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Master thesis

How obesity impacts the muscle fiber cross-sectional area and fiber type composition in an untrained, sedentary population

Master's degree in training physiology

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Forord

Jeg vil sende en stor takk til Håvard Hamarsland for at du lever og ånder for styrketrening og muskelfysiologi, og at du alltid har vært tilgjengelig om jeg har lurt på noe. Du har vært en viktig bidragsyter for å få denne oppgaven fullført, og fått meg til å innse at jeg må jobbe på uansett hvor god tid jeg har trodd at jeg har hatt. Takk for at du lot meg være med på prosjektet, og at jeg fikk lov til å pirke i litt lårmuskulatur sammen med deg på biopsirommet. Jeg vil i tillegg si tusen takk til Daniel Hammarström for å være veldig behjelpelig med all statistisk hjelp i R Studio, og svare på alle mine dumme spørsmål om alt fra grafer til pipelines.

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Til slutt vil jeg takke alle medstudenter for å ha holdt ut med meg gjennom 5 år, dere er gull verdt hele bølagjengen.

Summary

Introduction: Obese individuals possess excess adipose tissue interacting with the skeletal muscle, resulting in an alteration in the contractile properties of the muscle tissue resulting in reduced muscle quality and function.

Purpose of study: The aim of the study was to investigate how the degree of obesity impacted the muscle fiber type CSA and composition in an overweight sedentary population after a 10-week resistance training intervention.

Method: 29 men and women between 30-60 years of age were recruited and completed 10 weeks of 2 weekly supervised resistance training sessions. Biopsies and ultrasound of m. vastus lateralis, DXA scans and isokinetic knee extensor strength tests were conducted prior to supplementation period, prior to intervention period and after intervention period. Results were analysed in RStudio.

Results: The results indicated no significant increase in muscle fiber type CSA for type I nor II between the timepoints, but there was a significant increase in the intervention period in comparison with the control period of CSA for both fiber types. There was a significant increase in isokinetic strength and muscular growth measured as muscle thickness amongst the participants.

Conclusion:
Based on these findings there's reason to believe that in relation to individuals with a BMI within recommended values, the increased chronic inflammation due to obesity, in correlation with inactivity, leads to an increased muscle fiber type CSA, shift in fiber type composition with increased ratio of fiber type II as well as a reduction in relative muscle strength.

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Theory

Obesity has developed to be one of the leading public health challenges in the 20th century, where more than 1,9 billion of the world's population over 18 years are affected as of 2020 (Mohammed, Sendra, Lloret, & Bosch, 2018). The amount of obese people/individuals in the world are drastically increasing, with a notable escalation within the last few decades (Blüher, 2019). Norway has proven to be one of the four countries in western Europe with the greatest increase in occurrence of obesity amongst adults, and a report from 2019 showed that approximately 1 in 5 adult Norwegians are overweight (Aamo, Lind, Myklebust, Stormo, & Skogli, 2019). Obese individuals possess excess adipose tissue interacting with the skeletal muscle, resulting in an alteration in the contractile properties of the muscle tissue resulting in reduced muscle quality and function (Hill & Tallis, 2019; Stuart, Lee, South, Howell, Cartwright, et al., 2017). Being obese is associated with several chronic diseases like diabetes, hypertension, cardiovascular disease, liver disease and different cancers, which all contribute to a deterioration in the quality of life and life expectancy (Blüher, 2019; Gutin, 2018). Depending on the severity of the disease and comorbidities, obesity is considered to decrease the life expectancy with approximately 2-10 years (Whitlock et al., 2009). Previous research has observed that one of the most important factors of obesity treatment is physical exercise, if it's performed regularly and properly. Studies have shown that physical activity contributes to an increased energy expenditure and fat loss, as well as protecting against the loss of lean body mass, reducing obesity-related cardiometabolic health risks, and increases the overall mental wellbeing (Ahtiainen et al., 2016; Okay, Jackson, Marcinkiewicz, & Papino, 2009).

Body-mass index (BMI) is one of the standards by which obesity and healthy weight is measured and defined, as it calculates one's body size by dividing the person's weight with the height squared (kg/m^2). The calculation is more of an indicator than a direct measurement of both the amount of total body fat and your overall physical health, as it is not a precise measurement for predicting whether the observed lean body mass is either muscle tissue, organs, bones, body water or adipose tissue. Nevertheless, it can be a good indication for predicting whether an individual is within healthy weight limits or not (Calle, Thun, Petrelli, Rodriguez, & Heath, 1999). This unit of measurement predicts that healthy weight limits are with a BMI between 18 and 25, BMI over 25 is considered overweight while a BMI equal to or greater than 30 is categorized as obese.

There's an abundance of factors as to why people gain weight and become overweight, and research has shown that one of the leading causes is increased inactivity (United Nations General Assembly, 2011). Additionally, studies have shown that genetics, increased energy consumption and medicinal causes, as well as demographic and socio-economic conditions have proven to be dependable factors (DeNies et al., 2014; Okay et al., 2009). Obesity entails other physiological adaptations like increased fat mass, both total, visceral and intramuscular fat mass, a heightened inflammatory milieu and an enhanced risk of metabolic syndrome (Fantuzzi, 2005; Thompson, Jarvie, Lahey, & Cureton, 1982). In addition, previous studies have shown a correlation between increased BMI and the probability of developing lifestyle diseases, also known as non-communicable diseases (NCD). Overweight people are particularly vulnerable to cardiovascular diseases like hypertension and stroke, type 2 diabetes as well as an increased risk for several types of cancer, including breast, colorectal, prostate and kidney cancer (Blüher, 2019; Kruger, Ham, & Prohaska, 2009). Additionally, evidence from previous studies suggest that overweight people may experience different psychological adaptations, where a reduced quality of life is eminent as well as increased mortality. Research has shown that even handling simple everyday tasks may suddenly seem daunting, which is often associated with a reduced overall motivation and lowered self-esteem (Blüher, 2019; Chin, Kahathuduwa, & Binks, 2016; Fantuzzi, 2005).

The principal cause of obesity and weight gain is the increased amount of adipose tissue due to a positive energy balance, where the person has a larger energy consumption than energy expenditure throughout the day. Adipose tissue is considered to be the largest endocrine organ in the body, secreting cell signalling proteins called adipokines. Amongst others they send out and respond to signals that modulate appetite and energy expenditure, as well as contributing to the regulation of the inflammatory response (Fantuzzi, 2005; Fasshauer & Blüher, 2015; Mancuso, 2016). This increase of adipose tissue and thus adipokines can accelerate the aging process by affecting cell signalling processes in the skeletal muscle, and thereby causing a decline in the contractile function of the skeletal muscle (figure 1) (Schiaffino & Reggiani, 2011). Previous research has shown that obesity impacts the contractile performance of the muscle the same way ageing does (David J Tomlinson, Robert M Erskine, Keith Winwood, Christopher Ian Morse, & Gladys L Onambélé, 2014). They have both been associated with chronic inflammation, muscle atrophy, reduced protein synthesis in response to exercise, fiber type shifting and impaired excitation-contraction coupling (Erskine et al., 2017; Hill & Tallis,

2019; Koster et al., 2011; Messa et al., 2020; Tomlinson, Erskine, Morse, Winwood, & Onambélé-Pearson, 2016).

Obesity can lead to increases in the absolute force and power of the weight-bearing muscles in the lower extremity due to the increased demand (Maffiuletti et al., 2007). However, if you normalize the force production and power output to the total body mass there's a decrease related to increased BMI (Abdelmoula et al., 2012; Hubal et al., 2005), which leads to a reduced muscle quality and fatigue resistance (Franchi et al., 2018). All these factors mediated by obesity may lead to reduced mobility, exercise capacity and respiratory muscle function (Okay et al., 2009; J. Tallis, James, & Seebacher, 2018).

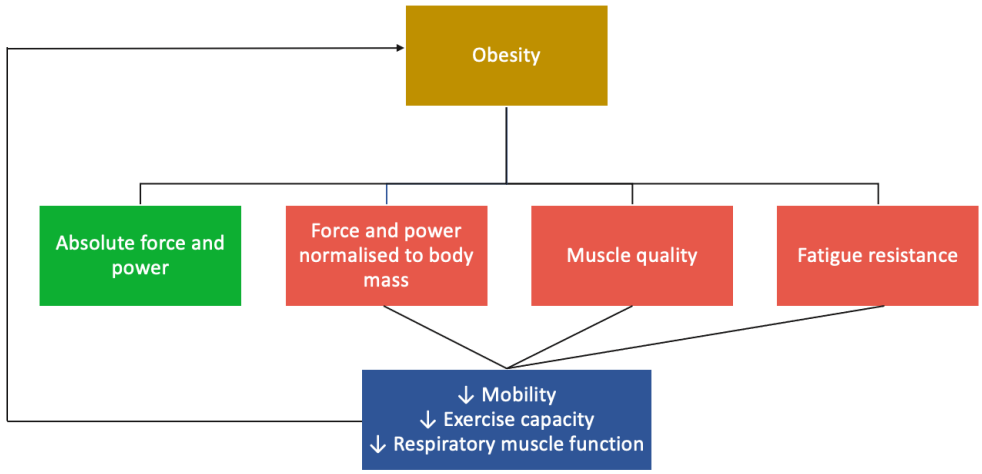

Figure 1 Summary of physiological adaptations to obesity in skeletal muscle. Obesity can lead to increased absolute force and power in muscles in the lower extremity, but not when normalised to body mass. This leads to decreased muscle quality and fatigue resistance. Due to these adaptations, obesity may result in reduced mobility, exercise capacity and respiratory muscle function.

Mammalian skeletal muscle is a heterogeneous organ composed of different muscle fiber phenotypes characterised by dissimilarities in their metabolic and contractile properties, where the various phenotypes are differentiated by their structural and functional characteristics. Previous studies have shown that using histochemical staining for pH-sensitive myosin adenosine triphosphatase (mATPase) activity has proven to be an efficient method to differentiate the fiber types based on which myosin heavy chain (MHC) isoform they express (Röckl et al., 2007; Schiaffino & Reggiani, 2011). This method has shown that the human skeletal muscle consists of two major fiber type classifications, the slow-twitch (type I) and fast-twitch (type II) fibers (Verdijk et al., 2010). Whilst there are at least 11 MHC isoforms encoded by different genes in mammalian skeletal muscle, only four genes have

been found in adult skeletal muscle: I, IIa, IIx and IIb (DeNies et al., 2014; Tanner et al., 2002). Even though humans possess the gene that encodes for the MHC IIb isoform, it is not expressed within skeletal muscle (DeNies et al., 2014).

The fiber type characteristics differ in their MHC isoforms and the enzymatic capacity they possess. Muscle fiber type I, containing the MHC isoform MHCI, is characterized as being a fatigue resistant, mitochondria-rich vascularized fiber with high oxidative capacity. They utilize mostly oxidative phosphorylation within the mitochondria as their metabolic pathway to produce adenosin triphosphate (ATP), and they can thereby withstand longer periods of energy demanding tasks. The fast-twitch type II fibers are subdivided into fast-oxidative (type IIa) and fast-glycolytic (type IIx) muscle fibers, based amongst others on the MHC isoforms they express and the concentration of mitochondria within the muscle fibre (Schiaffino & Reggiani, 2011). Type IIx mainly depend on anaerobic glycolysis in the cytoplasm of the cell as their cellular respiration to generate ATP as they have a lower concentration of mitochondria, and therefore a lower oxidative capacity than the type I and IIa fibers (Couturier et al., 2013). Like the type I fibers, type IIa have a lower shortening velocity, force and power production than type IIx (J. Tallis et al., 2018). In addition, the type IIa and IIx fibers generally contain a higher concentration of sarcoplasmic reticulum (SR) than type I fibers, located within close proximity to the t-tubules of the cell membrane. When the muscle fiber is stimulated by an action potential, the depolarization of the cell membrane triggers a release of calcium-ions from SR which facilitates the interaction between actin and myosin during contractions. Due to the increased numbers of SR located within the type II muscle fibers, they have a higher muscle contraction and relaxation speed than type I which in turn make them more explosive and powerful (Bourdeau-Julien, Sephton, & Dutchak, 2018; Dirk Pette & Staron, 1997). As type IIx muscle fibers depend on anaerobic glycolysis and thereby produce less ATP than the oxidative type I and type IIa fibers, they are more easily fatigued and can easily wear out within few minutes (Bourdeau-Julien et al., 2018).

The skeletal muscles are highly plastic tissues that adapts to the different tasks they are given to complete, whether that is to lift heavy weights, run fast or living a sedentary lifestyle (Lieber, Roberts, Blemker, Lee, & Herzog, 2017). The precise estimate as of how much of the muscle's phenotype is either predetermined by genetics or is caused by an adaptation to nutritional or physiological challenges at a molecular and phenotypic level is still unknown (Bourdeau-Julien et al., 2018; Staron, 1997). Previous studies have suggested that

approximately 40% is predetermined by genetics, while 45% may be a result of the stimulus that affects the skeletal muscle (DeNies et al., 2014). When there's reoccurring changes to the different fiber types' metabolic environment it can lead to phenotypic changes of the fibers that generate fiber type switching (Hather, Tesch, Buchanan, & Dudley, 1991). This is mediated by activation of various cell signalling and transcriptional mechanisms that stimulate an adaptive process changing the energy metabolism of the cell, increasing the activity level of enzymes involved in the metabolism that eventually results in fiber type transitions (Bourdeau-Julien et al., 2018).

In healthy adult human skeletal muscle, there's a consensus that one can predict a change in fiber type composition with fiber type transitions from type IIx ↔ type IIa ↔ type I (Jansson, Sjödin, & Tesch, 1978; D. Pette & Staron, 2000; Schiaffino & Reggiani, 2011; J. Tallis et al., 2018). Previous studies have shown that the factors that result in an increase in neuromuscular activity causes fast-to-slow fiber type transitions, while factors that decreases the neuromuscular activity leads to transitions from slow-to-fast fiber types. The alteration in the muscle fiber stimuli result in a change of the inner milieu of the fiber type affecting the fiber's functional elements like the enzyme activity, the elements of the Ca^{2+}-regulatory system and the contractile and regulatory proteins of the myofibrillar apparatus (Burkholder, Fingado, Baron, & Lieber, 1994; Dirk Pette & Staron, 1997). The majority of previous studies indicate that resistance training (RT) in healthy individuals typically results in muscle hypertrophy and a decrease in the muscle fiber type IIx content, with an increase in type IIa fibers (Methenitis et al., 2020; D. Pette & Staron, 2000; Stuart, Lee, South, Howell, & Stone, 2017). As opposed to this, researchers have indicated that a lack of exercise and immobilization of the muscles may facilitate changes between slow and fast twitch muscle fiber types with an increase in the type IIx fibers (Jansson et al., 1978; Wilson et al., 2012). It's worth to note that changes in the metabolic function of the muscle fiber can occur without fibre type transitions, where exercise training can promote metabolic changes without there being fiber type transitions (Farrell, Joyner, & Caiozzo, 2011; J. Tallis et al., 2018).

Obesity can affect the signalling pathways that regulates muscle fiber type and contractile function (figure 2) (Hill & Tallis, 2019; Kriketos et al., 1997; Layne et al., 2011). Obesity leads to an increased insulin secretion which can promote regulations in the metabolic environment within the fiber, activating cell signalling and transcriptional systems that promote altered metabolism, force production, power output and fatigue resistance

(Bourdeau-Julien et al., 2018; J. Tallis et al., 2018). Research has shown that two of the pathways that are affected the most by obesity and excess adiposity are calcium signalling to the muscle fiber and adenosine monophosphate-activated protein kinase (AMPK) activity, which is activated during energy depletion (Schiaffino & Reggiani, 2011). During exercise one observes a reduced ATP concentration due to an increased energy expenditure, where ATP is hydrolysed to either adenosine diphosphate (ADP) or AMP. The reduction in the ratio between ATP and AMP activates AMPK, which directly impacts a shift from fast to slow muscle fiber types in addition to causing exportation of class II histone deacetylases (HDAC) from the nucleus. HDAC suppress the transcription of myocyte enhancer factor 2 (MEF2); a protein that mediates the shift from fast to slow fibers. By exporting HDAC out of the nucleus, the supressing effect on MEF2 is lifted, and you get an increase in muscle fiber type transformation from fast to slow fiber types. Due to the increased concentration of insulin, obesity inhibits the activity in AMPK leading to a reduction in the exportation of HDAC from the nucleus. This alters the expression of MEF2 and thereby reducing the quantity of type I fibers. The other signalling pathway to augmented transcription of MEF2 is the calcium cycling, where increased energy expenditure result in an increased calcium concentration in the cytosol by influx of extracellular calcium or releasement from SR. Increased calcium concentration in the muscle fiber cytosol activates calcineurin (CnA), that causes nuclear factor of activated T-cells (NFAT) to enter the nucleus and increase the expression of MEF2. Increased adipose tissue alters the calcium cycling, leading to a decreased transcription of MEF2 altering the fiber type transitions to slow muscle fibers.

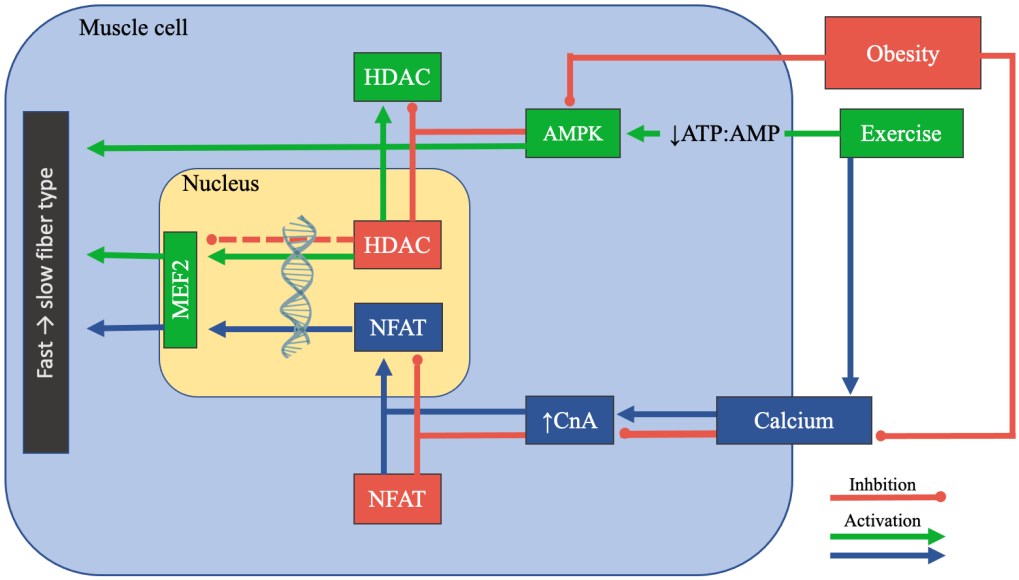

Figure 2 Summary of how obesity influence muscle fiber type transitions from fast to slow fiber type.
Due to increased energy expenditure, the ratio between ATP and AMP decreases, leading to an increase in activity of adenosine monophosphate-activated protein kinase (AMPK). AMPK directly impacts the fiber type transitions from fast to slow fiber types and promotes nuclear export of class II histone deacetylases (HDAC), a protein suppressing transcription of the myocyte enhancer factor 2 (MEF2) which enhances fast to slow fiber type transitions. Obesity inhibit AMPK activity, reducing the direct fast to slow fiber type transitions and export of HDAC from nucleus, leading to a decreased transcription of MEF2. Increased energy expenditure *additionally* enhances calcium concentration in the cytosol by influx from extracellular sources or release from sarcoplasmic reticulum (SR), activating calcineurin (CnA). Activation of CnA generates the nuclear factor of activated T-cells (NFAT) to enter the nucleus, stimulating transcription of MEF2 and slow fiber type expression. Obesity interferes with calcium signalling which mediates a decreased transportation of NFAT into nucleus.

There's a general consensus that obesity has a negative effect on the muscle quality and function, where increased adipose tissue may contribute to accelerated loss of muscle mass and strength with aging (Koster et al., 2011). Even though studies have examined the impact BMI has on intramuscular alterations, the literature on the correlation between the effects of RT on the muscle tissue in people with varied levels of obesity is limited. Therefore, the aim of the present thesis was to investigate how the degree of obesity impacted the muscle fiber type CSA and composition in an overweight sedentary population after a 10-week resistance training intervention.

Introduction

Obesity has developed to be one of the leading public health challenges in the 20th century, where more than 1,9 billion of the world's population over 18 years are affected as of 2020 (Mohammed, Sendra, Lloret, & Bosch, 2018). Obese individuals possess excess adipose tissue interacting with the skeletal muscle, resulting in an alteration in the contractile properties of the muscle tissue resulting in reduced muscle quality and function (Hill & Tallis, 2019; Stuart, Lee, South, Howell, Cartwright, et al., 2017). Studies have shown that physical activity contributes to an increased energy expenditure and fat loss, as well as protecting against the loss of lean body mass, reducing obesity-related cardiometabolic health risks, and increases the overall mental wellbeing (Ahtiainen et al., 2016; Okay, Jackson, Marcinkiewicz, & Papino, 2009).

The principal cause of obesity and weight gain is the increased amount of adipose tissue due to a positive energy balance. Adipose tissue is considered to be the largest endocrine organ in the body, secreting cell signalling adipokines. They send out and respond to signals modulating appetite and energy expenditure, as well as contributing to the regulation of the inflammatory response (Fantuzzi, 2005; Fasshauer & Blüher, 2015; Mancuso, 2016). This increase of adipose tissue and adipokines can accelerate the aging process by affecting cell signalling processes in the skeletal muscle, and thereby causing a decline in the contractile function of the skeletal muscle (Schiaffino & Reggiani, 2011). Previous research has shown that obesity impacts the contractile performance of the muscle the same way ageing does (David J Tomlinson, Robert M Erskine, Keith Winwood, Christopher Ian Morse, & Gladys L Onambélé, 2014), where they have both been associated with chronic inflammation, muscle atrophy, reduced protein synthesis in response to exercise, fiber type shifting and impaired excitation-contraction coupling (Erskine et al., 2017; Hill & Tallis, 2019; Koster et al., 2011; Messa et al., 2020; Tomlinson, Erskine, Morse, Winwood, & Onambélé-Pearson, 2016).

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Mammalian skeletal muscle is a heterogeneous organ composed of different muscle fiber phenotypes, differed by their MHC isoforms, where human skeletal muscle is composed by slow twitch (type I) and fast twitch (type IIa and IIx). Muscle fiber type I, containing the MHC isoform MHCI, is characterized as being a fatigue resistant, mitochondria-rich vascularized fiber with high oxidative capacity. Type IIx mainly depend on anaerobic glycolysis as they contain lower concentration of mitochondria. As type IIx muscle fibers depend on anaerobic glycolysis and thereby produce less ATP than the oxidative type I and type IIa fibers, they are more easily fatigued and can easily wear out within few minutes (Bourdeau-Julien et al., 2018).

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There's a general consensus that obesity has a negative effect on the muscle quality and function, where increased adipose tissue may contribute to accelerated loss of muscle mass and strength with aging (Koster et al., 2011). Even though studies have examined the impact BMI has on intramuscular alterations, the literature on the correlation between the effects of RT on the muscle tissue in people with varied levels of obesity is limited. Therefore, the aim of the present thesis was to investigate how the degree of obesity impacted the muscle fiber type CSA and composition in an overweight sedentary population after a 10-week resistance training intervention.

Method

This thesis is a part of a bigger project led by postdoc Håvard Hamarsland, called "Alpha and Omega of lifestyle therapy". The main intervention of the project is to examine the effect 13 weeks of contralateral RT combined with ingestion of n-3 polyunsaturated fatty acids, both before and during the intervention, has on the hypertrophy of the skeletal muscle in obese and lean individuals. This thesis will focus on the effects a RT protocol of 3x10 repetition maximum (10RM) has on the muscle fiber area and fiber type composition in obese and lean individuals, and relate these findings to changes in muscle mass and strength.

Study design

The main intervention was designed as an experimental study where the supplementation intervention was conducted as a double blinded randomized and placebo-controlled trial. The study was originally planned that the study was to be conducted in two complimentary rounds over two years, from June 2019 until March 2020 and June 2020 until April 2021, with 50 participants in each round. As a consequence of the coronavirus (COVID-19) becoming a global pandemic and shutting down the world for a couple of months, the intervention was put to a halt the spring of 2020. This further led to that approximately half of the individuals that participated in the first round did not have the opportunity to finish their post-testing and therefor had no results as to how their body had reacted to the intervention. To reach the golden mark of 100 participants completing the study, the intervention is planned to be prolonged for another year with a new group of participants from June 2021 until March 2022. In this master thesis I will implement the data from the first round of the main intervention as we do not have the results from the biopsies from the second round yet, as well as the third and final round of the project is not conducted.

Participants and recruitment

Untrained individuals (age 30-60) in Lillehammer and the surrounding areas were recruited through ads in the local newspaper, on Facebook or Inn.no (Figure 3). Inclusion and exclusion criteria for participation is listed in table 1. To enrol in the study, the participants had to sign an information letter of consent stating that they approved of the content of the study (Attachment 1).

Table 1 Inclusion and exclusion criteria for participation in the project

INCLUSION CRITERIA	EXCLUSION CRITERIA
Age 30-60	Challenges understanding Norwegian
Resistance training < once every other week, last six months	Instable cardiovascular disease
Endurance training < three hours a week	Injury/sickness preventing execution of heavy resistance training
	Muscle and skeletal disease preventing execution of heavy resistance training
	Oral use of steroids last two months prior to intervention
	Severe mental illness
	Allergies to local anaesthesia
	Smoking

Throughout the first round of the study there were in total 29 individuals who participated and completed the study, 11 overweight (BMI 30-43) and 18 normal weight (BMI <30). Bearing in mind that the individuals are aware of what their BMI, the two different groups are not blinded in this master thesis in contrast to the main intervention.

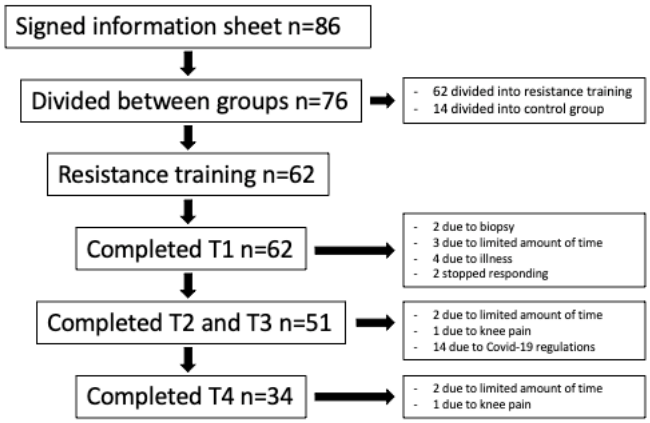

Figure 3 Flow chart of the flow of participants through each phase of the first round of the Alpha and Omega trial.

Training intervention

The intervention consisted of 3 weeks of testing and familiarization to RT followed by 10 weeks of two weekly, supervised sessions of RT (figure 4). By adding the familiarization period, the participants would get to know and learn the exercises in advance of the intervention. The main reason was to avoid injuries and secure that the increased strength to a greater extent was due to muscular adaptations and less due to neural adaptations, often observed at the beginning of a training period in untrained individuals (Sale, 1988). The training intervention consisted of a progressive contralateral training protocol for the legs, where the participants trained the two legs with different loads and repetitions. One leg would perform a higher resistance, but fewer repetitions (3x10RM), while the other leg would conduct a protocol consisting of lower resistance, but more repetitions (3x30RM). Which training protocol they were to train on which leg was randomized prior to the intervention. As the participants increased their strength, they would also increase their workload so that they always hit their achieved goal of either 10 or 30 repetitions for each set. The training protocol for the legs consisted of leg press, knee extensions and knee flexions. The participants also trained their upper body where they performed one bilateral and one unilateral exercise, but with the same load on both arms. The upper body exercises were bench press and dumbbell row (3x10RM). The training sessions were monitored by either the principal investigator, BSc- or MSc-students.

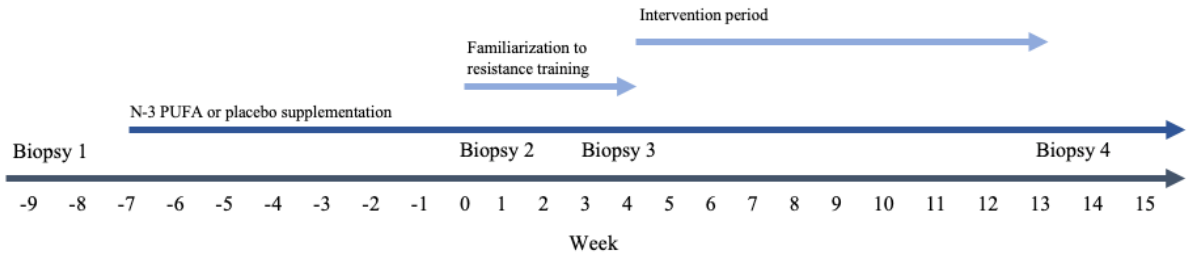

Figure 4 Timeline of the experimental design for the main intervention, with an overview of the relevant tests for this master thesis.

Measurements of outcome

The measurement of outcome in this master thesis includes using data from biopsies and immunohistochemistry staining slides to detect the muscle fiber area quantity of the different fiber types. The data was sampled at four different time points: i) before supplementation (T1), ii) before familiarization to RT (T2), iii) during the training intervention (T3) and iv)

after the training intervention (T4) (figure 4). The results in this thesis are only based on T1, T2 and T4, *renamed* "presup", "pre" and "post", respectively.

Obtaining of the muscle biopsy and processing

Percutaneous needle biopsies were obtained from m. vastus lateralis (VL) in both legs, in a fasted condition. Biopsy sampling was performed under antiseptic conditions, using local anaesthesia (Lidokain 10mg ml-1, Mylan Hospital A/S, Oslo, Norway) and a 12-gauge needle (Universal Plus®, Mermaid medical A/S, Stenløse, Denmark), operated with a spring-loaded biopsy gun (Bard Magnum®, Bard Norway A/S, Oslo, Norway). To obtain the adequate amount of muscle tissue required for all of the different analyses, there were sampled approximately three repeated clips (10-30 mg per clip) from each leg dependent on the amount of muscle tissue obtained from each clip. Each muscle sample was immediately dissected for visible connective tissue and blood in a saline-solution on ice (4°C; NaCl 0,9%, B. Braun). After the sampling, the muscle tissue was divided into aliquots for determination of different variables, where immunohistochemical determination of muscle fiber-type specific cross-sectional area (CSA), myonuclei and satellite cells where one of them. The participants were told to let the compression bandage stay on for at least one hour subsequent to the biopsy, and the band-aid for approximately 3 days. They were informed that the wound closure strips should stay on until it fell off by itself. To prevent infections, they were told to shower with something covering the wound, like a watertight band-aid or plastic wrap.

The muscle tissue required from the biopsy was fixed in a plastic screw-cap container with 4% Neutral Buffered Formalin (NBF) to prevent decay of the tissue. Using the formaldehyde fixation to preserve the tissue leads to a demineralization that aids the further sectioning of the tissue (Roelofs & De Bari, 2019). The tissue was stored in the container for a minimum of two hours before further processing. After the two hours the sample was analysed under a microscope where the muscle fibers were stripped from excess fat tissue, blood and connective tissue. Tissue processing cassettes were prepared with biopsy foam pads submerged in formalin to further secure the tissue. The muscle tissue was then placed in the longitudinal direction in the cassette and placed in a plastic container filled with formaldehyde for transportation to Lillehammer Hospital, department of pathology, for further processing.

Immunohistochemistry

The immunohistochemical methods were conducted at Innlandet Hospital Trust HF Lillehammer. The muscle tissue cassettes were fixated in formalin and placed in paraffin. The muscle samples were cut in cross section (4 µm) and incubated at 97 °C for 20 minutes prior to being put in distilled water for cooling. Visualization of the antibodies was conducted by incubation of suitable secondary antibodies: goat-anti-mouse Alexa Fluor (Thermo Fisher Scientific, Waltham, MA, USA). The fiber types and their cross-sectional area were determined by application of antibodies to the samples for determination of antigen expression in the fiber membrane (dystrophin; at.no. PA121011, Sigma-Aldrich, Saint-Louis, MO, USA), with subsequent secondary antibodies like Alexa Fluor 594 and 488 (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the muscle tissue samples were covered with a coverslip and glued together with EverBriteTM Hardset Mounting Medium and added DAPI (Biotium Inc., Fremont, CA, USA). The antibodies were diluted in 5% BSA.
Due to an error involving the staining of the muscle biopsies, we were unable to differentiate muscle fiber type IIa and IIx. The muscle fiber types are therefore grouped by muscle fiber type I and II in this master thesis.

Microscope/Morphometry

Once obtained from the hospital the coverslips containing the muscle tissue samples were stored in a dark refrigerator that maintained a temperature of 4 °C. The images were obtained by using a camera (Carl Zeiss AxioCam, München, Germany) mounted on a Axioscope-2 Mot Plus microscope (Carl Zeiss Light Microscopy, Göttingen, Germany) with the software Axio Vision 4.3 (Carl Zeiss Vision, München, Germany). The microscope was placed in a dark room to avoid disturbances from any light sources. Fluorescence antibodies are light sensitive and will fade when exposed to ambient light and should therefore be subjected to as little lighting as possible. Depending on the size of the muscle tissue sample on the coverslide, anywhere between 5 to 20 x10 Z-stack photos per sample to quantify the amount of muscle fiber type I and II as well as the mean of the fiber type CSA (figure 5). To ensure that one did not include the same muscle fibers for the images when the coverslip was moved under the microscope during the analysis, there was for every new image singled out one partial muscle fiber at the peripheral of the previous image that was used as a template for how the next photo should be aligned. To ensure the best possible quality the focus bar was reset between every image. The three different light settings for the FITC, Alexa and DAPI-filters were used for the different antibodies, and they all had their own channel by utilizing

the multidimensional auxiliary tool. Exposure, brightness and contrast were adjusted automatically, while the overexposure indicator was always activated. Prior to taking the images, all three filters were adjusted through the multidimensional tool using the "measure" option. In some cases, the automatic measurement option gave poor image quality due to highly concentrated spots with immunofluorescent light caused by irregularities and damage to the muscle tissue sections. To prevent this overexposure, the exposure was manually adjusted to achieve the best possible image quality. The camera setting was set to "fast" for all three images, as well merging them together as one at the end of the analysis.

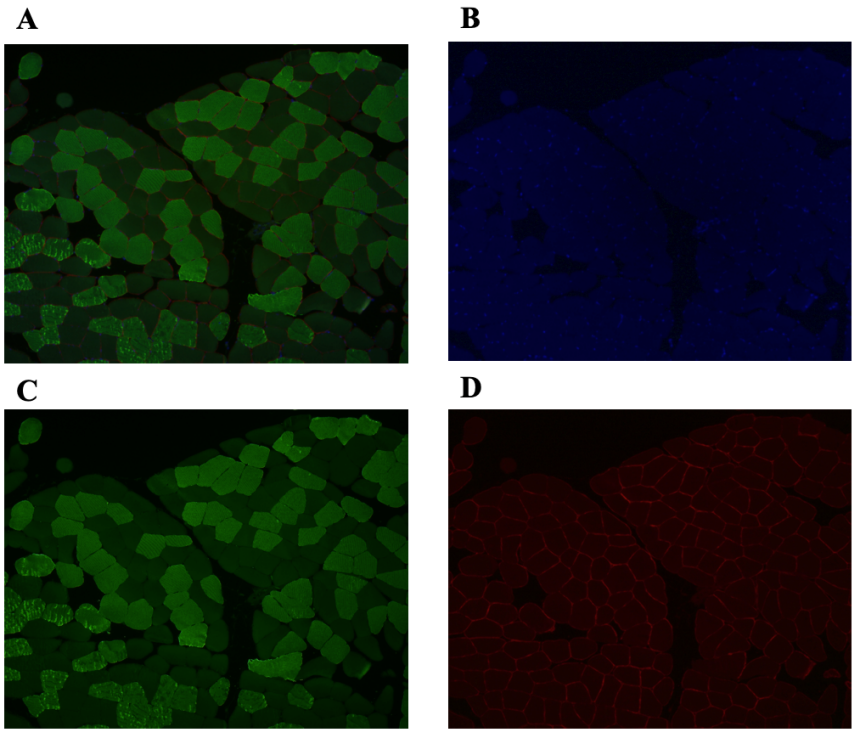

Figure 5 Representative cross section of a muscle biopsy of musculus vastus lateralis histochemically assayed for myofibrillar actomyosin adenosinetriphosphatase activity. A: Dystrophin/fiber type I/DAPI (collectively); B: Dystrophin; C: Fiber type I; D: DAPI.

The immunohistochemical analysis were further processed and completed for the muscle fiber type distribution and cross-sectional area of each fiber type with CellProfiler™ (McQuin et al., 2018), with modified methods (Attachment 2).

Muscle thickness

The muscle thickness of the VL was measured bilaterally while the participants were lying down using a 2-D B-mode ultrasound with a 50 mm linear array transducer (HD11XE, Philips, Bothell, WA, USA) right after the Dual-energy X-ray absorptiometry (DXA) scan at all timepoints (presup, pre and post). To secure an accurate placement of the probe for each sampling, a transparent sheet was placed on the thigh to highlight the measuring point of the ultrasound as well as surrounding skin characteristics like freckles and skin scarring. The probe was placed aligned with the muscle's fascicles without putting any external compression on the ultrasound probe. To lower the risk of erroneous measurement, the same person conducted the ultrasound imaging for every timepoint. There were obtained 3 images from both legs every time. To determine the muscle thickness, the distance from the muscle fascia to the VL was measured. The images were analysed using an imageJ plug-in described in a previous publication (Seynnes & Cronin, 2020).

Performance assessment

The Humac NORM isokinetic dynamometer (CSMi Medical Solutions, Stoughton, MA) was used to assess the maximal muscle strength of the knee extensor. Prior to data collection the dynamometer was calibrated according to the manufacturer's instructions. The participants were seated in an upright position, with the backrest set at 85°. The knee was aligned with the rotational axis of the dynamometer and the lever arm was tightly fastened approximately 5 cm above the lateral malleolus with an inelastic velcro band. The thigh of the tested leg was fastened with an inelastic band to the chair as well. The movement of the leg was restricted to a predetermined range of motion of 90°-0°. To minimize compensatory upper body movement, the participants were fastened with inelastic harnesses that kept their hips and trunk still during the testing. For every test, the participants started with testing their right leg before their left leg was tested straight after. The ankle of the opposite leg was confined behind a stationary ankle support to prevent having any impact on the results. They performed the test at three different velocities: 3 repetition at 60° per second, 3 repetition 240° per second and 2 isometric repetitions at a 60° angle in the knee joint. Before each test the participants performed a familiarization protocol that consisted of them performing submaximal repetitions at approximately 60% of maximum effort of the movement at the given velocity. Between each of the repetitions the participants were given a 1-minute rest period before they manually started the next repetition. The repetitions for the two isokinetic

movements were activated by bringing the lever arm down to a 90°-degree angle, while the trained personnel counted down from 3 to start the isometric testing protocol.

Ethical considerations

The main intervention is approved by the Regional Committees for Medical and Health Research Ethics, region south-east (REK), and is carried out in accordance with the Declaration of Helsinki (World Medical Association, 2013). All participants signed a written informed consent form prior to participating in the project (Attachment 1), as well as being informed of their right to withdraw from the project at any given time without being obligated to state a reason.

Muscle biopsy sampling was performed under well-established procedures by experienced personnel under medical supervision. Prior to the muscle biopsy sampling the participants were given both written and oral information about the post-procedure care of the incision site to minimize the risk of infection. If the participants reported or experienced any adverse effects to the local anaesthetics, they would be excluded from the study. 1-2 days following the sampling of muscle tissue, one may experience mild soreness surrounding the incision site that normalizes over time. *Training* is not associated with any high risk of participation, but to even lower the risk of injuries or other undesirable outcomes there was trained personnel present at every training session. Participants were given the opportunity to have access to their own test data upon request.

Statistics

Due to the large muscular and neural changes one experiences during the familiarization period, the pre values for the comparisons of training adaptations were set to after the familiarization period. All descriptive data is presented as means ± standard deviation (Williams, Bailey, & Mauger), unless otherwise stated. The data was analyzed in R, for full script of analysis follow this link: https://github.com/MarteJohannson/MasterThesis.git. To measure the effect of the intervention, a one-way analysis of variance (ANOVA) was utilized. A paired-samples t-test was conducted to compare the percentage change in muscle fiber type CSA, muscle thickness and strength between control and intervention period. Regression analysis were used to determine associations between BMI and continuous variables including muscle fiber type CSA, muscle thickness and muscle strength. For all analysis, differences were considered significant at $p<0,05$.

Results

Participant characteristics are provided in Table 2. 29 participants completed all biopsies in the first round of the study and were included in this analysis.

Table 2 Participant characteristics at pre

Variable	***Data***
n (♂/♀)	29 (15/14)
Age	42,2 ± 5,8
Weight	82,4 ± 96,5
BMI	30,0 ± 6,0

Effect of the intervention

Muscle fiber CSA and composition

There were no significant differences in type I ($F(2, 84) = 0{,}22$, $p = 0{,}81$) or type II ($F(2, 84) = 2{,}35$, $p = 0{,}1$) fiber CSA for absolute values between timepoints (figure 6). Comparing percent change in CSA between the control and intervention periods revealed a significantly greater increase in type II fibers (0,84 % decrease in control and 5,74 % increase in intervention period, $t(28) = 0{,}013$, $p < 0{,}001$), but not type I fibers (1,95 % increase in control and 13,15 % increase in intervention period, $t(28) = 0{,}144$, $p = 0{,}18$). We did not observe any alterations in fiber type composition from pre to post testing for fiber type I (44,5 % to 45,2 %, respectively) or type II (55,46 % to 54,81 %, respectively).

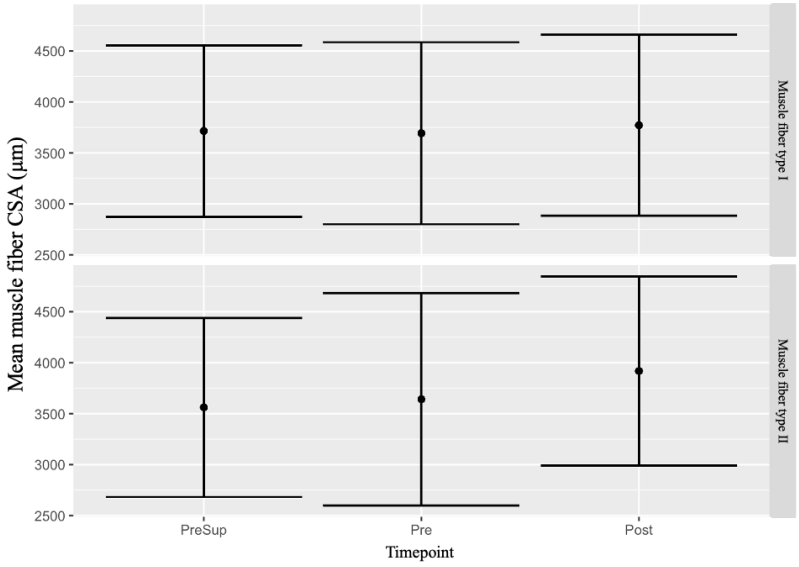

Figure 6 Displays the group mean values for adaptations of m. vastus lateralis muscle fiber type cross-sectional area (CSA) to 10 weeks resistance training in muscle fiber type I and II.

Muscle thickness

There was a significant difference in VL muscle thickness (F(2,84) = 4,34, *p*=0,016) for absolute values between timepoints (Figure 7). Comparing percent change in VL muscle thickness between the control and intervention periods revealed a significantly greater increase (increase of 1,85 % in control period and 12,32 % in intervention period, *t*(28) = 6,3, *p*<0,05).

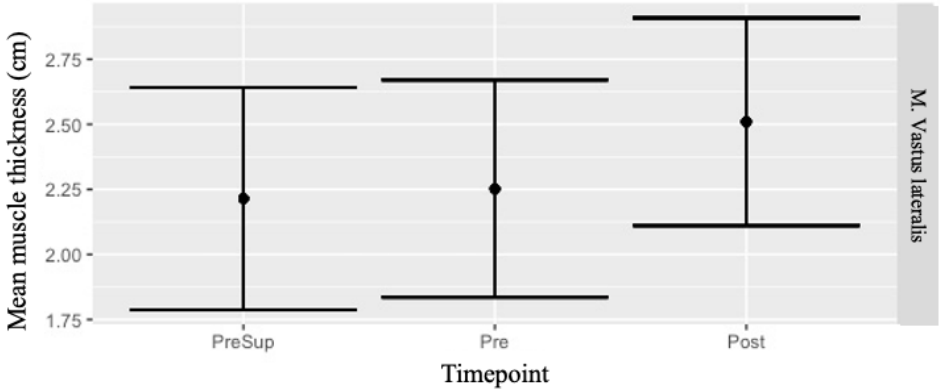

Figure 7 Displays the group mean muscle thickness values by each timepoint through the intervention.

Muscle strength

Isokinetic muscle strength for 0° per second had significant differences (F(2, 84) = 3,46, *p*=0,04), but 240° per second were not significantly different (F(2,84) = 1,21, *p*=0,30) for absolute values between timepoints (figure 8). Comparing percent change in peak torque between control and intervention periods revealed a significantly greater increase in the test of 0° per second (increase of 1,44 % in control period and 20,75 % in intervention period, *t*(28) = 5,7, *p*<0,05), and 240° per second (2,8 % decrease in control period and 14,72 % increase in intervention period, *t*(28) = 4,8, *p*<0,05).

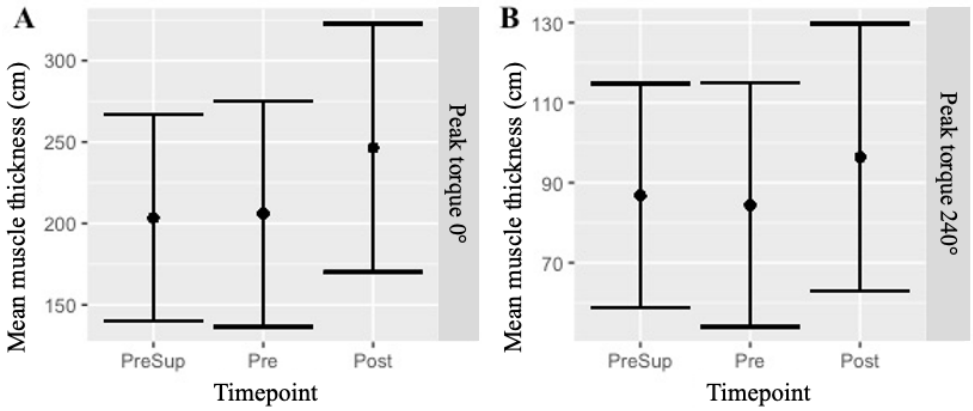

Figure 8 Displays the group mean isokinetic strength values by each timepoint through the intervention. Shows group mean values for peak torque (Nm) during isokinetic knee extension at 0° (A) and 240° (B) per second.

Effect of BMI

Muscle fiber cross-sectional area

Based on the present population, the percentage change of muscle fiber type CSA was significantly predicted by the individuals' BMI (figure 9). The results of the regression indicated that the BMI significantly predicted the muscle fiber type CSA for both fiber type I (figure 9A, R^2=0,23, F (2,26) =3,84, p=0,035) and fiber type II (figure 9C, R^2=0,43, F (2,26) =9,63, p=0,01). When filtering the CSA of the muscle fiber type by four different BMI categories, we observed a pattern with BMI for muscle fiber type I (figure 9B) and type II (figure 9D). The leaner BMI categories had a lower baseline level as well as an increased predicted change of muscle fiber type CSA than the higher BMI calculations.

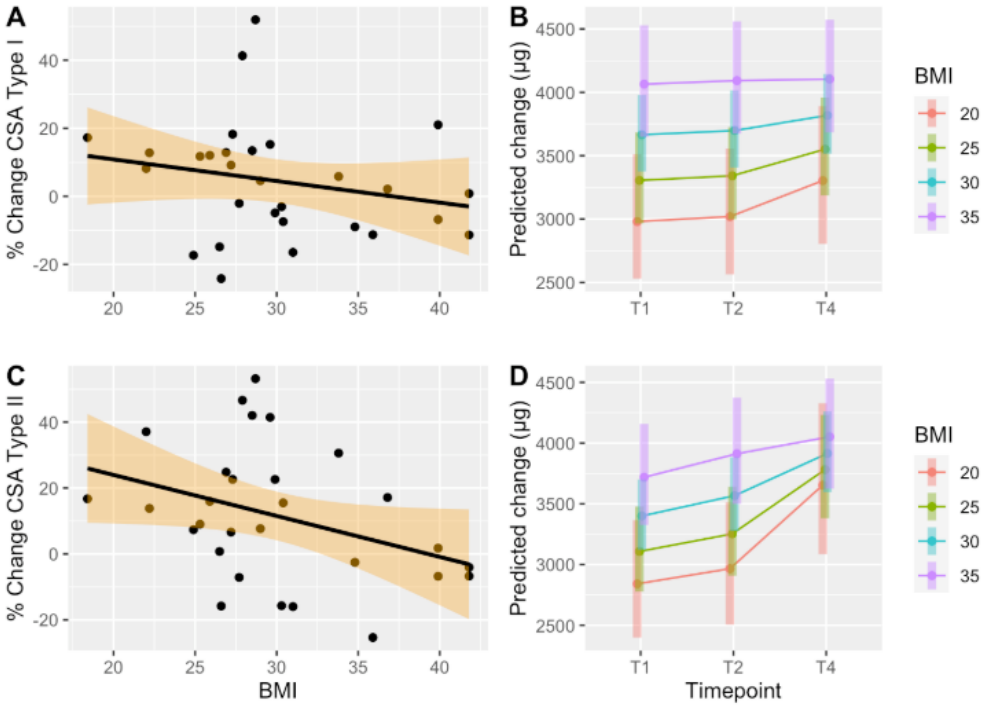

Figure 9 Displays effects of muscle fiber cross-sectional area (CSA) based on BMI as measured at timepoint presup, pre and post. Figure A and C shows the correlation between percentage change in CSA of muscle fiber type 1 and type II and increased BMI, respectively. Data are presented as individual respondent values, bar graph value indicate group mean value, and standard deviation values are presented by orange area. Figure B and D shows the predicted

Muscle thickness

A regression analysis was used to test if the muscle thickness of VL was significantly predicted by the participants' BMI. The results of the linear regression model indicated that the BMI significantly predicted the VL muscle thickness (figure 10) (R^2= 0,45, F (1,27) = 22,19, p=0,007). For every one-point increase in BMI, the increase in muscle thickness reduced with 0,9 %.

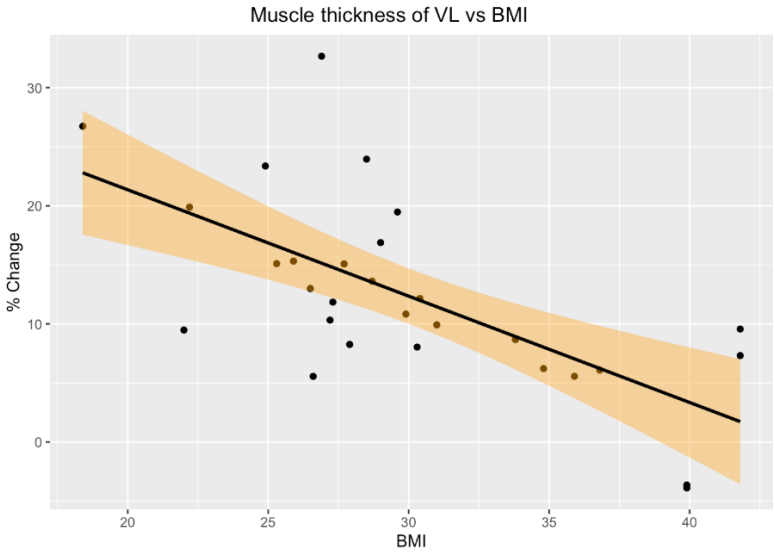

Figure 10 Displays the observed correlation of percentage increase in VL muscle thickness after the intervention period and increased BMI. Data are presented as individual respondent values, bar graph value indicate group mean value, and standard deviation values are presented by orange area.

Muscle strength

A linear regression model showed that an increased BMI value correlated with a lower percentage change of muscle strength after the RT intervention (figure 11). There was no significant correlation between change in isometric peak torque and BMI (R^2=0,06, F (1,27) = 1,60, *p*=0,217).

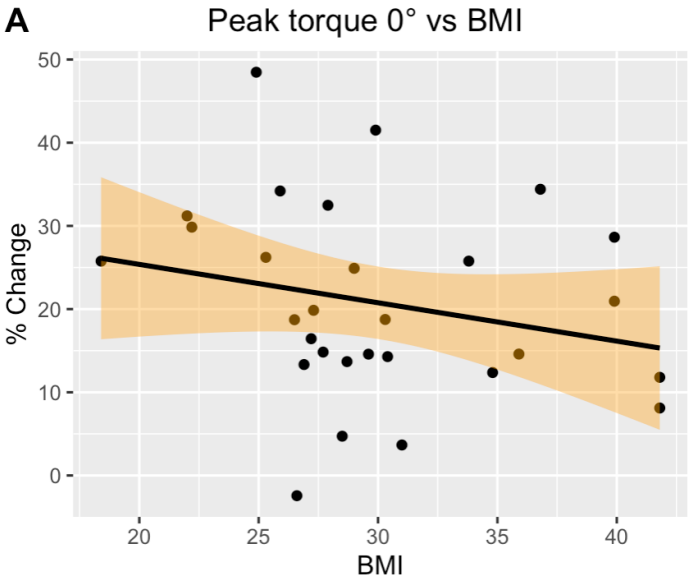

Figure 11 Displays relationship between percentage change of peak torque and BMI from pre to post testing. Data are presented as individual respondent values, bar graph value indicate group mean value, and standard deviation values are presented by orange area.

Discussion

The aim of the present study was to compare the effects of 10 weeks of RT on muscular growth and morphological alterations in VL in people with obesity. The results indicated no significant increase in muscle fiber type CSA for type I nor II between the timepoints, but there was a significant increase in the intervention period in comparison with the control period of CSA for both fiber types. This correlated with observations of increased isokinetic strength and muscular growth measured as muscle thickness.

Muscle fiber type cross-sectional area, thickness and strength

The increase in muscle thickness as a result of RT correspond with previous studies indicating that RT resulted in muscle hypertrophy (Chin et al., 2016; Stuart, Lee, South, Howell, & Stone, 2017). The present results indicated that the muscle fiber CSA values (figure 6) correlated with muscle thickness and strength values (figure 7 and 8A, respectively), where an increase in CSA of both muscle fiber type I and II correlated with higher VL muscle thickness and isometric strength of the knee extensor muscles post intervention. Franchi et al. (2018) assessed that the vastus lateralis muscle thickness was a reliable index of muscle CSA at a single timepoint, as well as concluding that increases in muscle thickness after a RT intervention predicted increased muscle CSA. Previous studies have also reported an increase in knee extensor muscle strength of sedentary adults of ~ 5-7 % after progressive RT interventions (Ahtiainen et al., 2016; Stuart, Lee, South, Howell, & Stone, 2017), corresponding with other studies reporting that RT positively influences the muscle strength, quality and mass in normal weight and obese individuals (Choi et al., 2015; Garcia-Vicencio et al., 2016; Verdijk et al., 2009). Ahtiainen et al. (2016) also found a significant correlation between relative RT-induced changes in muscle size and strength (p<0,01), where 287 untrained younger and older individuals obtained increased 1RM leg press and muscle thickness in response to 20-24 weeks of RT (~19-24 %, ~2-30 %, respectively), which conform with the results obtained in this thesis.

Effect of BMI

When body composition status was classified by BMI, it was predicted that the obese participants exhibited a greater muscle fiber CSA prior to the intervention than participants with normal BMI in type I (figure 9B) and type II (figure 9D). These findings are similar with results from a previous study that observed significant interactions between the BMI value

and muscle fiber CSA size in gastrocnemius medialis in a population of 100 trained and untrained women ranging from 18-80 years old (D. J. Tomlinson, R. M. Erskine, K. Winwood, C. I. Morse, & G. L. Onambélé, 2014). An increased BMI led to a lower percentage change in muscle fiber CSA for both type I and II (figure 9A and 9C, respectively) from pre to post testing, in comparison with the individuals with lower BMI. It was predicted based on the data from the present thesis that the participants with a BMI of 35 would reach a plateau at the pre-testing of the muscle fiber CSA for type I and II, whereas there was no increase in CSA of the muscle fiber post intervention in contrary to the lower BMI classifications (figure 9B and 9D, respectively). It appeared as if the lower the BMI classifications is, the smaller is the fiber type CSA prior to the intervention. The participants with the lower BMI classifications had the greatest predicted increases in fiber type CSA for both fiber types after the intervention period. In the type II muscle fibers, there was an enhanced predicted change of muscle fiber type CSA for the lower BMI classes in relation to type I.

The lowered predicted increase in muscle fiber CSA for obese individuals from pre to post might be due to the CSA being within close range to the maximal potential of the CSA prior to the RT intervention. A previous study observed a greater absolute muscle mass in obese individuals in relation to normal weight individuals prior to the RT intervention, but with the lowest increase after the intervention (de Oliveira Silva et al., 2018). A possible explanation as to why obese individuals have an altered muscle fiber type CSA size is a reduced muscle quality, where increased fat infiltration might alter the contractile function the muscle tissue, leading to a reduced intrinsic muscle strength (Erskine et al., 2017; Jason Tallis, Hill, James, Cox, & Seebacher, 2017). For untrained individuals with a high BMI-value it might be a good idea to normalize the quality of the muscle tissue they possess prior to focusing on increasing the muscle fiber type CSA. The results from the VL ultrasound displayed a significant correlation between the muscle thickness and BMI.

Results from the isometric strength test indicated that obesity led to increased absolute force production of the knee extensor muscles compared to normal weight participants. However, when looking at the relative strength values based on the total body mass of the individual, the increase of muscle strength was in favor of the participants with lower BMI. This correlates with previous studies that observed that obese individuals had a significantly lower maximum muscle strength than non-obese participants when normalized to CSA (D. J. Tomlinson et al., 2014). The weight bearing skeletal muscle of the lower extremity are subjected to a chronic training stimulus due to the excess body mass, which might promote increased contractile work during activities of normal daily living and elicit a physical training effect (Garcia-Vicencio et al., 2016; Hulston et al., 2018). This might also explain the reason as to why the muscle fiber CSA of type I and II are enlarged pre intervention in the participants with higher BMI values. The higher values at pre-testing might lead to a decreased training effect,

Contrary to the expected values one sees in the muscle fiber type CSA where the type II fibers are expected to be bigger than type I, the two different fiber types were approximately the same size (Burkholder et al., 1994). This similarity in sizing is usually expected in ageing muscle tissue, associated with muscle adaptations such as a decrease in total muscle mass and muscle fiber CSA (Hill & Tallis, 2019). Intramuscular fat can accumulate within the muscle fiber, and thereby reducing the contractile properties and enlarge the fiber (Rahemi, Nigam, & Wakeling, 2015). Data from this master thesis have similar results as a previous study researching age-related decline in muscle fiber type II CSA (Messa et al., 2020). Based on these findings, there is reason to predict there being a correlation between the observations of degenerative muscle quality between individuals with increased BMI and ageing individuals.

A previous study has stated that as a result of obesity, the most important mechanism that alters contractile function is the changes in muscle fiber type (J. Tallis et al., 2018). Since obesity affects the signaling pathways that regulates muscle fiber type transitions (figure 1), there is often an increased ratio of the type II fibers in contrast of type I. This alteration in muscle fiber composition may result in a lowered muscle quality and performance, which can be a substantial contributor to the negative cycle of obesity. Obese individuals respond worse to RT, which in addition to lowered self-esteem can lead to reduced participation in physical activity and in time a further weight increase. Jason Tallis et al. (2017) reported that obesity caused a significant reduction in fatigue resistance in mice, and a muscle-specific reduction in

contractile performance and muscle quality. They stated it was likely due to fiber type and metabolic profile, which might be related to changes in MHC expression and AMPK activity.

Strength training in treatment of obesity

Based on the results conducted in this thesis I would recommend obese individuals to implement progressive resistance training to reduce the risk of non-communicable diseases and increase life quality. I would also recommend that individuals who wish to be stronger and get a better muscle quality to lose som weight, due to the adipose tissue promoting an inflammatory response and lowering the RT adaptations one usually witnesses after a period of consistent RT. By reducing the BMI value, one may experience an increased relative force in the muscle and increased muscle quality, leading to an enhanced mobility and thereby bettering the overall life quality of the individual. It is possible to have an increase in muscle strength and other factors while maintaining a high BMI value, but due to the many health risks that accompany the increased values I would recommend a program consisting of both RT and a lowered caloric intake until one has reached an ideal BMI value within healthy limits.

Conclusion

Analysis of muscle size, quality and strength changes after 10 weeks of progressive resistance training in the knee extensors in 29 untrained, sedentary men and women demonstrated the following: 1) Increased BMI predicts increased muscle fiber type CSA in type I and II, muscle thickness and strength; 2) Obese individuals have a higher absolute muscle thickness and strength, but lower relative strength than individuals with normal weight; 3) Obesity leads to increased muscle fiber type CSA, where elevated BMI values predicts high muscle fiber type CSA as well as lowered progress in fiber type CSA in relation to consistent RT.

Based on these findings there's reason to believe that in relation to individuals with a BMI within recommended values, the increased chronic inflammation due to obesity, in correlation with inactivity, leads to an increased muscle fiber type CSA, shift in fiber type composition with increased ratio of fiber type II as well as a reduction in relative muscle strength.

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Attachments

Attachment 1 – Information letter to participants

Alfa og Omega i livsstilsterapi

- Styrketrening og omega-3 supplementering for forbedret helse og muskelfunksjon med i individer med overvekt og friske kontroller

Dette er et spørsmål til deg om å delta i et forskningsprosjekt for å undersøke hvordan økt fettmasse og omega-3 supplementering påvirker muskelmassen ved styrketrening. Du får dette informasjonsskrivet fordi du har vist interesse for studien.
For å delta i studien må du være mellom 30 og 60 år og være utrent (trene styrke mindre enn en gang i uken og utholdenhet mindre enn 3 timer i uken). Personer med ustabil kardiovaskulær sykdom, sykdom eller skade som hindrer tung styrketrening, sykdom i muskel- skjelettsystemet, alvorlige mentale lidelser, allergi mot lokalbedøvelse, røykere eller personer som har brukt medisiner eller preparater med steroider de siste to månedene vil bli ekskludert fra studien.
Fedme rammer hver femte voksne person i Norge og er forbundet med en rekke helseutfordringer. Blant disse utfordringene er tap av muskelmasse, -kvalitet og funksjon, noe som bidrar til å redusere livskvaliteten. Fedme gir også en rekke andre fysiologiske endringer som kan bidra til å redusere responser på livsstilterapi med trening. Personer med fedme oppnår ikke de ønskede forbedringene i muskelfunksjoner og helsetilstand som typisk medfølger slik terapi. Vi vet lite om hvorfor, men det er trolig flere grep som kan tas for å øke effekten av treningen. Vi kan endre kroppens indre miljø, slik at den blir mottakelig for trening. Dette kan for eksempel gjøres gjennom endringer i kosthold. Vi kan også ta i bruk alternative treningsmetoder som omgår den iboende motstanden mot vekst i muskulaturen. Sannsynligvis vil en kombinasjon av slike terapier (kombinasjonsterapi) føre til bedret trenbarhet. Hovedmålet med denne studien er å skaffe kunnskap om hvordan livsstilterapi kan optimaliseres for å omgå de fysiologiske utfordringene knyttet til fedme. Dette skal vi gjøre gjennom å kombinere inntak av et omega-3 supplement med to ulike styrketreningsprotokoller. De to protokollene gjennomføres på hvert sitt bein innad i deltakerne. Det ene beinet vil da trene 3 sett med 10 repetisjoner og det andre vil trene 3 sett med 30 repetisjoner. Sammenligningen innad i en deltaker fjerner forskjeller i genetikk,

kosthold og livsførsel mellom treningsprotokollene og gjør det lettere å finne eventuelle forskjeller.

Hva innebærer PROSJEKTET?

Deltakere i prosjektet skal deles i to grupper: en intervensjonsgruppe og en referansegruppe. Intervensjonsgruppen skal innta enten omega-3 eller placebo, gjennomfører alle tester og gjennomføre 13 uker med styrketrening. Referansegruppen skal gjennomføre noen av testene og skal ellers fortsette å leve sitt vanlige liv. For intervensjonsgruppen består prosjektet av tre perioder (se figur 1). Periode 1 går over 7 uker hvor du inntar omega-3 tilskudd eller placebo uten å gjøre andre endringer i livsførselen din. Supplementeringen med omega-3 eller placebo fortsetter også gjennom de to neste periodene. Periode 2 er tilvenning til styrketrening og varer i 3 uker. Periode 3 er et styrketreningsprogram på 10 uker hvor hele kroppen trenes to ganger per uke. I periode 2 og 3 får du personlig oppfølging av en av våre bachelor- eller masterstudenter på alle økter. Før og etter hver av periodene gjennomføres en rekke tester for å måle effekten av omega-3 supplementeringen og styrketreningen (se tabell 1). I periodene med trening vil det være to oppmøter i uken og øktene vil vare ca 1 time. I ukene med testing vil det være 2-3 oppmøter i uken. Det vil være mulig å trene både på dagtid og ettermiddag. Deltakere i referansegruppen vil få tilbud om å livsstilveiledning etter endt prosjektdeltakelse og vil få tilbud om en periode med veiledet styrketrening.

Gjennom prosjektperioden kan du ikke bruke kosttilskudd som inneholder omega-3. Antall fiskemiddager skal begrenses til en middag med hvit fisk per uke.

I prosjektet skal vi innhente og registrere opplysninger om deg gjennom følgende tester (se figur 1 for tidspunkter)

Tabell 1: Oversikt over tester og tidspunkt for intervensjonsgruppene og referansegruppen

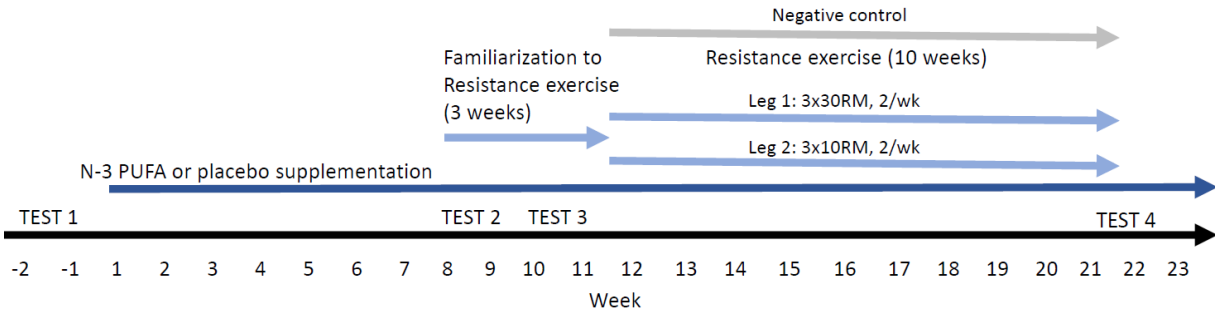
Intervensjonsgruppe	**Referansegruppe**

• Styrketester i beinpress og kneekstensjon (2xT1, 2xT2, T3, T4)
• Utholdenhetstester
  ○ 6 minutters step test (TEST 1, 2, 4)
  ○ Sykkeltest på ett bein (TEST 2, 4)
• Måling av kroppssammensetning med DXA-scan (TEST 1, 2, 3, 4)
• Måling av muskeltverrsnittsareal og fettinfiltrasjon med MR (TEST 2, 4)
• Måling av midjeomkrets (TEST1, 2, 4)
• Måling av muskeltykkelse i låret med Ultralyd (TEST 1, 2, 3, 4)
• Oral glukosetoleransetest (TEST 1, 2, 4)
• Blodprøver (TEST 1, 2, 3, 4)
• Biopsier (TEST 1, 2, 3, 4)
• Inntak av deuterium for måling av muskelproteinsyntese i lårmuskulaturen (tre siste ukene av treningsperioden)
• Blodtrykk (TEST 1, 2, 4)
• Spørreskjema om helse, muskel- og skjellet-plager (TEST 1, 2, 4)
• Kostregistreingsskjema (TEST 1, 2, 4)
• Avføringsprøver (TEST 1, 2, 4)
	• Styrketester i beinpress og kneekstensjon (2xTEST2, TEST4)
	• Måling av kroppssammensetning med DXA-scan (TEST 2, 4)
	• Måling av midjeomkrets (TEST1, 2, 4)
	• Oral glukosetoleransetest (TEST 2, 4)
	• Blodprøver (TEST 2, 4)
	• Biopsier (TEST 2, 4)
	• Spørreskjema om helse, muskel- og skjellet-plager (TEST 2, 4)
	• Kostregistreingsskjema (TEST 2, 4)

Testene vil fordeles på to testdager som vil ta ca 2-3 timer hver. Testdag 1 må gjøres på dagtid da flere av testene (blodprøve, DXA og biopsi) denne dagen må gjøres fastende. Testdag 2 kan gjøres på dagtid og ettermiddag.

Hensikten med de ulike testene

Flere studier finner at personer med overvekt ser ut til å ha en redusert evne til å bygge muskler og bli sterkere ved styrketrening. Det er også mulig at de to ulike treningsprotokollene (3x10 og 3x30) vil gi ulik effekt. For å undersøke disse spørsmålene måler vi effekten av styrketrening og omega-3 på styrke (beinpress og to typer kneekstensjon), utholdenhet (6 minutters step-test og ettbeins sykling) og muskelmasse (DXA, ultralyd, MR og muskelvekst ved hjelp av deuterium og muskelvekst på cellenivå i biopsiene) med flere ulike tester. Videre ønsker vi å undersøke effektene av styrketreningen på flere helsevariabler knyttet til overvekt og risikofaktorer for diabetes og hjerte-karsykdom (oral glukosetoleransetest, blodprøver, blodtrykk midjemål og fettmasse). Biopsiene fra låret kan hjelpe oss å forklare mekanismene (for eksempel: hvilke gener som slås av og på og hvordan cellene virker) bak endringene og eventuelle forskjeller vi finner i styrke og muskelvekst. I tillegg til det som skjer inne i muskelfibrene vil muskelveksten være avhengig av det miljøet som er rundt muskelen. To viktige bidragsytere til dette miljøet er betennelse, som ofte er økt ved overvekt, og kommunikasjon fra andre vev via signaler som inngår i det vi kaller metabolomet. Betennelsesstatus og metabolomet blir målt i blodprøvene. To viktige bidragsytere til både betennelse og metabolomet er fettvev og bakteriene i tamen, som begge påvirkes negativt av overvekt. Tidligere studier viser at omega-3 kan ha en positiv effekt på tarmbakteriene og fettvevet og derigjennom bidra til bedre helse og bedre forhold for muskelvekst. For å forstå hvordan tarmbakteriene påvirkes av trening og omega-3 og igjen potensielt påvirker treningseffekt tar vi også avføringsprøver. Kosthold er en faktor som påvirker effekten av trening samt de fleste andre målene i denne studien. Vi gjør derfor 3 runder med kostregistrering gjennom studien. Overvekt fører ofte med seg plager blant annet i form av muskel- og skjelettplager, endret mage- tarmfunksjon og kan også påvirke livskvaliteten. Ved hjelp av flere spørreskjema ønsker vi å undersøke om styrketrening i kombinasjon med omega-3 kan redusere muskel- og skjelettplager, gastrointestinale plager og forbedre livskvaliteten.



Figur 1: Oversikt over studien

Mulige fordeler og ulemper

Totalt vil det tas 4 biopsier fra hvert bein i intervensjonsgruppen og 2 i hvert bein for referansegruppen. Noen vil synes denne typen vevsprøver er ubehagelig. Man blir typisk støl i muskulaturen i 1-2 dager etter biopsien. Inngrepet vil etterlate små arr, som hos de fleste forsvinner med tiden. I svært få tilfeller vil biopsitakning kunne føre til at følelsen i huden rundt biopsien forsvinner over en lengre periode. Biopsitaking er også forbundet med en viss infeksjonsfare. Risikoen for disse komplikasjonene er svært liten ved bruk av prosedyrene som benyttes i dette prosjektet. Biopsiene tas fra lårmuskelen på utsiden av låret ca midt mellom kneet og hoften. Vi setter først en dose lokalbedøvelse (samme type som hos tannlegen) før vi steriliserer området. Selve biopsien tas med en nål med en diameter på 2,1 millimeter som føres inn i lårmuskelen. For å få nok vev må vi inn 2-3 ganger i samme hull ved hvert testtidspunkt. Du vil få klare instrukser om hvordan du skal behandle såret i etterkant av prøvetagningen. Blodprøvene i studien anses ikke å ha noen risiko.

For å kunne måle hvor raskt nye proteiner bygges inn i muskulaturen må du i løpet av de tre siste ukene i prosjektet innta en større og to mindre doser med tungtvann. Det er ingen kjente helsekonsekvenser ved inntak av de dosene som anvendes i studien, men lett svimmelhet kan forkomme. For å unngå dette vil dosen fordeles over flere inntak og du vil følges opp av testpersonalet i perioden hvor svimmelhet kan inntreffe.

Styrketreningen vil mest sannsynlig føre med seg helsemessige forbedringer. I tillegg forventer vi en gjennomsnittlig økning i muskelmasse på ca. 2 kg for deltakerne i studien. Deltakelse i studien vil kunne gi mer kunnskap og erfaring med styrketrening og kan bidra til å etablere trening som en rutine i hverdagen. Deltagelse i studien vil gi mulighet til å gjennomføre en rekke tester du ellers ikke ville hatt tilgang til.
Skulle vi oppdage noe som avviker fra det vi forventer og/eller gir oss mistanke om helseproblemer vil det bli tatt initiativ til videre medisinsk oppfølging.

Frivillig deltakelse og mulighet for å trekke sitt samtykke

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte Håvard Hamarsland (tlf: 93445916, mail: havard.hamarsland@inn.no) eller Stian Ellefsen (tlf: 97666521, mail: stian.ellefsen@inn.no).

Hva skjer med OPPLYSNINGENE om deg?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet i hensikten med prosjektet. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert. Du har også rett til å få innsyn i sikkerhetstiltakene ved behandling av opplysningene.
Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger gjennom en navneliste. Det er kun prosjektmedarbeiderne i studien som har tilgang til denne listen.
Opplysningene om deg vil etter endt prosjekt flyttes over i en generell biobank (se senere) og anonymisert innen 31.12.2028.

Hva skjer med prøver som blir tatt av deg?

Alle blod- og vevsprøver, samt øvrig informasjon som innhentes i prosjektet, inklusiv informasjon som blir utledet fra det biologiske materialet, vil bli lagret i kodet tilstand i en forskningsbiobank tilknyttet prosjektet og vil ved prosjektslutt bli overført til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» (REK-id:

213483), situert ved Høgskolen i Innlandet/Sykehuset Innlandet. TrainOME-prosjektet er igangsatt for å avdekke sammenhenger mellom individers tilpasningsevne til trening, også kalt trenbarhet, og kroppslige/cellulære særtrekk. Gjennom den generelle biobanken skal prøvene analyseres sammen med prøver fra en rekke andre prosjekter, hvor den overordnete målsettingen er å studere faktorer som er bestemmende for generell trenbarhet. Dette innebærer generell analyse av cellebiologiske og genetiske trekk som for eksempel cellers form/utseende/evne til å dele seg og vokse, arvematerialets sammensetning (inklusiv DNA-sekvens og epigenetisk modifisering), proteinsyntese, proteinforekomst og -funksjon, RNA-uttrykk og -regulering, hormonforekomst, kroppens indre miljø (metabolomet), og mange flere mål. Det biologiske materialet vil bli anonymisert innen 31.12.2038, hvorpå det vil bli destruert innen fem år. Forskningsdata som har blitt utledet av materialet vil deretter bli oppbevart i anonymisert tilstand på sikker server på ubestemt tid, sammen med øvrige data innhentet i prosjektet. Professor Stian Ellefsen er hovedansvarshavende for forskningsbiobanken.

Noen analyser skal gjøres hos samarbeidspartnere ved andre institusjoner. Analyse av muskelproteinsyntese skal gjøres ved universitetet i Birmingham i England. Analyse av muskelcellenes evne til å vokse, spesialisere seg og dele seg skal gjøres ved Universitet i Oslo (cellene holdes i live etter biopsitaking og er gjenstand for eksperimenter på laboratoriet). Prøvene som blir sendt til våre samarbeidspartnerne vil være kodet. Det vil dermed ikke være mulig å finne tilbake til din identitet basert på prøvene alene. Eventuelle restmaterialer fra analysene vil enten bli destruert eller returnert til oss etter at analysene er gjennomført (senest innen 31.12.2026).

Genetiske undersøkelser

Det vil bli innhentet informasjon om din genetiske sammensetning. Denne informasjonen skal primært gi innsikt i sammenhengen mellom individuelle responser på styrketrening, målt som muskelvekst, og individuell genetisk variasjon. Altså å forstå hvorfor noen responderer bedre på styrketrening enn andre. Dette perspektivet er forankret i målsettingen med den generelle biobanken "Trainome - humane cellers tilpasning til trening og miljø" (REK-id: 2013/2041), hvortil prøvene skal overføres etter prosjektlutt. Forståelse for hvilken rolle ulike gener spiller for muskelvekst er på et tidlig stadium. Det er derfor ikke mulig å gi genetisk veiledning basert på analysene i studien. Det skal ikke gjøres analyser som kobler enkeltmutasjoner til bestemte helseutfordringer. Genetiske data er unike og er derfor i prinsippet ikke anonyme, selv om koblingsnøkkelen som kobler deg til dine data blir slettet. Alle genetiske data (inkludert transkriptomdata) skal oppbevares på sikker server hos Tjenester for sensitive data (TSD).

Forsikring

Som deltaker i studien er du forsikret gjennom Høgskolen Innlandets forsikring hos Gjensidige.

OppfølgingsPROSJEKT

Det kan bli aktuelt med et oppfølgingsprosjekt for å undersøke reproduserbarheten i treningsrespons. I den sammenheng vil deltakere kunne bli kontaktet igjen etter endt studie med informasjon om oppfølgingsstudien.

Økonomi

Studien og biobanken er finansiert gjennom forskningsmidler fra Høgskolen i Innlandet og Sykehuset Innlandet. Det finnes ingen økonomiske egeninteresser og alle som deltar som forskere og prosjektmedarbeidere, mottar kun vanlig lønn i løpet av prosjektperioden. Rimfrost AS har bidratt med omega-3 og placebo til studien. Rimfrost AS har skriftlig frasagt seg alt ansvar og rett til å påvirke resultat eller publikasjoner som resulterer fra prosjektet.

Godkjenning

Regional komité for medisinsk og helsefaglig forskningsetikk har vurdert prosjektet, og har gitt forhåndsgodkjenning (2019/818)

Etter ny personopplysningslov har behandlingsansvarlig Høgskolen innlandet og prosjektleder Håvard Hamarsland et selvstendig ansvar for å sikre at behandlingen av dine opplysninger har et lovlig grunnlag. Dette prosjektet har rettslig grunnlag i EUs personvernforordning artikkel 6 nr. 1a og artikkel 9 nr. 2a og ditt samtykke.

Du har rett til å klage på behandlingen av dine opplysninger til Datatilsynet.

KONTAKTOPPLYSNINGER

Dersom du har spørsmål til prosjektet kan du ta kontakt med Håvard Hamarsland, tlf: 93445916, epost: havard.hamarsland@inn.no.

Personvernombud ved institusjonen er Anne Sofie Loftshus (anne.lofthus@inn.no).

Jeg samtykker til å delta i prosjektet og til at mine personopplysninger og mitt biologiske materiale brukes slik det er beskrevet

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Sted og dato

Deltakers signatur

Deltakers navn med trykte bokstaver

FORESPØRSEL OM AVGIVELSE AV VEVS-OG BLODPRØVER TIL EN GENERELL FORSKNINGSBIOBANK

The TrainOme – humane cellers tilpasning til trening og miljø

Dette er en forespørsel til deg om du ønsker å bidra med vevs-og blodprøver i den generelle forskningsbiobanken the TrainOME.

Hva er The TrainOME?

The TrainOME er en generell forskningsbiobank som er godkjent av regional etisk komité (REK) og som legger til rette for oppbevaring av biologisk materiale som skal benyttes til forskning og kartlegging av sammenhengen mellom trenbarhet og cellulære egenskaper. Biobanken inkluderer vevs- og blodprøver fra en rekke enkeltstående forskningsprosjekt, som hver og en har blitt vurdert av regional etisk komite. Hvilke analyser som vil bli gjort på dine prøver vil i sin helhet være definert i den prosjektspesifikke prosjektprotokollen. For ytterligere informasjon, ta kontakt med hovedansvarshavende for forskningsbiobanken, Stian Ellefsen (epost: stian.ellefsen@inn.no; tlf: 61288103).

Hva skjer med prøvene og informasjonen om deg?

Prøvematerialet vil bli oppbevart i låsbar fryser på låst lagerrom, situert ved Høgskolen i Lillehammer/Sykehuset Innlandet. Alle opplysninger og prøver vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Denne vil bli oppbevart adskilt fra øvrige data, enten i låst skap lokalisert til låsbart kontor eller på sikker server tilhørende Høgskolen i Lillehammer og vil kun være tilgjengelig for autorisert personell. Det vil ikke være mulig å identifisere deg i resultatene som kommer ut av biobanken når disse publiseres. Deler av materialet vil kunne bli sendt til utlandet for analyse. Merking vil i slike tilfeller være begrenset til identifikasjonsnummer; dvs. de vil bli sendt i kodet tilstand. Ubenyttet materiale vil bli returnert til Lillehammer i etterkant av analysene. Det biologiske materialet vil bli anonymisert innen 31.12.2038, hvorpå det vil bli destruert innen fem år. Høgskolen i Lillehammer ved administrerende direktør er databehandlingsansvarlig.

Dine rettigheter

Det er frivillig om du vil la ditt biologiske materiale inngå i The TrainOME-biobanken og du kan når som helst trekke tilbake ditt samtykke uten at du trenger oppgi grunn for dette. Hvis du sier ja til innlemmelse i biobanken, har du rett til å få innsyn i opplysninger som er registrert på deg og også rett til å få korrigert eventuelle feil som oppdages. Du vil etter loven ha krav på jevnlig informasjon om hvordan materialet blir benyttet. Om du trekker ditt samtykke, vil ditt biologiske materiale samt

utledete data bli slettet, med mindre opplysningene allerede inngår i analyser eller har blitt brukt i vitenskapelige publikasjoner.

Prosjektkoordinator eller øvrige prosjektmedarbeidere kan kontaktes når som helst i arbeidstiden:
Stian Ellefsen (hovedansvarshavende), tlf: 61288103, epost: stian.ellefsen@inn.no
Bent Rønnestad (prosjektkoordinator), tlf: 61288193, epost: bent.ronnestad@inn.no
Gunnar Slettaløkken (prosjektkoordinator), tlf: 61288182, epost: gunnar.slettalokken@inn.no

Samtykke til deltakelse i den generelle forskningsbiobanken

Jeg bekrefter med dette å ha lest informasjonsskrivet knyttet til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» og samtykker til at mine vevs- og blodprøver kan inngå i biobanken:

Sted:…… Underskrift: ……

Dato: …../…. 20…..

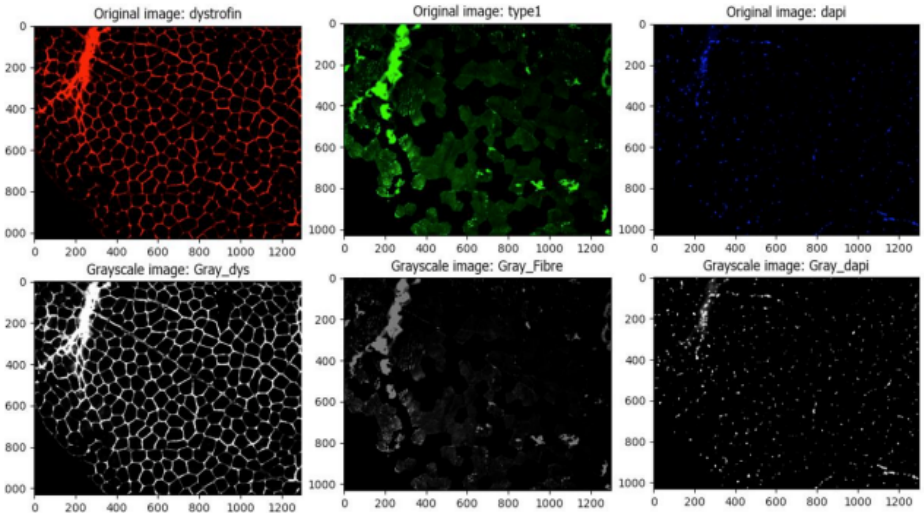
Attachment 2 – CellProfiler

- Three images were captured and merged to one for each biopsy.
- The images we wanted to analyze were entered into CellProfiler, and are labeled according to the timepoint, participant number and the correct leg.
- C0 is fiber type, C1 is dystrophy and C2 is nucleus.
- The number that comes after the C determines what type of filter that has been used, the number after the T states what timepoint it is. The number following FP states which participant the biopsy belongs to, while the letter after VL (R or L) states the correct leg. These data were used by CellProfiler to categorize the results in the output file.

The order of the steps and what they do

1. ColorToGray

- Converts a photo with multiple colour channels to one or multiple scales of grey. This step is used for both dystrophin, fiber type and nucleus.

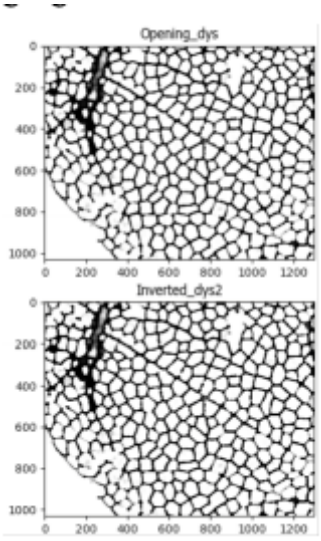


2. Invert

- Inverts the photos.
    - Correct illumination calculate
        - Calculates a lighting function that's used to correct uneven lighting/shadowing, or to reduse uneven bakgrounds in the photos
    - Closing
        - Disk

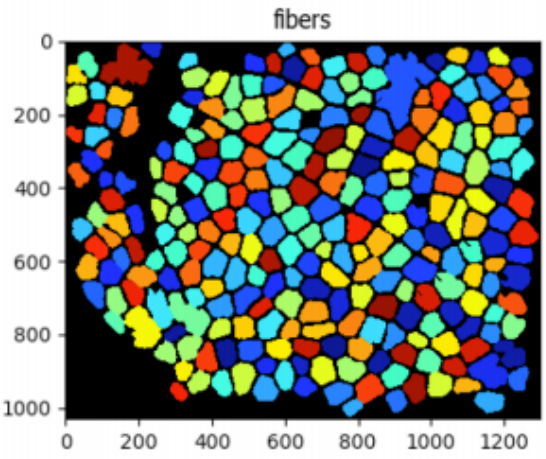
- Used to remove "pepper noise" and merge small light specks. Empty voids were filled to make it easier to observe the network, and to not get any unwanted fibers in that area later on. The settings were adjusted so that it found structures that formed a disk with a maximal size of 7.

- EnhanceOrSupressFeatures
  - Enhances or reduces certain image functions (e.g., specks and circle formations, and makes it possible to enhance subsequent identification of the image)
  - Used "neurites" to detect the kind of shape we are looking for and enhance the intensity of them, which are long thin objects. The program will attempt to find them and enhance them. The intensity was set to 10, as well as selecting the "line-structures".
- Opening
  - Does the opposite of "closing"; removes white specks and merges the black specks as well as enhancing the intensity. Size was set to 10.
- ImageMath
  - Performs various mathematical formulas of to or more photo intensities with a constant for individual photo intensities. Enhanced the blackness in the photo by 20.



3. IdentifyPrimaryObjects

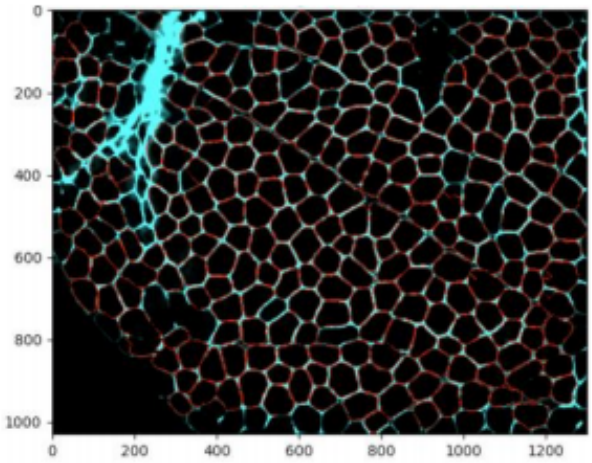
- Identifying biological objects of interest.
    - Threshold
        - Produces a black and white photo based on a threshold that's been set in advance. The threshold level was set to "global", and the method was "outso". "Two-classes" was chosen as the threshold when the levels of the grey scales could easily be divided into only two different levels.



4. OverlayOutlines

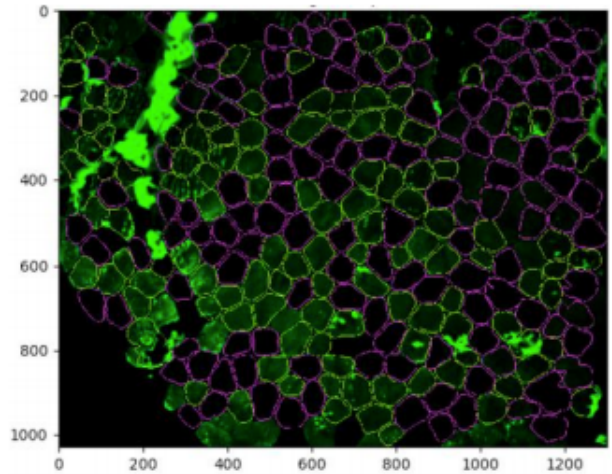
- Places contours of different objects on top of the wanted photo.
    - ExpandOrShrinkObjects
        - Expands or shrinks objects with a defined distance. In this case they were expanded.
    - MeasureObjectSizeShape
        - Measures multiple areas and forms by the identified objects.
    - FilterObjects
        - Removes objects based on measurements produced by a different module. Every object that did not satisfy the specified parameters were excluded.

- The measurement chosen to filtrate was "eccentricity" which was set to >0,85, and "FormFactor". FormFactor tells us the shape of the cell. If a cell looks long and slim, we are certain it is not dissected correctly and will therefore be excluded from the analysis.



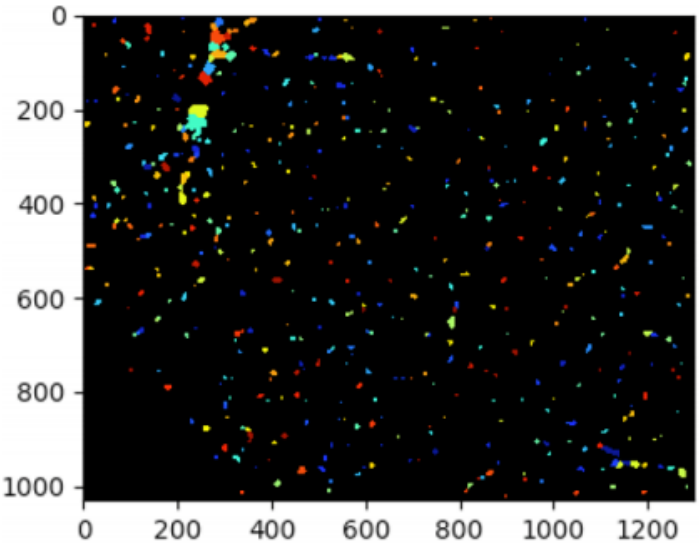
5. MeasureObjectIntesnity

- The first step was to use MeasureObjectSizeShape, which always uses the final image step conducted prior to this one as its template.
- MeasureObjectIntensity measures the intensity for the identified objects. For the nuclei, this module will test the intensity function for every object based on one or more similar grey scale photos. Measurements are received for every object.
    - FilterObjects
        - After measuring the intensity, FilterObjects was used again. This round it was used to filtrate out the measurements from "MeanIntensity", which tells us the mean pixel intensity within an object.



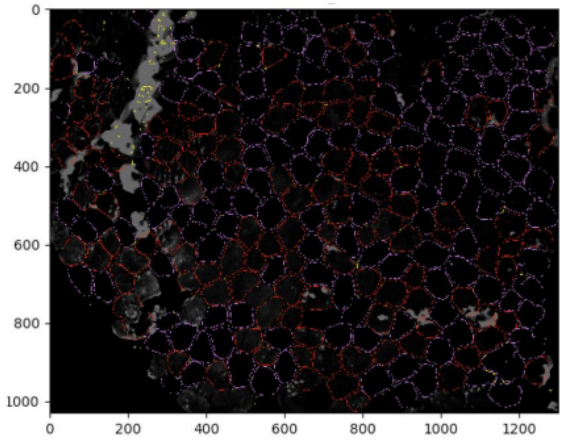
6. IdentifyPrimaryObjects

- Identifying the objects of what we're interested in analyzing. For nuclei, the size was set to 40 to make sure every nuclei was accounted for. The challenge was that many of the nuclei were within close proximity to one another, and was there merged into one nuclei in the analysis. "Minimum close entropy" was chosen as a method for the threshold. This makes the distribution of the different intensities that defines the fore and background used as estimates for the distribution that produces the intensity to the pixels in the fore and background.
- ShrinkToAPoint; Alters every nuclei into one pixel that’s in the center of the nuclei. If the pixel is within the cell it counts as one myonuclei.



7. OverlayOutlines

- Places new contours of different objects over the wanted image.
    - RelateObjects
        - Eventually we relate the different objects to what we want to analyze. This module lets us attach subordinate objects to bigger objects. This is an important step for counting the number of nuclei that's connected to the different fibers, and to calculate the mean values for the number of myonuclei attached to every fiber type.



8. Export to spreadsheet

- The final step was to export the results from the analysis in CellProfiler to an Excel spreadsheet