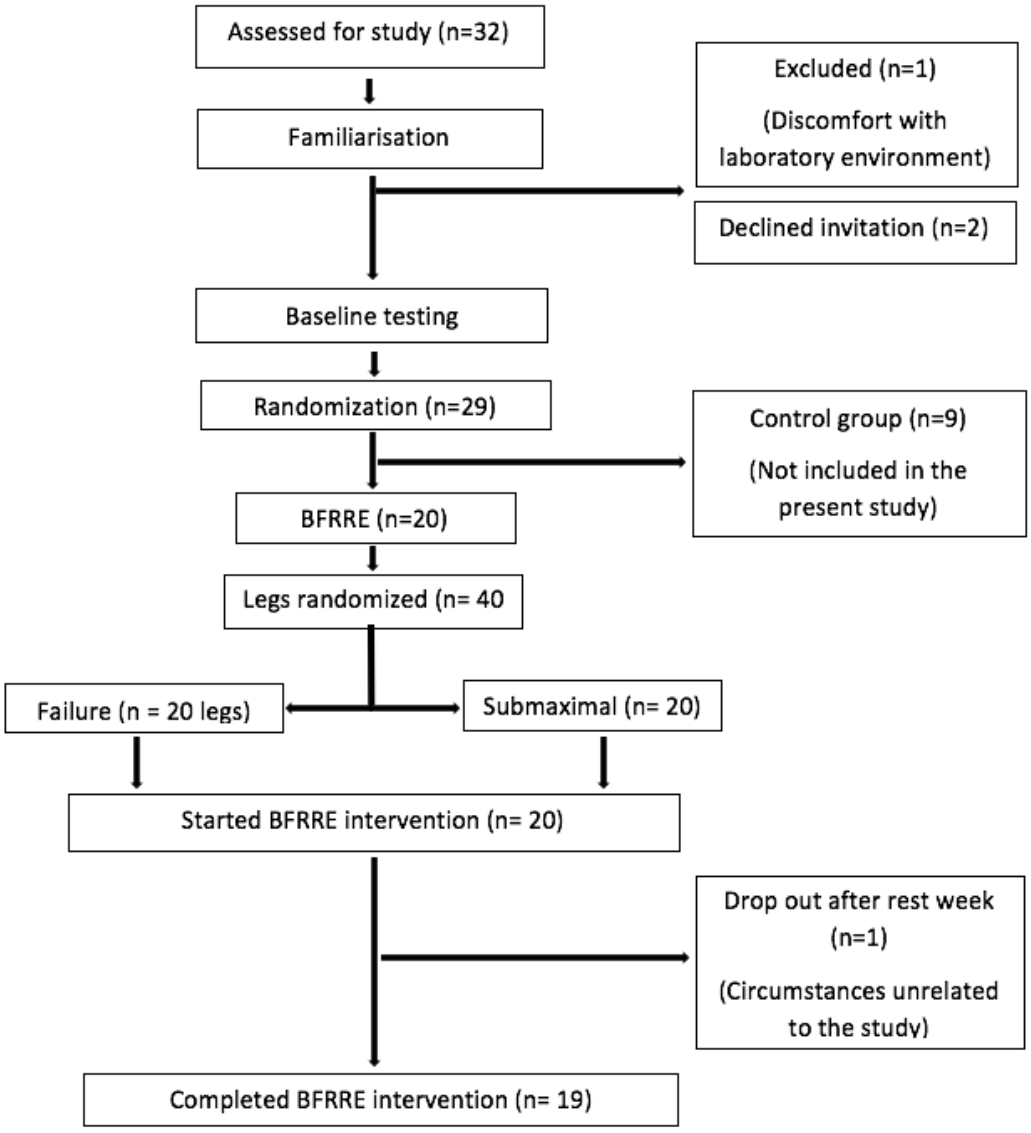

Figure 2: Flow chart illustrating the recruitment process and the participation throughout the intervention. BFRRE = Blood flow restricted resistance exercise.

The main intervention period of "Occlusion 6" consisted of two times 7 bouts over 5 days, separated by 10 days of rest. The training intervention was executed under a period of two weeks, including 14 BFRRE bouts in 10 days (7 bouts in five days followed by 10 days of recovery, before another five days of training, consisting of 7 bouts). General measurements including height, weight and limb occlusion pressure were collected before intervention start (baseline) along with ultrasound- and strength- measurements, which was collected again 17 days (post 17) after the end of the intervention (Figure 2). Post 17 was selected as the source for post-measurements due to the greatest increase of muscle thickness observed this day. Oxygen saturation measurements and ultrasound pictures to determine the amount of subcutaneous fat were taken during the third training session, three days into the intervention.

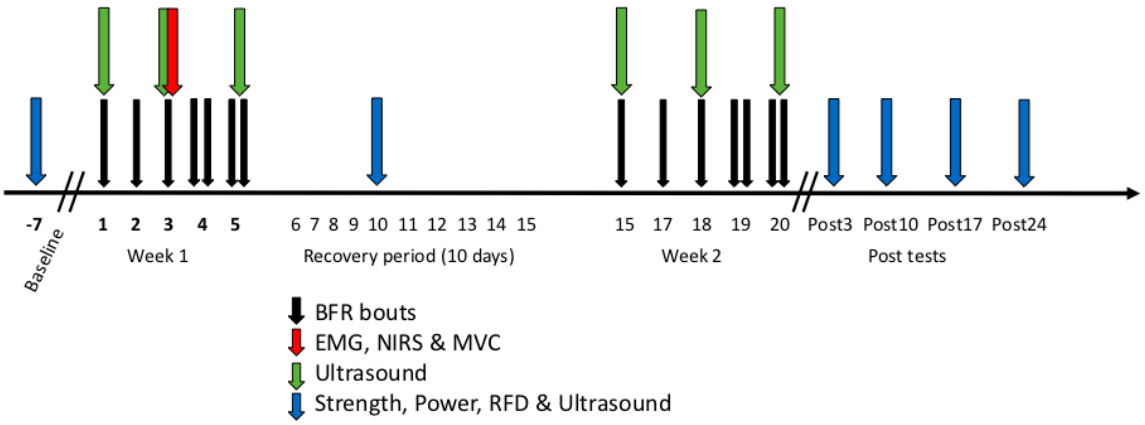

Figure 3: Overview over the study course of "Occlusion 6". One arrow representing one test/training session. BFRRE = blood flow restricted resistance exercise, EMG = electromyography, NIRS = near infrared spectroscopy, MVC = maximal voluntary isometric contraction, RFD = rate of force development.

The present study obliged with the standards set by the Declaration of Helsinki and was approved by Norwegian centre for research data (NSD) and the ethical committee of the faculty (FEK) ahead of initiation. All subjects received oral and written information regarding the aim and methods of the study, and prior to inclusion had to sign a written informed consent (appendix 2).

4.2 Blood flow restriction resistance exercise

All subjects started their training sessions with a standardized warm up on a stationary ergometer bike (Wattbike Pro/Trainer, Wattbike Limited, Nottingham, England) for 5 minutes at 100 watts. Subjects in the failure group performed four sets of knee extensions (with progression the first three days, to avoid excessive fatigue) to concentric failure at 20% of 1 RM with 30 seconds of rest as described in Nielsen et al (2012). The first two training sessions in the failure group were conducted with submaximal training loads based on the rate of perceived exertion (RPE, 6-20) 15 and 18 respectively. Subjects in the submaximal group performed four sets of submaximal knee extensions at 20% of 1 repetition maximum (1 RM), with 20-, 10-, 10-, 10 repetitions, (also interspersed with 30 seconds rest). Training load was not adjusted throughout the intervention. Right leg was always trained first, and subjects had a five-minute rest before training the left leg.

Ahead of each training session all participants in the failure and submaximal group placed a 145 mm wide cuff (Delfi Medical, Vancouver BC, Canada) with a pressure zone of 135 mm placed on the proximal part of the thigh. The cuff was coupled to a tourniquet system apparatus (Zimmer A.T.S.750, Warsaw, IN, USA), and the pressure was set to 100 mmHg for both men and women, remaining inflated through all set- and rest- periods and deflated after the final set. All training was conducted in a unilateral knee extension machine (G- and F200 Leg Extension, David health solutions LTD, Helsinki, Finland). The subjects were instructed to sit upright with their back against the back support and to hold on to the handles. The seat was adjusted so that the lateral epicondyle of the knee aligned with the rotation axis of the machine. Thereafter, the subject was instructed to strap up to the knee extension machine and place the foot pedal right over the ankle joint. Motion range was set from 90° to 10° (0= full extension). Each repetition in both protocols were completed in a pace of 1.5 seconds during each of the concentric- and eccentric phase, controlled by using a metronome (Metronome beats, Stonekick, St. Albans, England). Strong verbal communication was given during each training session to motivate the subjects. The average training load on the failure leg was 20% of 1 RM or 11.3±3.9 kg and 11.3±3.5 kg on the submaximal leg. Training load remained the same throughout the intervention. The occlusion pressure was 155±22 mmHg on the failure leg and 155±23 mmHg on the submaximal leg, relatively to a limb occlusion pressure of respectively 66±8% (min: 50%, max 77%) and 66±8% (min 49%, max 77%) at 100 mmHg.

4.3 Instruments

4.3.1 General measurements

General measurements were taken as a part of the baseline testing before the initiation of the intervention. Height measurements were taken using a standard metric measurement tape (Kawe, Height measurement tape, Germany), and a bodyweight measuring scale (Seca, Model 713, Hamburg, Germany) were used to measure weight. Arterial limb occlusion pressure (LOP) was measured by an experienced practitioner using a digital tourniquet system with automatic regulation of pressure (Zimmer A.T.S. 2000, Warsaw, IN, USA) and a doppler (Dopplex D900, Huntleigh Healthcare Ltd., UK). Subjects were placed in a supine position, which demonstrated by Sieljacks et al. (2017) has a significant correlation with measurements taken in a seated position as long as a wide pressure cuff is used. The tourniquet was placed around the proximal part of their thigh, was inflated to 100 mmHg and gradually increased 5 mm HG in steps until auscultatory pulse of arteria tibialis posterior was no longer present. The lowest pressure where the pulse was no longer present. Two measurements were taken from each leg and a third was made if the two values differed with >5mm Hg. The average between the two closest values was considered as LOP.

4.3.2 Near-Infrared Spectroscopy

Tissue oxygenation was measured using Near-Infrared Spectroscopy (NIRS) (ForeSight Elite, Casmed, Branford, USA). This apparatus uses an infrared light source and detector that quantifies the amount of oxygenated haemoglobin found in the small blood vessels and intracellular myoglobin. Together this provides information about the local oxygen delivery and utilization. Three sensors (ForeSight Elite Large sensor, Branford, USA) with 25 mm between light sources, each connected to the NIRS apparatus were used for measuring different areas of muscle tissue and surrounding areas; one attached on the distal part of m. Vastus Lateralis, one on the middle part of m. Vastus Lateralis and one on the m. Vastus Medialis. The subjects were instructed to take place in a chair and to extend the knee to localize and palpate the m. Vastus Lateralis and m. Vastus Medialis to further determine the attachment area of the sensors. Recording areas were determined by a trained examiner based on the placement of the EMG sensors, the pressure cuff and individual´s anatomy of the thigh. One sensor was placed on the distal part of m. vastus lateralis, one on the proximal part of m. vastus lateralis and one on the oblique fibers of m. vastus medialis. For optimal attachment of sensors, subjects were shaved and cleaned with alcohol. The sensors were prepped for attachment using adhesive gel. A blue-, red-, or black- coloured kinesiotape was applied to

cover the sensors to keep potential light disturbance out and keep them steady in place. Baseline values was determined as the lowest, most steady values of tissue oxygenation for each sensor before warmup and inflation of the pressure cuff was initiated. Warmup consisted of 15 repetitions of knee extensions with a load of 5 kg without blood flow restriction. Pressure was inflated a few seconds ahead of the initiation of set 1. The pressure and cuff were released for a five-minute, seated recovery period which allowed an opportunity to observe the post exercise reoxygenation of the muscles after the completion of the 4^{th} set of knee extensions. Subjects were instructed to sit completely still. Recovery was followed by reattaching and inflating the pressure cuff again with the aim of completely restrict both venous and arterial blood supply for seven minutes, to reach the individual "full occlusion". NIRS-measurements were disconnected first when the subjects reached baseline levels of tissue oxygenation saturation and the pressure cuff again was released. Measurements were taken consistently from connecting the sensors to the NIRS apparatus, and every two seconds through warm up, all 4 sets of training, 3 sets of rests, the recovery period after training and throughout the full occlusion period.

The lowest level of oxygenation during each of the training sets 1-4 during BFRRE was registered along with the highest value for each of the three periods of rest. Oxygenation measurements were quantified as percent of maximal oxygenation. All values were adjusted to each of the subject's individual baseline value and minimal value reached during full occlusion. Area Under the Curve refers to the post exercise tissue oxygen levels and was used as an indication of the work conducted during the training session. Area under the curve was set to include all values from the point where tissue oxygen saturation reached and exceeded the baseline level after training, to where the SO_2-values again reached the baseline values after recovery.

4.3.3 Ultrasonography

Measurements of muscle thickness on vastus lateralis were performed by one trained examiner and conducted using a brightness mode (B-mode) ultrasonography device (Logic Scan 128 CEXT-1Z kit, Telemed, LT). Focus, depth, dynamic range, power, gain and frequency are different settings used to best identify collagenous tissue which is used to define the outlying part of the muscle, these were adjusted in Echo Wave 2. Participants were instructed to lie supine on a bench with knees fully extended and relaxed muscles of the lower

limb during the measurements. The measurements were taken distally (40%) and proximally (60%) of the lateral epicondyle of the knee to the great trochanter major. A transparent sheet was placed over the thigh to mark scars, moles, birthmarks etc. and intended marks for the ultrasound pole before the first ultrasound session to ensure reliability. Four still/panoview-pictures were taken of vastus lateralis, resulting in 8 pictures per leg in total in each session. Additionally, two pictures were taken of m. vastus medialis oblique fibers on the acute testing day for quantifying the amount of subcutaneous fat. Ultrasound measurements comes with some uncertainties, therefore, baseline values for muscle thickness was determined by the average of the pictures taken at baseline and from the first training session. The analysing of ultrasound pictures was conducted using ImageJ (version 1.46r, National Institutes of Health, USA). Three vertical lines per picture between the inner edge of the superficial and deeper aponeurosis was used for muscle thickness measurements. Similarly, three vertical lines per picture between the outer and the inner edge of the superficial aponeurosis was used for measuring subcutaneous fat.

4.3.4 One-Repetition Maximum

Before the start of the training intervention subjects performed familiarization tests, and a pre-test (baseline test) where the highest value of each test was set as baseline value. Familiarization test were also used for the registration of the individual settings for the knee extension machine. Strength tests were performed at baseline to determine the training load of 20% of 1 RM.

1 repetition maximum (1 RM) was tested in the same machine as the BFRRE. Participants went through a specific warm up consisting of two repetitions at 50% and 70% of 1 RM and one repetition at 80%, 90%, and 95% of 1 RM respectively, interspersed with 1 min rest between sets. An increase of load (≥0.25 kg) between 1 RM attempt was chosen by a trained test instructor until concentric failure was reached. The knee joint had to reach an angle of 10° for the repetition to be approved (0° = full extension). Markings on the knee extension machine´s display were used to ensure acceptable lifts. Between each 1 RM attempt there was 2 minutes of recovery and minimum of 30 seconds rest between each leg. Right leg was always tested first. Strong verbal communication was given during each 1 RM attempt. 1 RM was tested after testing maximal voluntary contraction (MVC).

4.4 Statistical Analysis

All data is presented as mean and standard deviation (SD). Differences between the failure and submaximal protocols in StO_2 during training session 3 was investigated using a series of independent samples t-test. Normal distribution of the data was analysed using histogram, a comparison of mean and standard deviation and consider the skewness to take variance into account. The relative change in muscle thickness from baseline to post 17 in percent and mean StO_2 during training session 3, expressed in percent was both analysed using Spearman correlation. Area under the curve was first calculated using Medcalc Software (version 7.4.4.0, Belgium) and further analysed using Spearman correlation. The α-level was set to <0.05 in all analyses, except the repeated independent samples t-tests used to investigate differences between the submaximal and failure protocols in StO_2, where the α-level was set to <0.01 due to multiple testing. Statistical analyses were performed in SPSS (version 24, IBM, Chicago, IL, USA). Figures were made with GraphPad (GraphPad Prism 7.03, GraphPad Software, Fay Avenue, CA, USA).

5 Method discussion

5.1 Study design

The primary objective of the present study was to investigate the level of StO_2 during a failure and a submaximal BFRRE protocol in three muscle areas (vastus lateral distal, vastus lateralis proximal and vastus medialis). A randomized controlled trial (RCT) along with a within-subject design was accordingly considered as an appropriate design. When investigating the hypothesizes regarding casual relationships, RCTs are rated as the "gold standard". It would be impossible to fully implement the relationship between cause and effect in the present study to reality due to the many confounding variables that possibly affects the outcome. We did however try to control for and identify as many variables possible. Test leaders were also acting as motivators and instructors during training bouts, this may have affected the training and the participants. The Hawthorne effect (Thomas, Silverman, & Nelson, 2015), concerns a change in personal behaviour due to the applied attention to the participants. Test leaders were also present and in interaction with the participants during training bouts, this may have affected the training and the participants which in turn may decrease the internal validity. Whilst this matter should be considered when assessing external validity, the within-subject design in the present study outweighs the potential effect on internal validity. A within-

subject design is beneficial for doubling the number of participants, in terms of being able to use both legs. Even though a negative cross-transfer effect has been observed in some within-subject studies involving physiological parameters such as strength (Madarame et al., 2008), this is likely to be inconsequential in comparison to differences in diet, activity and sleep which likely is present in studies comparing variables between subjects (MacInnis, McGlory, Gibala, & Phillips, 2017). The statistical power will be positively affected as a result.

The NIRS sensors were subjectively placed on each individual participant based on already attached EMG-sensors and tourniquet. Irregularities in measurements between each leg and between each individual is a consequence affecting the reliability and internal validity of the present study (Esaki et al., 2005).

Several post-tests of muscle thickness were included in the intervention; day 3, 10, 17 and 24 days after cessation of training. However, only data from post-test 17 was used in the present study. Data collected during post-test 17 was selected due to the greatest gains in muscle thickness of the post tests. When investigating exercise physiology, repeated measurements are frequently carried out (Thomas et al., 2015). Thus, one might speculate if data from post-test day 3, day 10 and day 24 would supplemented the present study with a more accurate picture of the change in muscle thickness.

5.2 Study Sample

The subjects of the present study were within an age range of 18-41 years old (24.4±7.1) (mean±SD). Oguri et al. (2008) found a difference in StO_2 between endurance- and strength trained athletes, suggested to be a result of the strength trained athletes' abilities to extract more oxygen within the working muscles and thus achieve different StO_2 values. Physiological differences in relation to sporting backgrounds is not a variable of great concern in regard to the StO_2 measurements present study as the subjects confirmed the absence of any systematic strength training six months prior to the study (≤1 session per week). We suggest that findings in the present study might be generalized to groups of people, similar to the one included in the present study; untrained men and women within the age range of 18-45 years old. The subjects were recruited to participate on a voluntary basis, which potentially may have been influenced by motives such as being previously strength trained or having interests in exercise or fitness. Also, despite instructions to avoid other resistance exercise on

the legs during the intervention, one might suspect that this potentially can have been ignored by some of the participants. Considering the high efforts required performing high intensity low load BFRRE, we suggest that this is a training method for particularly motivated people, people specifically interested in resistance exercise and/or as a part of a training programme or rehabilitation programme fitted to a specific athlete.

5.3 Training protocols

The aim of the study was to investigate the level of StO_2 during a submaximal and a failure protocol of BFRRE. The submaximal protocol of the present study was adjusted in repetitions ahead of research intervention, based on previous unpublished research conducted by our research group. Several fails to complete the previous submaximal protocol of 30-15-15-15 led to the change into 20-10-10-10 to ensure a submaximal performance. The change was tested in a pilot study on 11 participants to ensure that the submaximal protocol was not too close to the failure protocol. Significantly greater number of repetitions and with only two participants who failed to complete the submaximal protocol during the first week of training in the present study, we consider the present submaximal protocol successful in providing submaximal stimuli.

The degree of muscle activity, blood flow and oxygen consumption are thought to differ among individual muscles and also within a single muscle (Bhambhani., 2004; Miura et al. 2011; Van Beekvelt et al. 2001). Individualized pressure was not applied in the present study due to the overall intention of the research project which involved to replicate certain elements from previous research within our study group. Particularly to obtain more reliable data and to get a better insight of the StO_2 during different BFRRE protocols in the future, individualized pressures should be applied. NIRS measurements are known to be affected by blood flow and blood volume (Ferrari, Mottola & Quaresima, 2004). The present study showed significant differences in StO_2 between the sites of measurements (vastus lateralis distal, vastus lateralis proximal and vastus medialis). We speculate that this may be a reflection of differences in blood flow within and between the muscles. This is supported by Sjøgaard, Keins, Jørgensen & Saltin (1986), who suggest two potential reasons for the heterogeneously distributed blood flow observed in contracting muscles: 1) Varied intramuscular pressure due to unequal recruitment of muscle fibers in different parts of the muscles. The difference in oxygen consumption by the muscles induced by the greater

muscle fiber recruitment will result in difference in StO_2 (Esaki et al., 2005). 2) Different muscle architecture (short bulging ones such as fibers close to the knee joint, compared to long, slender fibers further away from the joint (Esaki et al., 2005).

It is considered important to monitor and regulate exercise intensity to include subjective responses from the participants. Rating of perceived exertion (RPE) is often used to describe this (Scott et al., 2014), however, the present study does not include data about the participants perceived exertion (RPE) and delayed onset muscle soreness (DOMS). The total evaluation of the participants experiences of the exercise along with the physical outcome is therefore insufficient. One can argue that this would limit the ability to prescribe the exercise protocols for the appropriate population.

5.4 Measurements

Reliability is the ability of a test or an instrument to provide consistent or reproducible data (Tanner & Gore, 2012). Validity is known as the degree to which a test or instrument is supposed to measure (Thomas et al., 2015). It is important to strive for both a strong validity and reliability of tests to ensure a strong internal validity of the study. The test leaders were all trained and familiar with the equipment, testing procedures and protocols, with perhaps the exception of the NIRS-measurements: The introduction and familiarisation with the NIRS were done only a few days ahead of the testing, and somewhat briefly, due to a late delivery of the equipment. The NIRS-examiner was however supervised and assisted by a more experienced researcher.

5.5 Tissue Oxygen Saturation

The light path and metabolism of subcutaneous fat is different from that in muscle tissue. Therefore, as the subcutaneous fat increases, measurements by NIRS will lead to an underestimation of StO2 (Van Beekvelt et al 2001; Nasseri et al 2016). The findings of positive significant correlations between the amount of subcutaneous fat and degree of StO_2 in vastus lateralis proximal and vastus lateralis distal in the present study indicates and reinforces previous findings that tissue oxygen saturation measurements are affected by the amount of subcutaneous fat (Ruiter et al., 2005). The present study has taken into consideration the impact of subcutaneous fat on NIRS-measurements by normalizing the StO_2 values of each subject to their respective values of baseline and full occlusion. To further

enhance the result in future studies, it is possible to apply a relative occlusion pressure based on each individuals' limb occlusion pressure, and thigh circumference (Fahs et al., 2012) (Lixandrão et al., 2018). In respect of the overall aim of the current project, to replicate findings from Nielsen et al. (2012), therefore, the absolute pressure was kept. One might also speculate if the placement of other equipment, such as electromyography sensors and the pressure tourniquet potentially have affected the outcome of the NIRS-measurements.

The NIRS instrument used in the present study (described in 4.3.2) comes with four cables, allowing for measurements at different sites, which provide a better picture for the local StO_2 in different muscles and within one muscle. We considered performing leg extensions with the NIRS-sensors and cables somewhat challenging as the moving leg and the weight of the cables occasionally pulled on the sensors. Other NIRS instruments available include wireless options, considered reliable and suitable for StO_2 measurements during exercise (Shadgan, Reid, Gharakhanlou, Stpublisher-ids, Macnab, 2009). The NIRS instrument, Fore-sight Elite (described in 4.3.2) is however, adapted to measurements of muscle tissue specifically, tested for validity (MacLeod, Ikeda, Cheng, Shaw, 2013) and considered to provide accurate monitoring of skeletal muscles used in the present investigation.

The NIRS-sensors used (described in 4.3.2) were of single-use purposes. In the NIRS-measurements of the present study, a set of 12 sensors were re-used to avoid excessive costs. A consequence to occasional connection issues between the NIRS sensors and the connective cable and/or proper sensor attatchement was missing data from 4 sensors. The data of StO_2 was extrapolated to predict missing values. This was the case for vastus lateralis proximal for three subjects and vastus medialis for one subject respectively. It is addressed by Esaki et al. (2005) that StO_2 measured by NIRS does not represent oxygenation in the entire limb, but only in a specific location. Variation in the level of StO_2 at different locations within the vastus lateralis and rectus femoris has been showed (Quaresima, Colier, Van Der Sluijs & Ferrari, 2001) and consistency of probe placements is essential for appropriate comparisons. Due to the single measurement occasion of StO_2 for each leg there was no need to remove and reapply the sensors. The StO_2 measurements for each specific location is therefore considered reliable.

5.6 Muscle size and subcutaneous fat

Ultrasound was used for measurements of thickness of muscles and subcutaneous fat in the present study. A method requiring less time and money than the "gold-standard" of magnetic resonance imaging (MRI) and computed tomography (CT) (Sanada, Kearns, Midorikawa & Abe, 2006). Current literature supports ultrasound as an adequately reliable method to measure the morphology of muscles *in vivo* (Franchi et al., 2018; Scott et al., 2012). One adequately trained examiner, who was aware of the potential errors that comes with ultrasound measurements conducted all measurements in the present study. This aids in eliminating errors that may threaten the validity and reliability of the measurements, such as: operator viability, the way the muscle grows (Noorkoiv, Nosaka & Blazevich, 2010), muscle swelling from other activities performed ahead of measurement (Reeves et al., 2010), the influence of the participant's hydration level (Ward & Lieber, 2005), compression from the transducer and/or poor manual processing of images (Reeves, Maganaris & Narici, 2004). When analysing the data however, two different examiners took part due to logistical issues; one conducted the analyses of muscle thickness, the other the analyses of subcutaneous fat. This is a weakness in the processing of data but is likely of minimal impact as one examiner trained the other and they both used the same computer program.

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Appendix

Content

Appendix 1

PART II
Research Paper

Tissue oxygen saturation during different blood flow restriction exercise protocols

The following paper written after the standards of the journal:
"Medicine & Science in Sports and Exercise"
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Tissue oxygen saturation during two different blood flow restriction exercise protocols

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Tissue oxygen saturation during different blood flow restriction exercise protocols

ABSTRACT

The primary objective of the present study was to investigate the level of tissue oxygen saturation (StO_2) during a failure and a submaximal blood flow restricted resistance exercise (BFRRE) protocol in three muscle areas; vastus lateralis distal (VLd), vastus lateralis proximal (VLp) and vastus medialis (VM). Secondarily the association between StO2 during session number three of BFRRE and muscle thickness 17 days post intervention. I addition, the correlation between StO_2 and the amount of subcutaneous fat. **Methods:** Twenty untrained men and women aged 18-45 yrs old performed high intensity, low load, unilateral leg extensions with BFRRE. Each subject had one leg randomized to follow a failure protocol with four sets to concentric failure, the other leg to follow a submaximal protocol consisting of 20, 10, 10 and 10 repetitions. A pressured cuff of 145mm was inflated to 100 mmHg to induce partial blood flow restriction and training load was set to 20% of 1 RM. Near-Infrared Spectroscopy was used to quantify StO_2 during training. Ultrasonography was used to measure muscle thickness in VLd and VLp and subcutaneous fat in VLd, VLp and VM. **Results:** No significant difference was observed between the submaximal and failure groups in StO_2 level for VLd, VLp or VM. There was a significant correlation between amount of subcutaneous fat and StO_2 in VL distal ($r=0.52$; $p<0.001$), and VL proximal ($r=0.58$; $p=0.001$). StO_2 during BFRRE was not correlated to muscle thickness 17 days post intervention. **Conclusion:** The submaximal and failure protocols induce similar levels of StO_2. Subcutaneous fat should be taken into account in NIRS-measurements. More research is needed regarding how different BFRRE protocols may affect the level of hypoxia.

Keywords: kaatsu; occlusion; hypoxia; near infrared spectroscopy;

INTRODUCTION

Increases in muscle growth and strength is most commonly known to be promoted by high intensity resistance exercise using loads of >60% of 1 repetition maximum (1RM) (1). However, more recent studies have investigated resistance exercise with intensities as low as 20% of 1RM in combination with vascular blood flow restriction (2). Blood flow restriction resistance exercise (also called Kaatsu) has become an increasingly popular resistance training option used to sustain or gain muscle mass in a variety of groups such as athletes (3), astronauts (4) and in rehabilitation (5) to mention a few. The reduced blood flow associated with blood flow restriction resistance exercise (BFRRE) has been shown to induce a hypoxic condition in the muscle cell (6). Which, in turn is hypothesized to increase the accumulation of metabolites and results in metabolic stress, one of the proposed mechanisms for the muscular adaptions seen in BFRRE, the relationship is however, still under investigation (7,8). Factors stimulating magnitude metabolic stress includes number of repetitions and sets of exercise, length of inter set rest periods, speed of movement, load, occlusion pressure and degree of hypoxia are also under investigation (2, 9, 10). Despite the clear evidence-based indications that BFRRE induce an intermuscular hypoxic condition (11), there is still questions regarding how hypoxia affect hypertrophy seen in BFRRE. No one has to our knowledge compared a submaximal and a failure BFRRE protocol in regard to the changes in StO_2 during exercise.

Measurements of StO_2 are potentially underestimated as a consequence of greater amounts of subcutaneous fat (12). It is suggested to take this into consideration and adjust for it by normalizing the data to baseline values (13). However, it is debated whether this action is enough to ensure reliable data outcomes (14). Data about the level of hypoxia during two BFRRE protocols of different volumes and intensities, and how these relates to changes in muscle mass may provide information regarding to role of hypoxia in BFRRE.

The main objective of the present study was to investigate the level of StO_2 during and a submaximal protocol (20-10-10-10 repetitions) and a failure protocol (four sets to voluntary concentric failure) of knee extensions with low load BFRRE. The secondary objectives were: 1) To investigate the association between the level of StO_2 during one session of BFRRE and the change of muscle thickness after a period of BFRRE. 2) To investigate the association between StO_2 during a session of BFRRE and the amount of subcutaneous fat.

METHODS

PARTICIPANTS. Thirty-two healthy untrained men and women were recruited to the present study. Two participants declined further participation after the familiarization period due to time commitment, and one was excluded due to medical problems inhibiting the participant from performing tests. Twenty-nine participants completed baseline testing and were further randomized to either the BFRRE group (n=20) or the non-exercising control group (n=9). Only the BFRRE group is included in the present study (N=20). None of the participants had conducted regular strength training in the last six months (≤1 session per week). All subjects received oral and written information regarding the aim and methods of the study, and prior to inclusion had to sign a written informed consent. The present study obliged with the standards set by the Declaration of Helsinki and was approved by Norwegian centre for research data (NSD) and the ethical committee of the faculty (FEK) ahead of initiation.

STUDY DESIGN. The present study was conducted as a randomized controlled experimental trial (RCT) and took place at the University of Agder. A with a within-subject design was used to compare the BFRRE protocols. All subjects got their legs randomized, one to perform the exercise with the failure protocol and one to the submaximal. The main intervention period consisted of two times seven bouts over five days, separated by ten days of rest. The training intervention was executed under a period of two weeks, including 14 BFRRE bouts in 10 days (7 bouts in five days followed by 10 days of recovery, before another five days of

training, consisting of 7 bouts). General measurements including height, weight and limb occlusion pressure were collected before intervention start (baseline) along with ultrasound- and strength- measurements. The latter was collected again 17 days after the completion of the intervention. Oxygen saturation measurements and ultrasound pictures to determine the amount of subcutaneous fat were taken during the third training session, three days into the intervention. The third training session was the "acute testing day" and was the occasion for the main collection of data in the present study.

BLOOD FLOW RESTRICTED RESISTANCE EXERCISE. All subjects started their training sessions with a standardized warm up on a stationary ergometer bike (Wattbike Pro/Trainer, Wattbike Limited, Nottingham, England) for 5 minutes at 100 watts. Subjects in the failure group performed four sets of knee extensions (with progression the first three days, to avoid excessive fatigue) to concentric failure at 20% of 1 RM with 30 seconds of rest as described in Nielsen et al (2012). The first two training sessions in the failure group were conducted with submaximal training loads based on the rate of perceived exertion (RPE, 6-20) 15 and 18 respectively. Subjects in the submaximal group performed four sets of submaximal knee extensions at 20% of 1 repetition maximum (1 RM), with 20-, 10-, 10-, 10 repetitions, (also interspersed with 30 seconds rest). Training load was not adjusted throughout the intervention. Right leg was always trained first, and subjects had a five-minutes rest before training the left leg. Ahead of each training session all participants in the failure and submaximal group placed a 145 mm wide cuff (Delfi Medical, Vancouver BC, Canada) with a pressure zone of 135 mm placed on the proximal part of the thigh. The cuff was coupled to a tourniquet system apparatus (Zimmer A.T.S.750, Warsaw, IN, USA), and the pressure was set to 100 mmHg for both men and women, remaining inflated through all set- and rest- periods and deflated after the final set. All training was conducted in a unilateral knee extension machine (G- and F200 Leg Extension, David health solutions LTD, Helsinki,

Finland). Motion range was set from 90° to 10° (0= full extension). Each repetition in both protocols were completed in a pace of 1.5 seconds during each of the concentric- and eccentric phase, controlled by using a metronome (Metronome beats, Stonekick, St. Albans, England). Strong verbal communication was given during each training session to motivate the subjects. The perceived exertion was measured directly after completed set according to Borg CR10 scale and ratings was according to Borg RPE.

LIMB OCCLUSION. Arterial limb occlusion pressure (LOP) was measured by an experienced practitioner using a digital tourniquet system with automatic regulation of pressure (Zimmer 200) and a doppler (Hokanson MD6 bidirectional Doppler). Subjects were placed in a supine position, with the tourniquet was placed around the proximal part of their thigh, was inflated to 100 mmHg and gradually increased 5 mm HG in steps until auscultatory pulse of arteria tibialis posterior was no longer present as described previously in was measured as previously described by Sieljacks et al. (2016). The lowest pressure where when the pulse was no longer present. Two measurements were taken from each leg and a third was made if the two values differed with >5mm Hg. The average between the two closest values was considered as LOP.

NEAR-INFRARED SPECTROSCOPY. Tissue oxygenation was measured using Near-Infrared Spectroscopy (NIRS) (ForeSight Elite, Casmed, Branford, USA). Three sensors (Fore-sight Elite, Large Sensor, Branford, USA) with 25 mm between light sources, each connected to the NIRS apparatus were used for measuring different areas of muscle tissue and surrounding areas. One sensor was placed along the muscle fibre length of vastus medialis, one approximately placed on the distal part of vastus lateralis, and one placed on the proximal part of vastus distalis. Recording areas were determined by a trained examiner based on the placement of the EMG sensors, the pressure cuff and individual´s anatomy of the thigh. For optimal attachment of sensors, subjects were shaved and cleaned with alcohol. The sensors

were prepared for attachment using Tensive conductive adhesive gel. A blue-, red-, or black-coloured kinesiotape was applied to cover the sensors to keep potential light disturbance out and keep them steady in place. Baseline values was determined as the lowest, most steady values of tissue oxygenation for each sensor before warmup and inflation of the pressure cuff was initiated. Warm-up consisted of 15 repetitions of knee extensions with a load of 5 kg, without blood flow restriction. Pressure was inflated a few seconds ahead of the initiation of set 1. The pressure and cuff were released for a five-minute, seated recovery period after completion of the 4^{th} set, which allowed an opportunity to observe the post exercise reoxygenation. Subjects were instructed to sit completely still. Recovery was followed by reattaching and inflating the pressure cuff again with the aim of completely restrict both venous and arterial blood supply for seven minutes, to reach the individual "full occlusion". Measurements were taken consistently from connecting the sensors to the NIRS apparatus, and every two seconds through warm up, all 4 sets of training, 3 sets of rests, the recovery period after training and throughout the full occlusion period. The lowest level of oxygenation during each of the training sets 1-4 during BFRRE was registered along with the highest value for each of the three periods of rest. Oxygenation measurements were quantified as percent of maximal oxygenation. All values were adjusted to each of the subject's individual baseline value and minimal value reached during full occlusion. Area Under the Curve refers to the post exercise tissue oxygen levels and was used as an indication of the work conducted during the training session. Area under the curve was set to include all values from the point where tissue oxygen saturation reached and exceeded the baseline level after training, to where the StO_2-values again reached the baseline values after recovery.

ULTRASONOGRAPHY. Measurements of muscle thickness on vastus lateralis were performed by one trained examiner and conducted using a brightness mode (B-mode) ultrasonography device (Logic Scan 128 CEXT-1Z kit, Telemed, LT). Focus, depth, dynamic

range, power, gain and frequency are different settings used to best identify collagenous tissue which is used to define the outlying part of the muscle, these were adjusted in Echo Wave 2. Participants were instructed to lie supine on a bench with knees fully extended and relaxed muscles of the lower limb during the measurements. The measurements were taken at distal (40%) and proximal (60%) the lateral epicondyle of the knee to the great trochanter major. A transparent sheet was placed over the thigh to mark scars, moles, birthmarks etc. and intended marks for the ultrasound pole before the first ultrasound session to ensure reliability. Four still -pictures were taken of vastus lateralis resulting in 8 pictures per leg in each session in. Additionally, two pictures were taken of vastus medialis on the acute testing day for quantifying the amount of subcutaneous fat. The analysing of ultrasound pictures was conducted using ImageJ (version 1.46r, National Institutes of Health, USA). Muscle thickness measurements was set as the mean value of two pictures, where each picture was measured by the average of three vertical lines between the inner edge of the superficial and deeper aponeurosis was used to measure muscle thickness. The outer and the inner edge of the superficial aponeurosis was used for measurements of subcutaneous fat.

STATISTICAL ANALYSIS. All descriptive data are presented as mean and standard deviation (SD). The StO_2 data were normalised to baseline and full occlusion values and analysed as change of the variable from baseline. Statistical analyses of muscle thickness were conducted with the percentage change of the variable from baseline. Subcutaneous fat was analysed in millimetres (mm). Differences between the failure and submaximal protocols in StO_2 during training session 3 was investigated using a series of independent samples t-test. Normal distribution of the data was analysed visually using histograms, a comparison of mean and standard deviation and consider the skewness to take variance into account. The correlation between the relative change in muscle thickness from baseline to post 17 in percent and average StO_2 during training session 3, expressed in percent was analysed using

Spearman correlation. Area under the curve was first calculated using Medcalc Software (version 7.4.4.0, Belgium) and further analysed using Spearman correlation. The α-level was set to <0.05 in all analyses, except the repeated independent samples t-tests used to investigate differences between the submaximal and failure protocols in StO_2, where the α-level was set to <0.01 due to multiple testing. Statistical analyses were performed in SPSS (version 24, IBM, Chicago, IL, USA). Figures were made with GraphPad (GraphPad Prism 7.03, GraphPad Software, Fay Avenue, CA, USA).

RESULTS

PARTICIPANTS. Twenty participants took part in the intervention (n=20), ten males and ten females. Subjects were aged 24.4 ± 7.1 years, weighted 80.5 ± 24.5 kilograms and measured 170.6 ± 9.2 centimetres in height. One participant dropped out after the rest week due to circumstances unrelated to the study. Subjects had a body mass index (BMI) of 26.3 ± 5.7. Baseline values for muscle thickness and subcutaneous fat for the failure and submaximal groups are presented in table 1.

BLOOD FLOW RESTRICTED RESISTANCE EXERCISE

Total limb occlusion pressure was 155±22 mmHg in the failure leg, 155±23 mmHg in the submaximal leg, the occlusion pressure applied during exercise corresponded to a relative limb occlusion pressure of 66±8% (min: 50%, max: 77%) and 66±8% (min: 49%, max 77%) at 100 mmHg, respectively. Training load throughout the intervention was on average 11.3±3.9 kg on the failure leg and 11.3±3.9 kg on the submaximal leg (20% 1RM). While training load was kept constant, total number of repetitions performed and thus the total amount of work increased proportionally. Throughout the intervention, participants in the failure protocol performed an average of 78 ± 21 repetitions per bout, which was significantly more than the submaximal protocol with their average of 50 ± 1($p < 0.05$). The participants performed an average of 46±14, 13±5, 10±4, 10±4 repetitions during set 1-4 respectively with

the failure leg. The average repetitions performed per bout was 78±21. The prescribed number of repetitions for the submaximal leg were successfully completed by all participants except two: One participant failed to complete the final sets in session 1,2 and 6 and missed 2, 9 and 2 repetitions respectively. Another failed to complete set 2-4 in session 1 and 2, missing 20 and 24 repetitions respectively. All participants completed the total of 50 reps within the 4 sets prescribed in the submaximal protocol during session number 3, whilst the average number of repetitions performed across all 4 sets for the failure group was 76 ± 15 ($p < 0.05$). In set 1-4 separately, this corresponded to 46±10, 12±7, 10±5, 8±4 repetitions respectively.

TISSUE OXYGEN SATURATION. On analysis of the measurements taken during each exercise set and rest, no significant difference in StO_2 was observed between the failure and submaximal groups in any of the muscle areas measured (vastus lateralis proximal, vastus lateralis distal and vastus medialis) (figure 1). The failure group showed a relative decrease in StO_2 of 33(27) % in vastus lateralis proximal, 53 (25) % in vastus lateralis distal and 64 (23) % in vastus medialis from baseline to set 1. Similarly, the submaximal group decreased StO_2 from baseline to set 1 of 31(18) % in vastus lateralis proximal, 51(20) % in vastus lateralis distal and 56 (26) % in vastus medialis. Thereafter the StO_2 both during sets and rest periods, clearly displayed a recurring pattern of reduced saturation during exercise (figure 1). The inter set rest periods showed an increase of a similar degree. This applied to all three areas of measurements (vastus lateralis proximal, vastus lateralis distal and vastus medialis) in both the submaximal and failure group. There was a significant difference in average StO_2 during exercise between the different muscle areas measured: Vastus lateralis proximal 67(25) % showed a higher level of StO_2 during exercise than vastus lateralis distal 48(21) % ($p<0.05$), vastus lateralis proximal 67(25) % showed a higher level of StO_2 than vastus medialis 35(24) % ($p<0.05$) and vastus lateralis distal 48(21) % showed a higher level of StO_2 than vastus medialis 35(24) % ($p<0.05$)). There was a significant difference between the submaximal and

failure groups for the time exposed to hypoxia (failure: 5.3 minutes [5, 5.6]; submax: 4 minutes [4,4]), p=<0.001. The significant negative correlation found between StO_2 during and after exercise for vastus lateralis proximal r=-0.40 (p=0.013) (figure 3) did not apply to the muscles vastus lateralis distal (r=-0.21) or vastus medialis (r=0.13).

MUSCLE THICKNESS

No significant correlation was observed between the average tissue oxygenation during training session 3 and the change in muscle thickness from baseline to post 17 in the locations measured (vastus lateralis proximal (p=0.63) and in vastus lateralis distal (p=0.47)).

SUBCUTANEOUS FAT

Significant correlations between the amount of subcutaneous fat measured and the average StO_2 during session 3 in vastus lateralis distal (figure 2 a) r=0.52 (p=0.01) and vastus lateralis proximal (figure 2 b) r=0.58 (p<0.001) were found. No such correlation was observed between subcutaneous fat and StO_2 in vastus medialis (p=0.44).

DISCUSSION

The pattern of SaO_2 level during a single session of BFRRE showed a significant, although similar, decrease in O_2 saturation from baseline to set 1 in both the submaximal and the failure group. This was followed by a non-significant, recurring pattern from rest number 2 and all points of measurements throughout the final set number 4. Average StO_2 from baseline through to set 4 was significantly different between the muscles. There was a significant correlation between amount of subcutaneous fat in vastus lateralis and average StO_2 during BFRRE; however, this was not the situation with respect to vastus medialis. There was no significant correlation was seen between StO_2 during a session of BFRRE and the change in muscle thickness from baseline to 17 days post intervention.

TISSUE OXYGEN SATURATION. A few studies have investigated the pattern of StO_2 during single sessions of BFRRE, of which some has compared StO_2 during BFRRE with

non-occluded low- or high load resistance exercise training (2, 7, 9). However; to our knowledge, no-one has compared two different low-load BFRRE protocols regarding acute and post exercise StO_2. The re-occurring StO_2 pattern is also observed after investigating the StO_2 in the oblique fibers of vastus medialis during knee extensions with loads of 50 % of 1 RM (7). Ganesan and co-workers (7) also observed that the group which performed knee extension to failure with blood flow restriction was associated with nearly 10% lower average StO_2 during the two inter-set rest periods prescribed, compared to the two non- blood flow restricted groups. The present study reports similar mean levels of StO_2 in both exercise protocols, with resting values continuously decreased throughout the session in both groups.

The low StO_2 through the rest periods is a phenomenon which seem to be characteristic for BFRRE and is discussed to be a possible contributor to the stimuli of hypoxic signalling (2, 7). The rest periods during non-occluded exercise will in contrast, provide a possibility for the muscles to regain normal blood flow and recover in between sets, hence the increase in StO_2 seen in the non-blood flow restricted groups (7). This is underpinned by Fujita et al. (14) who observed that the difference between low load BFRRE (20% 1RM) and high intensity conventional resistance exercise (70% 1RM), was the continuously low StO_2 during inter-set rest periods for the BFRRE group and the recovery of StO_2 into resting levels in the high- intensity group. Both the failure and the submaximal StO_2 patterns seen in the present study seem comparable to the StO_2 pattern BFRRE observed by Ganesan and co-workers (7). Despite the observed decrease in oxyhemoglobin recovery slope during BFRRE, Ganesan and co-workers (7) did not observe any overall reduction in oxyhemoglobin in either exercise or rest. It is further stated that this observation suggest BFRRE does not induce a limited O_2 delivery. Ganesan and co-workers present two possible theories for the hypoxic signaling in BFRRE: 1) The impact of the low StO_2 observed during recovery or 2) the magnified O_2 availability and extraction (7).

Tissue oxygen saturation during different blood flow restriction exercise protocols

The no difference in SaO_2 between the protocols in the present study suggests that it is not necessary to conduct low load BFRRE to failure to achieve the low StO_2 observed during recovery or a greater intermuscular O_2 extraction. However, in light of the significant difference in time under hypoxia which was observed between the protocols, significant difference in time of which each of the legs were under hypoxia, one might speculate if the failure protocol induces a greater amount of metabolic stress, and thus an increased recruitment of muscle fibers compared to the submaximal one. Cayot and co-workers (24) investigated the effect of various durations under occlusion on neuromuscular activation and SaO_2 during low-volume isometric exercise. This was done by applying the blood flow restriction either 5 minutes prior to exercise, immediately before exercise, or not at all (control group). Various submaximal intensities were tested (20, 40, 60, 80 % of maximal voluntary contraction). The results indicate that the application of blood flow restriction prior to exercise, and thus the increase of time under occlusion can enhance the exercise-induced metabolic stress. Similarly, the present study indicates that a longer duration with applied occlusion may affect the extent of hypoxic stimuli and thus can be assumed to induce a greater metabolic stimulus. Despite this, Cayot and co-workers (24) suggest that the duration of occlusion alone is not likely to be the primary stimulus for the increased neuromuscular activation frequently associated with BFRRE.

The significant differences in StO_2 between the different muscles or areas of the same muscle seen in the present study have also been noted by Esaki et al. (12). The reasons for such differences in StO_2 were also discussed and two potential explanations are mentioned (12): 1) When the exercise intensity increases O_2 consumption increase proportionally and 2) differences in intermuscular pressure as a result of muscle contraction may affect the O_2 delivery and result in differences in StO_2 within or between agonists.

The latter explanation is, however, excluded as a reason in Esaki et al. (12), based on their own findings which showed a lower StO_2 in the rectus femoris muscle than in vastus lateralis distal and vastus lateralis proximal during unilateral knee extensions. One may speculate if the latter theory is related to the possible different muscle activation in various muscles or areas of muscle during the specific phases of contractions. Sadamoto et al. (15) recognized contradictive results to Esaki and colleagues (12) as the same exercise showed that a greater increase of intramuscular pressure, indicating a lower StO_2 was found in vastus lateralis, not rectus femoris. This compliment the results of the present study, which shows that low load BFRRE might initiate a similar or greater decrease in StO_2 compared to traditional high load exercise.

The reactive hyperaemia post BFRRE enhances the delivery of nutrients to the working muscles and is hypothesized to be the mechanism stimulating these processes. Gundermann et al. (30) tested this hypothesis by a randomized crossover study with two trials: Low-intensity resistance exercise with blood flow restriction and low-intensity resistance exercise with a pharmacological vasodilator infusion into the femoral artery immediately after exercise to simulate the reactive hyperaemia after blood flow restriction exercise. It is concluded by Gudermann et al. (30) that reactive hyperaemia is not the primary mechanism stimulating mTORC1 signalling and muscle protein synthesis following BFRRE. However, they do highlight the possibility for it to be a supplementary variable (Gundermann et al., 30). To further establish to what extent reactive hyperaemia affect mTORC1 signalling and muscle protein synthesis more research is required.

SUBCUTANEOUS FAT. The findings of positive significant correlations between the amount of subcutaneous fat and degree of StO_2 in vastus lateralis proximal and vastus lateralis distal indicates and reinforces previous findings that tissue oxygen saturation measurements are affected by the amount of subcutaneous fat (12). The present study has

taken into consideration the impact of subcutaneous fat on NIRS-measurements by normalizing the StO_2 values of each subject to their respective values of baseline and full occlusion and included absolute values for StO_2 measurements as recommended (7). The authors justify this by the relatively thin (3-6 mm) overlying tissue observed on the vastus medialis oblique fibers in the participant in the present study (results not shown), the time-resolved approach of measuring StO_2, which is thought to be more sensitive to absorptions in deep tissues, and the level of total hemoglobin recorded. The measurements were consistent between subjects and indicated similar distribution of muscle in the sample volume. In contradiction the overlying tissue of vastus medialis in the present study averaged 16 mm and vastus lateralis 12 mm.

The complex light propagation within tissue, individual variations in subcutaneous fat thickness and the maximal depth of NIRS (approximately half the distance between the light source and detector) are all factors known to potentially influence the measurements (12). It is also found that the light path and metabolism of subcutaneous fat is different from that in muscle tissue. Therefore, as the subcutaneous fat increases, measurements by NIRS will lead to an underestimation of StO2 (28). In a study by De Ruiter and colleagues (11) it was observed a significantly lower degree of StO_2 in rectus femoris compared to vastus lateralis and vastus medialis during knee extensions. Due to the significantly greater amount of subcutaneous fat observed in the same muscle (rectus femoris) compared to vastus lateralis and vastus medialis, the StO_2 was corrected for adipose amount of subcutaneous fat to avoid underestimations. Liaxandrao and colleague (17) states that subcutaneous fat reduces the signal intensity of NIRS by approximately 20 percent when using a source-detector separation of 30-40 mm. It should also be mentioned that regional differences in muscle oxygenation and metabolism in different areas within or between agonists has been registered. As shown in the present study; despite the attempt to adjust for subcutaneous fat by normalizing each subject's

values to its relative baseline and full occlusion values, significant correlations with StO_2 was still found. The individual anatomical differences can result in underestimations or overestimations of StO_2 and potentially provide a different training response for the subjects when not using individualised occlusion pressure (stimuli). There are suggestions of applying a relative occlusion pressure based on each individuals' limb occlusion pressure, and thigh circumference (13,17). Both non-individualised and individualised prescriptions for occlusion pressure applications have been shown to provide sufficient blood flow restriction to the working muscle (17). To what extent this provides an equally sufficient local hypoxia is in need of further investigation. An application of a relative occlusion pressure was not done in the present study due to the overall aim of the main study which was to investigate the difference between a failure protocol previously used in the study by Bjørnsen and colleagues (31) with a submaximal protocol.

MUSCLE THICKNESS. A combination of resistance training and exposure to systemic hypoxic conditions or vascular occlusion induces muscular hypertrophy due to hypoxia (8). Nishimura et al. (19) report that a none-exercising group exposed to hypoxic environments showed no significant improvement in muscle hypertrophy (measured as cross-sectional area). Based on this, the researchers state that hypoxia must be combined with exercise to induce hypertrophic responses. Despite the significant change in muscle thickness in the present study, no significant association with StO_2 during a single session of low load BFRRE and the change in hypertrophy (muscle thickness) 17 days post intervention in vastus lateralis (distal or proximal) was observed. It should be noted that although the level of StO_2 in vastus lateralis during exercise in the present study was found to be significantly lower than baseline, there are not presented measurements of other mechanisms substantiating as to what extend the applied hypoxic stress contributed to metabolic stress during exercise, such as the muscle fiber activation or level of pH within the muscles.

As previously mentioned, the hypoxic stimuli may also have been different between the subjects in the present study, due the absolute occlusion pressure applied during exercise. Along with a somewhat unspecific individual placement of the NIRS- sensors, this may have affected the quality of StO_2 measurements. This indicates that the level of hypoxic stimuli applied throughout the intervention is different between the subjects, resulting in possibly different outcomes of change in muscle thickness. Most importantly it is not known if the StO_2 observed acute in session number 3 is representative for the entire intervention (14 sessions). Therefore, we do not know in the present study, to what extent hypoxic stimuli was contributing to the change in muscle thickness. This study substantiates conclusions of previous research: More research is needed to investigate the real influence of hypoxia on hypertrophic responses (19, 20). Also, investigation of how the StO_2 level affects muscle growth is required to determine a dose-response effect. Monitoring of the StO_2 during all sessions in the intervention of future studies would also be beneficial to ensure representative hypoxic stimuli throughout the intervention.

PERSPECTIVES. The findings of the present study are comparable to other studies investigating the acute effects of BFRRE in regard to StO_2 patterns (2,7). One may speculate if the duration of hypoxia achieved through BFRRE potentially play a greater role than the level of hypoxia in enhancing metabolic stress, greater muscle fiber activation and the drive of some of the physiological adaptions resulting in greater muscle thickness (Loenneke 12, Patterson 13). We argue that future research incorporating measurements of muscle fiber activation (through electromyography) and components related to muscular stress, such as pH along with StO_2 measurements would likely aid in a better understanding of how different BFRRE protocols affect metabolic stress. The results of the present study show no difference between the failure or the submaximal protocols the non-significant association with muscle thickness after a period of high intensity BFRRE. One possible reason for this may be the

standardised pressure applied during the BFRRE which likely have provided different stimuli for the individual subjects. StO_2 measurements were taken during the third training session only. Lacking control over the StO_2 on the remaining 13 training bouts of the intervention give reason to speculate if the obtained data is representative for all sessions. We suggest that future research should include StO_2 measurements during all bouts of training. Based on the results of the present study, we argue that the similar hypoxic stimulus achieved by both the submaximal and failure protocols indicate that either may be used to induce muscular growth. Previous findings by Wernbom et al., (5) suggests that the rate of perceived exertion increase with exercise intensity. Thus, we argue that there is a likeliness for subjective experiences of a more strenuous failure protocol due to the intensity of the protocol compared to the submaximal one. This give reason to suggest the submaximal protocol for prescription to populations similar to that of the present study (untrained men and women aged 18-45 yrs). Athletes and highly motivated individuals may benefit further from the failure protocol as a part of a training programme, in combination with conventional high load resistance training (24), or in rehabilitation.

STRENGTHS AND LIMITATIONS.

One strength of the present study is the adherence of the subjects throughout the intervention as well as the control of the supervised individual exercise sessions and testing. Methodological differences between previous and present research including type of exercise, load, duration of interest rest periods, number of repetitions in sets, duration of the eccentric and concentric phases of the exercise, hypoxia induced locally (through occlusion pressure). Any of which may affect the hypoxic muscle environment, metabolic stress, and muscular adaptions and therefore should be considered when comparing different literature. The greatest limitation of the present study is the lack of control over StO_2 during the entire intervention and therefore it is difficult to know whether the data collected on the third

training session is representative for all the remaining sessions of the intervention. Another limitation is the lack of power calculations with the StO_2-measurements. A test-retest such as coefficient of variation (CV) should be conducted to ensure reliable measurements of the NIRS-instrument. The randomized design used in the present study aids in avoiding selection-bias and thereby strengthen the internal validity. The use of the same sized cuff across all subjects is a weakness of the study, as evidence suggest that the cuff size should be based on thigh circumference to ensure the intended stimuli to be similar between the subjects (13, 18). The placement of NIRS sensors was not precisely consistent between the subjects due to the already applied electromyography sensors and the size of the cuff. The individual differences in thigh circumference offered a varied amount of thigh surface area available for the NIRS-sensors to be applied.

CONCLUSION. The level of StO_2 in the submaximal and failure protocols was similar in degree, although the failure protocol promoted hypoxia for a longer time period. StO_2 during a single bout of BFRRE and muscle thickness 17 days post intervention showed no association. This is a topic for further investigation and we suggest monitoring StO_2 throughout an entire intervention period as a precaution to achieve a similar hypoxic stimulus between the participants. Subcutaneous fat should be accounted for when investigating StO_2 in the future., preferably by individualizing the occlusion pressure. More research is needed regarding how different BFRRE protocols affect the level of hypoxia.

ACKNOWLEDGEMENTS OF INTEREST. The University of Agder supported the present study by a grant. The authors wish to direct our gratitude to all the subjects for their time and commitment to the present study. There are no conflicts of interest. The results of the present study do not constitute endorsement American College of Sport Science, and are presented honestly, clearly and without falsification, fabrication, or inappropriate data manipulation.

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TABLES AND FIGURES

Table 1: Muscle thickness and subcutaneous fat for m. vastus lateralis distal and m. vastus medialis for the failure and submaximal groups at baseline. (Mean (SD)). *P-value indicates differences between the failure and submaximal groups. (#mm, millimetres).

Figure 1: Tissue oxygen saturation during BFRRE session 3 in A) m. vastus lateralis proximal, B) m. vastus lateralis distal and C) m. vastus medialis. The failure protocol is pictured in red circles and submaximal group in blue squares. Values are presented as mean with 95% CI.
*Indicates a significant decrease observed in tissue oxygen saturation (StO_2) from Baseline to set 1 in all muscles; vastus lateralis proximal, vastus lateralis distal and vastus medialis ($P<0.01$).

Figure 2: Correlation between subcutaneous fat and tissue oxygen saturation (StO_2) in A) m. vastus lateralis distal and B) m. vastus lateralis proximal.

Figure 3: Correlation between tissue oxygen saturation (StO_2) during and after training in m. vastus lateralis proximal.

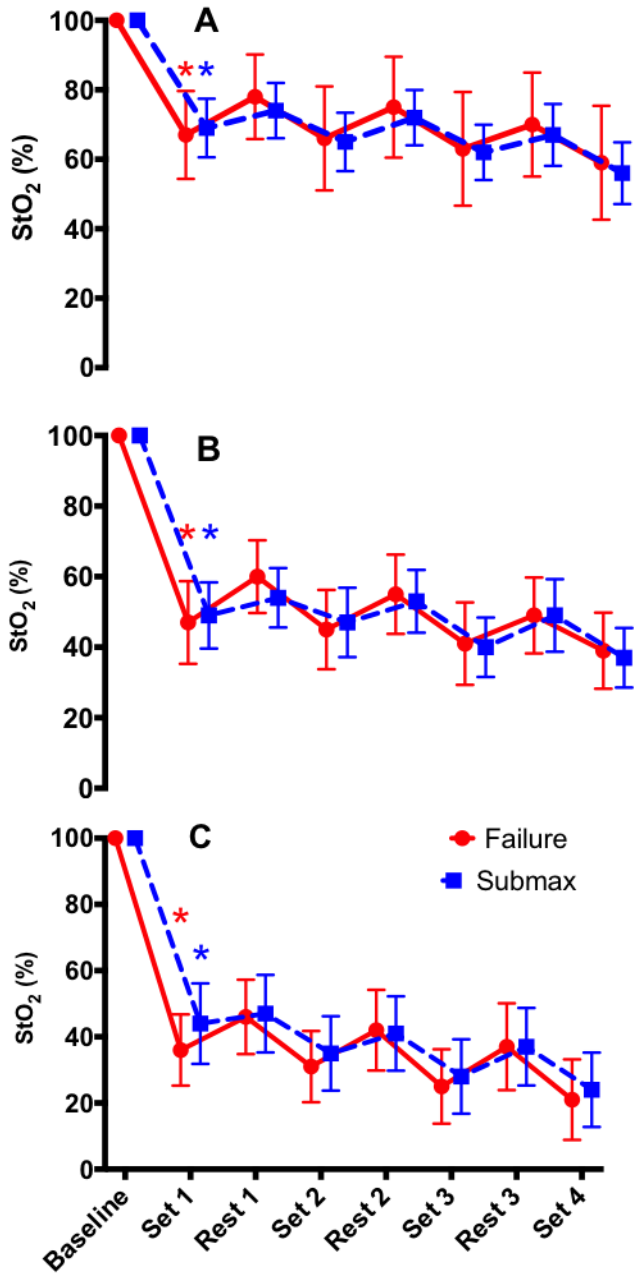
Tissue oxygen saturation during different blood flow restriction exercise protocols

Table 1:

	Failure (n=20)	**Submax (n=20)**	**P-value**
Muscle Thickness (mm):			
Vastus Lateralis (distal and proximal)	24 (3)	23 (5)	0.34
Subcontaneous Fat (mm):			
Vastus Lateralis distal	12.1 (4.6)	12.7 (4.8)	0.67
Vastus Medialis	16.0 (5.4)	16.0 (5.8)	0.98

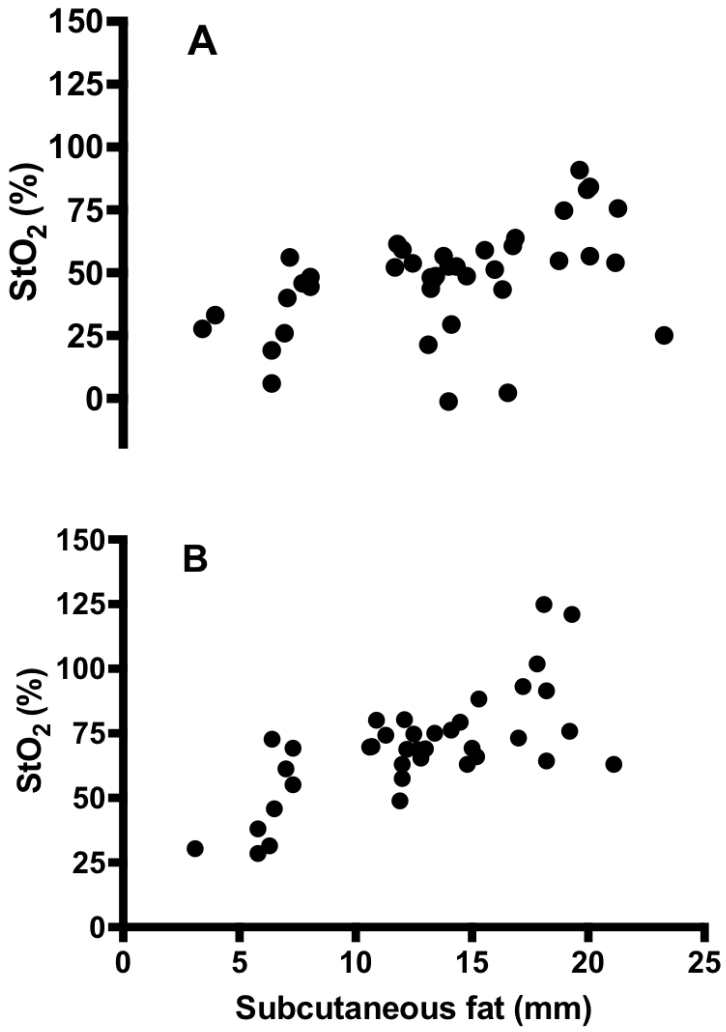
Tissue oxygen saturation during different blood flow restriction exercise protocols

Figure 1:



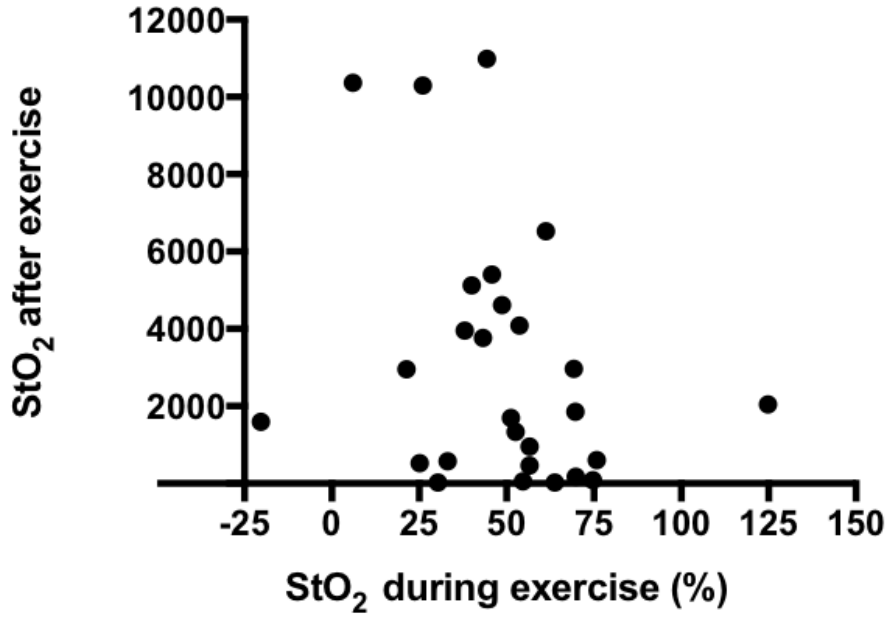
Tissue oxygen saturation during different blood flow restriction exercise protocols

Figure 2:



Tissue oxygen saturation during different blood flow restriction exercise protocols

Figure 3:



Appendix 2

Thomas Bjørnsen
Serviceboks 422
4604 KRISTIANSAND S

Vår dato: 20.10.2017 Vår ref: 55323 / 3 / LH Deres dato: Deres ref:

Tilrådning fra NSD Personvernombudet for forskning § 7-27

Personvernombudet for forskning viser til meldeskjema mottatt 14.08.2017 for prosjektet:

55323	*Styrketrening med redusert blodstrøm, utmattelse eller standardisert protokoll?*
Behandlingsansvarlig	*Universitetet i Agder, ved institusjonens øverste leder*
Daglig ansvarlig	*Thomas Bjørnsen*

Vurdering

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

Vilkår for vår anbefaling

Vår anbefaling forutsetter at du gjennomfører prosjektet i tråd med:

- opplysningene gitt i meldeskjemaet og øvrig dokumentasjon
- vår prosjektvurdering, se side 2
- eventuell korrespondanse med oss

Meld fra hvis du gjør vesentlige endringer i prosjektet

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

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

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

Vi tar kontakt om status for behandling av personopplysninger ved prosjektslutt

Ved prosjektslutt 31.12.2022 vil vi ta kontakt for å avklare status for behandlingen av personopplysninger.

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

Vennlig hilsen

Marianne Høgetveit Myhren

Lise Aasen Haveraaen

Kontaktperson: Lise Aasen Haveraaen tlf: 55 58 21 19 / Lise.Haveraaen@nsd.no
Vedlegg: Prosjektvurdering

Personvernombudet for forskning

Prosjektvurdering - Kommentar

Prosjektnr: 55323

FORMÅL
Formålet med prosjektet er å sammenligne en protokoll til utmattelse og en standardisert treningsprotokoll med 20-, 10-, 10- og 10 repetisjoner.

UTVALG OG REKRUTTERING
Utvalget består av friske menn og kvinner i alderen 19-45 år, som ikke har trent styrketrening systematisk de siste 6 månedene (mer enn 1 gang per uke).

Deltakerne rekrutteres gjennom annonsering i ulike medier i lokalsamfunnet, og via plakater ved Universitetet i Agder. Potensielle deltakere viser interesse ved å kontakte forsker, og blir deretter kalt inn til informasjonsmøte. Det opprettes ikke kontakt mellom informanter og forsker før de har ytret ønske om å delta i prosjektet. Personvernombudet mener rekrutteringsformen er tilstrekkelig for konfidensialitet og at frivilligheten ved deltagelse ivaretas gjennom prosessen.

INFORMASJON OG SAMTYKKE
Utvalget informeres skriftlig og muntlig om prosjektet og samtykker til deltakelse. Informasjonsskrivet vi har mottatt er stort sett godt utformet, men periode for oppbevaring av datamaterialet må endres fra 15 til 5 år, jf. epost fra forsker datert 12.10.2017. Kontaktopplysninger til daglig ansvarlig (Thomas Bjørnsen) og prosjektleder (Sveinung Berntsen) må også legges til.

Videre ber vi om at følgende setning fjernes: «Representanter fra kontrollmyndigheter i inn- og utland kan få utlevert studieopplysninger og gis innsyn i relevante deler av din journal. Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal». Dette fordi fagfellevurderte tidsskrift kun vil få innsyn i anonymiserte data, jf. epost fra forsker datert 20.09.2017.

Revidert informasjonsskriv skal sendes til personvernombudet@nsd.no før utvalget kontaktes.

DATAMATERIALET
Data samles inn gjennom standardiserte tester som er godt etablerte ved Universitetet i Agder. Forsker vil samle inn målinger av kroppssammensetning, muskelstyrke, -størrelse, og power.

Personvernombudet vurderer at det behandles sensitive personopplysninger om helseforhold, jf. personopplysningsloven § 2 nr. 8 c.

INFORMASJONSSIKKERHET
Personvernombudet legger til grunn at forsker etterfølger Universitetet i Agder sine interne rutiner for

Appendix 3

Forsøkspersoner søkes

UNIVERSITETET I AGDER

Kom i gang med beintrening!

Alt er gratis!

Bakgrunn for studien

Tidligere studier har vist kraftig muskelvekst på få dager selv med relativ lett motstand når blodtilførselen til muskelen reduseres (okklusjonstrening)

Fordeler med deltakelse

- Tett oppfølging av fagpersoner
- Tilgang på alle personlige testresultater
- Analyse av muskelmasse og fettprosent
- Innblikk i forskningsverdenen

Studien innebærer

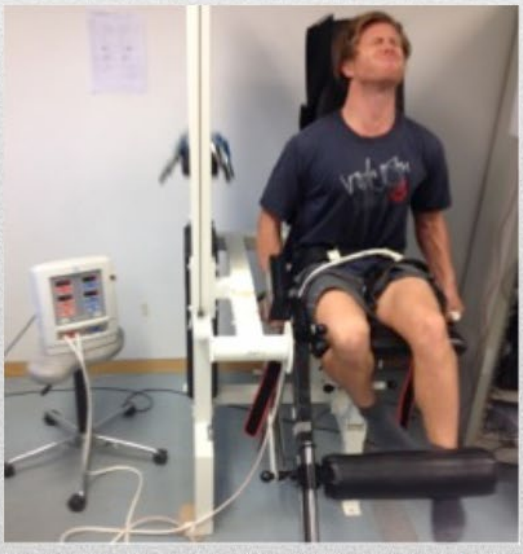
- To treningsperioder med syv økter (15 min per økt)
- Testing før, under og etter treningsperioden
- Testing innebærer
  - Muskeltykkelse
  - Muskelstyrke
  - Muskelaktivering

Kriterier for å være med

- Mann eller kvinne mellom 18-45 år
- Ingen bruk av kosttilskudd under testperioden
- Ikke trent regelmessig styrke på bein de siste 6 månedene (≤ 1 økt i uka)
- Tilgjengelig for trening og testing på Spicheren, september – november 2017

Eventuelle ulemper med deltakelse

- Treningen kan medføre en følelse av sårhet/stølhet i muskulaturen.



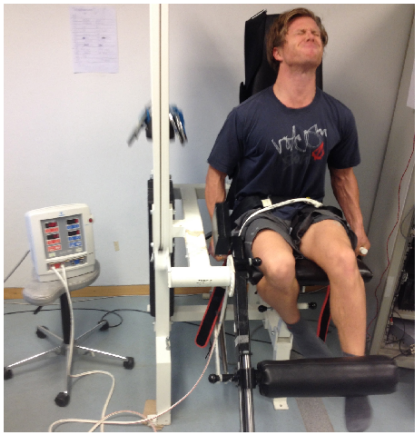
Interesserte bes ta kontakt med masterstudenter:

Kolbjørn Lindberg	**90870067**	**kalind.93@gmail.com**
Eller:		
Njål V. Sandnes	**90520752**	**njaal991@gmail.com**

Appendix 4

Forsøkspersoner søkes

Gratis beintrening



Fordeler med deltakelse

- Tett oppfølging av fagpersoner
- Tilgang på alle personlige testresultater
- Analyse av muskelmasse og fettprosent
- Innblikk i forskningsverdenen
- Hver økt tar kun 15min (!)

Ta kontakt ved stand i kantina eller:
Kalind.93@gmail.com

Appendix 5

Forespørsel om deltakelse som forsøksperson

Styrketrening med redusert blodstrøm

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

1) Du må være mann eller kvinne i alderen 18 - 45 år.

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

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

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

Bakgrunn og hensikt med forsøket

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

Gjennomføringen av forsøket

Tissue oxygen saturation during different blood flow restriction exercise protocols

Forsøket går ut på at du trener 7 treningsøkter på 5 dager i 2 perioder. De to treningsperiodene er avskilt med 10 dager hvile. Til sammen vil det bli 14 økter på 10 dager. Treningen består av sittende kneekstensjoner (forsiden av lårene), mens en trykkmansjett er plassert øverst på låret (i lysken).

Du vil bli trene begge bena, men med forskjellige protokoller. Det ene benet vil trene med 4 sett til utmattelse, mens det benet vil trenes submaksimalt nært utmattelse, tilfeldig valgt bein. Vi ønsker å se hvilken protokoll som er mest effektiv for muskelvekst, maksimal styrke, muskelpower samt muskelaktivering.

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

Før forsøket

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

Styrketrening med redusert blodstrøm

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

Treningsøkten består av 4 serier med 20 % av maksimal motstand til utmattelse på et ben, eller 4 sett med 20-, 10, 10 og 10 repetisjoner på det andre benet, i et kneekstensjonsapparat. Det vil være 30 sekunder pause mellom seriene. Blodstrømmen til arbeidende muskulatur vil være begrenset med ca. 50 % pga. trykkmansjetten.

Første treningsdag vil kreve det lengste oppmøtet. Her blir det tatt diverse tester (styrke, power, ultralyd, antropometri (høyde, vekt), elektromyografi)

Eventuelle ulemper ved å delta

- Deltakelse i prosjektet vil kreve mye tid og oppmerksomhet i treningsukene. Du må møte ved Universitetet/Spicheren totalt ca. 22-23 dager denne høsten (september – november 08.11).
- Trening som gjennomføres kan medføre en viss risiko for muskelskader, og følelse av sårhet/stølhet i muskulaturen.
- Trening med redusert blodstrøm kan oppleves som meget ubehagelig, men det er ikke knyttet mer risiko til denne typen trening sammenlignet med tradisjonell styrketrening.

Personvern

Vi vil kun lagre informasjon om deg under ditt forsøkspersonnummer. Undervis i forsøket vil vi oppbevare en kodeliste med navn og forsøkspersonnummer. Denne kodelisten vil være innelåst, slik at det er kun forskerne tilknyttet studien som har adgang til den. Representanter fra kontrollmyndigheter i inn- og utland kan få utlevert studieopplysninger og gis innsyn i relevante deler av din journal. Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. Alle som får innsyn i informasjon om deg har taushetsplikt. Innsamlet data vil bli anonymisert etter 15 år (kodelisten destrueres).
Alle prøver vil analyseres "blindet", det vil si at forskerne som utfører den enkelte analysen ikke vet hvilken forsøksperson prøven kommer fra. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Innsynsrett og oppbevaring av materiale

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

Informasjon om utfallet av studien

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

Tissue oxygen saturation during different blood flow restriction exercise protocols

Forsikring

For skade på mennesker som oppstår under medisinske forsøk, gjelder pasientskadelovens regler tilsvarende (hfl. § 50). Staten er selvassurandør for universitetene.

Finansiering

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

Publisering

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

Samtykke

Hvis du har lest informasjonsskrivet og ønsker å være med som forsøksperson i prosjektet, ber vi deg undertegne "Samtykke om deltakelse" og returnere dette til en av personene oppgitt nedenfor. Du bekrefter samtidig at du har fått kopi av og lest denne informasjonen.
Det er frivillig å delta og du kan når som helst trekke deg fra prosjektet uten videre begrunnelse. Alle data vil, som nevnt ovenfor, bli avidentifisert før de blir lagt inn i en database, og senere anonymisert.

Dersom du ønsker flere opplysninger kan du ta kontakt med

Kolbjørn Lindberg på tlf: 90870067, eller på mail: kalind.93@gmail.com
Njål Varhaug Sandnes på tlf: 90520752, eller på mail: njaal991@gmail.com

Vennlig hilsen
Prosjektgruppen for okklusjonstrening

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)