Inland Norway University

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Master Thesis

Ingesting glucose during resistance training blunt accumulation of protein expression MuRF1 in vastus lateralis in healthy trained adults..

IDR3005_1

2021

Acknowledgemente

"The resistance that you fight physically in the gym and the resistance that you fight in life can only build a strong character."
- Arnold Schwarzenegger

The year 2020 gave strength a new meaning. The whole world went into lock down, a pandemic sending the world in a silent, isolated panic. While I am still writing my paper in 2021 we are still in lock down.

I would firstly like to thank the Inland Norway University of Applied Sciences who tried their best, and still do, to make sure of the safety for their student. Even though they sent everybody home, they also adapted professionally fast. The school's fast adjustment gave a sense of security; I did not halve less to, even though I had most of my 3'rd semester at home.

I would like to thank Chris and Max; their involvement and enthusiasm in laboratory work have been motivating, and without them my analysis in laboratory work would be blunt.

To my fellow Students; Even though everything did not go as planned, we can say that not even a pandemic is going to stop us from getting educated.

To my Teachers; Thank you for your adaption and adjustment of lectures. It does not matter where you teach us from, it is just as motivating, engaging and informative. You have really shown that the teaching platform does not depend on where you are, but on how motivated we are in learning.

And a big thank you to Daniel and Stian for guiding throughout the year. Your professionally knowledge is extremely motivating. And thank you to the Ribose team for letting me in. A hardworking team with teachers and students helping and working with each other throughout the year. It has been an educating year with a steep learning process, and further enhanced my curiosity in physiology and health in the human body.

Considering Covid-19

This study is a part of a bigger project under the project Ribose.

The study was supposed to have at least 20 subjects, but we had a hard time getting subject recruited because of continuing closing down of the school. Another limitation was that of tests. We took blood samples, that were to be analysed at the hospital in Lillehammer, but during the project, the hospital had to change their protocol for analysing and we did not get the results in time for submission of this thesis. The subjects also consume deuterium oxide (D2O, heavy water) to measure rates of RNA and protein synthesis in form of spit tests which were to be analysed in Denmark. This also did not get back in time for analysis and might have been important for interpretation of the outcome of the project.

Abstract

Purpose: The purpose of this study was to investigate the effect of ingesting liquid glucose during resistance exercise on protein expression of muscle RING finger 1 (MuRF1) on healthy trained adults after 5 training sessions.

Method: Thirteen healthy trained participants performed six sessions of heavy-load resistance training with ingestion of glucose or placebo in this within subject randomized clinical trial. The subjects concluded a three-week intervention, consistent of familiarization phase the first week, and two weeks of resistance exercise. The participants legs were randomized in either glucose (GLU) or placebo (PLA), ingested during or after training session. Blood samples and biopsies were taken before and after intervention. The outcome measures were protein expression on MuRF1, nutrition status, muscle strength, training volume and glucose levels in blood. All data is analyzed in R Studio.

Results: Intake of glucose during resistance training led to a 26% [-0.4, -0.6] lower accumulation of protein expression MuRF1 in GLU compared to PLA (48% [0.03, 0.07] increase in post). The intervention led to an increase on total volume (PLA: 19% [0.1, 0.25], GLU: 18% [0.08, 0.24]. There was a time effect on isometric test (PLA: 8% [0.007, 0.16], GLU: 4% [-0.03, 0.12] on test 2, isokinetic 60sek (PLA: -18% [-0.25, -0.10], GLU: 9% [-0.16, -0.01] with significant difference between condition (p=0.03), and isokinetic 240sek (PLA -7% [-0.14, -0.01], GLU -5.5% [-0.1, 0.009]) on test 3. There was no difference between condition on restitution effect on isometric and isokinetic tests, but a time effect on isokinetic 60sek after 23 hours training session (p=0.02)

Conclusion: Ingesting glucose supplement during resistance training decreases protein content of MuRF1 in skeletal muscle

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1. Theory

Resistance training is performed with the purpose to increase performance in sporting competitions or to improve health and well-being in daily life. Specifically, resistance training improves muscular strength, size, and power in order to enhance athletic performance as results from morphological and neural adaptations, as well as a mean to achieve health improvements through blood pressure improved glucose tolerance, or insulin sensitivity (Folland, Williams, 2007; Kraemer, Nicholas, French, 2002; Ratamess, et al. 2009). Many of the beneficial effect's resistance training exhibits on athletic performance and well-being are due to training-induced muscle hypertrophy. Hypertrophy is affected by protein synthesis and protein breakdown and can only occur through a positive net balance of proteins (Biolo, et at. 1995; Kumar, et al. 2009; Ogawa, et al. 2006; Tipton, Wolfe, 2001). If the net balance is in a negative state, protein breakdown exceeds protein synthesis, which leads to muscle loss (Biolo, et at. 1995; Kumar, et al. 2009; Tipton, Wolfe, 2001). The process of breaking down and building up protein structures is determined by protein turnover (Tipton, Wolfe, 2001). This process is affected by catabolic and anabolic stimuli which respectively controls the rate of breakdown and synthesis (Philips, et al. 1997; 2002). Resistance training elevates both mechanisms, and it is the fractional rate between breakdown and synthesis that determine an increase or reduction in muscle mass (Philips, et al. 1997; 2002). Ingesting protein and carbohydrate increase the rate of synthesis (Biolo, et al. 1995), while other have found it does not affect protein synthesis (Fujita, et al. 2009; Koopman, et al. 2007). Co-ingesting carbohydrate and protein supplement during resistance exercise has been found to blunt protein breakdown (Beelen, et al, 2008; Roy, et al. 1997). The ubiquitin proteasome pathway (UPP) is the main pathway for protein breakdown in skeletal muscle (Peris-Moreno, et al. 2020). MuRF1 is an E3 ligase found in UPP that is myofibrillar specific and high level of insulin can blunt E3 ligases though phosphorylation by Akt/mTOR (Peris-Moreno, et al. 2020).

1.1 Protein turnover

On a cellular level, actin and myosin are the primary proteins that increase in size and results in muscle hypertrophy (Levers, et al. 2015). To induce this response there are two major signalling pathways, insulin-like growth factor 1 (IGF-1), which up-regulate phospoinositide-3-kinase-Akt (Akt) and protein kinase B-mammalian target of rapamycin (mTOR)

(Schiaffino, et al. 2013). The other pathway that regulates muscle mass is myostain-Smad3 that acts as a negative regulator that works as a co-operator for up-regulation of the myofibrillar degrading specific E3 ligase MuRF1 (Peris-Moreno, et al. 2020; Schiaffino, et al. 2013). Protein turnover is the balance between protein syntheses and breakdown, and an important factor in maximizing adaptions to training and muscular growth (Biolo, et at. 1995; Kumar, et al. 2009; Tipton, Wolfe, 2001). Studies shows that after resistance exercise protein synthesis is elevated for up to 48 hours while protein breakdown only remains elevated for up to 24 hours before returning to baseline values (Philips et al. 1997;2002; Yang, et al. 2006). Protein supplements in form of amino acids is often used to provide sufficient amino acids to ensure a positive net balance in protein turnover, but this alone without physical exercise is not enough, as it has been shown that chronic inactivity leads to elevated markers of protein breakdown even with a normal dietary intake (Ogawa, et al. 2006; Macnaughton, et al. 2014; Tipton, et al. 1999). Observation with co-ingesting carbohydrates and protein supplements after resistance exercise decrease markers for protein breakdown in both untrained (Yang et al. 2006) and trained participants (Churchley, et al. 2007). This demonstrates the complex relationship between nutrition and resistance exercise to keep protein turnover in a positive state. This way, resistance exercise contributes to protein turnover and nutrition status will determine if the net balance is in favour for synthesis or breakdown (Macnaughton, et al. 2014; Philips, et al. 1997). The question then becomes if inhibiting protein breakdown during resistance exercise can increase the net gain of proteins in any significant way. There is a very real theoretical possibility to due to the increase in insulin levels when ingesting glucose, acutely activates the IGF-1 pathway and up-regulates Akt and mTOR, which in turn up-regulates protein synthesis but also downregulates the pathway for muscle protein breakdown (Peris-Moreno, et al. 2020; Yoon, 2017). Indeed, observation have found that insulin can blunt expression of the ubiquitin specific E3 ligase MuRF1 (Glynn et al., 2010).

1.2 MuRF1

The formal term for protein breakdown is proteolysis, which is a fundamental biological process providing amino acids for synthesis to vital organs, tissue, or repairing and remodelling (Alberts, et al. 2019; Pasiakos, Carbone, 2014). Proteases are the enzymes that control proteolysis, which hydrolyse peptide bonds splitting them into smaller chains, and then into individual amino acids (Alberts, et al. 2019; McArdle, Katch, Katch, 2015). Proteasomes are the protein complexes that degrade proteins (Alberts, et al. 2019). Proteasomes resides in

the cytosol and nucleus within the cell, in a cylindrical form made from proteases (Alberts, et al. 2019). Proteasomes unfolds protein complexes marked for degradation by ubiquitin, and cuts them into short peptides (Alberts, et al. 2019). There are three pathways that degrade protein: autophagy, calpain calcium dependent cysteine protease, and the ubiquitin proteasomal pathway (Tipton, et al. 2018) In skeletal muscle, protein degradation is regulated primarily by the ubiquitin proteasome pathway (UPP) (Myung, Kim, Crews, 2001). The ubiquitin function as a marker that target cytosolic and nuclear proteins for rapid breakdown (Myung, Kim, Crews, 2001). When a protein is set for degrading, tagged with ubiquitin, the ubiquitin conjugating cascade (carboxyl group of Gly-76) is activated by ubiquitin-activating enzyme (E1) (Myung, Kim, Crews, 2001). The activated ubiquitin is then transferred to a thiol group of an activated site Cys residue (E2) by transacylation reaction (Myung, Kim, Crews, 2001). Then the ubiquitin attaches to protein substrate directly by itself, with E2, or together with ubiquitin ligases, (E3) (Myung, Kim, Crews, 2001). In more colloquial terms, when a protein is tagged with ubiquitin, the ubiquitinated protein enters the proteasome and degrades into smaller peptide units (McArdle, Katch, Katch, 2015). It is also believed that proteins with specific types E2 and E3 recognize specific proteins set for degradation (Myung, Kim, Crews, 2001).

In 2001, two papers identified two E3 ligases associated with muscle atrophy; Trim63 also known as muscle RING finger 1 (MuRF1) and FBX032 (MAFbx/astrogin 1) (Bodine, 2001; Gomes, et al. 2001). MuRF1 ligases are associated with skeletal muscle atrophy and is believed to be the main regulator of muscle mass through the FoxO families (Ogawa, et al. 2006; Peris-Moreno, et al. 2020). The current belief is that MuRF1 binds to titin located at the M-line where it has access to myosin and actin, to facilitate breakdown of myofibrillas (Peris-Moreno, et al. 2020). In skeletal muscle, MuRF1 is elevated under a fasted or/and physical inactive state and resistance exercise has shown to reduce the mRNA expression of MuRF1 (Mascher, et al. 2008; Ogawa, et al 2006). When FoxO is knocked out in mice, it has shown to lead to muscle sparing and blunting in insulin signalling of the Akt pathway, concluding that MuRF1 is highly important for the regulation of muscle mass (O'Neill, et al. 2018). When blood glucose increases, insulin is released and inhibits protein breakdown through Akt and mTOR, which down-regulates FoxO families (Peris-Moreno, et al. 2020;). In addition to inhibiting protein breakdown trough Akt, it has been hypothesized that insulin inhibits the activation of AMPK, which stimulates the expression of MuRF1 (Deng, et al. 2015).

1.3 Measure protein breakdown

There are several methods used to measure protein breakdown in the human body. Arteriovenous balance (AV-balance) is one way, butt his method requires trained personnel and equipment that is not easily obtainable, and do not measure protein breakdown per se., but Supplements and resistance training measure blood flow across the muscle to ascertain protein breakdown, synthesis, and net balance of amino acids (Pasiakos, Carbon, 2014). Another way to measure protein breakdown is urinary excretion of 3-methyilhistidine (3-MH), which is often used in combination with, or without AV-balance (Pasiakos, Carbone, 2014). This is a valid method to measure muscle breakdown as 3-MH are residues derived from the breakdown of actin and myosin and requires less equipment than AV-balance (Pasiakos, Carbone, 2014). On the other hand, 3-MH method has been criticized because participants must be in a strict dietary control, as ingestion of animal meat can affect the excretion of myosin and actin residue (Pasiakos, Carbone, 2014). Even if participants are under a strict meat-free dietary control, it does not imply "true" results on protein breakdown during resistance exercise, it has been shown that protein from dietary intake is important for a positive net balance and protein supplements can further enhance synthesis (Beelen, et al. 2010; Macnaughton, et al. 2014; Miller, 2007). QRT-PCR to analyse mRNA expression and western blotting for protein content are the other valid methods (Pasiakos, Carbone, 2014). These methods of measurement have a greater availability compared to other methods, such as measuring the AV blood flow to gather evidence of protein breakdown (Tipton et al., 2018). While these methods fail to represent cumulative changes in the muscle, they will give a snapshot of the intramuscular milieu at a specific point in time (Pasiakos, Carbone, 2014). Th reasoning behind using a within-participants study design in conjunction with western blotting for protein markers, is that the design will remove any biological differences and hopefully give strong evidence on the difference between conditions (Hammarström et al., 2019). it has also been found that changes in biological markers after only two weeks were indicative of future training response which gives the basis for the length of this study (Hammarström et al., 2019).

1.4 Supplements and resistance training

Ingesting amino acids through a protein rich meal or supplement stimulates muscle protein synthesis (Miller, 2007). Also, in conjunction with resistance exercise ingesting 40 grams (g) of essential amino acids after is sufficient to accelerate protein synthesis and blunt protein breakdown (Glynn, et al. 2010; Macnaughton, et al. 2014). The timing of ingesting carbohydrates may be an important factor to minimize markers for protein breakdown. A study examining supplements right before and after training has shown to decrease markers of protein breakdown in urinary samples, stating that the timing of supplement intake is important to maximize protein turnover by increasing protein net balance with amino acids, and minimizing protein breakdown (Kume, et al. 2020). Reducing protein breakdown is in fact the main way carbohydrates improve the protein net balance, when not ingested with amino acids (Børsheim, et al., 2004). Ingesting 100 grams of carbohydrates alone, without protein supplements, after resistance training can improve net protein balance, but does not elevate the net balance for synthesis to a positive state (Børsheim, et al. 2004). Also, observation has been found that a smaller amount of glucose intake can blunt expression of MuRF1, and as little as 30 grams of glucose seems to be sufficient (Glynn, et al. 2019). With a normal nutrition intake, the evening before resistance exercise, ingesting both carbohydrate and protein supplements improves whole body protein synthesis (Beelen, et al. 2008).

Ingesting a carbohydrate-only supplement directly after, and 1 hour after resistance exercise results in an up-regulation of blood plasma glucose, and down-regulation of protein breakdown (Roy et al., 1997) and different studies have looked at the effects of ingesting both carbohydrate and protein together on training. There are some questions regarding the importance of inhibiting protein breakdown on the anabolic response of the fractional rate in protein turnover (Glynn et al., 2010). Ingesting a mixture of carbohydrates and amino acids after resistance exercise resulted in a greater acute response in net protein balance than ingesting carbohydrates alone (Borsheim et al., 2004). This effect was also observed to last for more than an hour (Borsheim et al., 2004). There is also evidence to suggest different timing of ingestion in relation to exercise could affect the net balance in protein gain. Nutritional intake before resistance exercise resulted in lower protein breakdown than eating right after or staying fasted when measuring 3-MH excretion (Kume et al., 2020). Even when accounting for nutritional imbalances between subjects by standardizing a meal the night before, ingesting protein and carbohydrates together during exercise still resulted in elevated protein synthesis (Beelen et al., 2008). This underlines that supplementation surrounding

exercise has value in its own right, and not just as an antidote for underlying nutritional imbalances. When co-ingesting carbohydrates and essential amino acids between exercise sets, 3-MH urinary markers for protein breakdown in myofibrillas is reduced by 27%, whereas for participant without supplements, 3-MH markers increased by 56% (Bird, S. P., Tarpenning, K. M., Marino, F. E., 2006).

Therefore, this thesis will examine if the timing of glucose intake, either during resistance exercise or in the evening reduces MuRF1 protein content after 5 training sessions. The hypothesis of this thesis is therefore stated as:

There is a significant reduction on protein markers of MuRF1 when ingesting glucose during resistance exercise.

2. Introduction

Resistance training improves muscular strength and size, as results from morphological and neural adaptations (Folland, Williams, 2007; Kraemer, Nicholas, French, 2002; Ratamess, et al. 2009). Hypertrophy is affected by protein synthesis and protein breakdown and can only occur through a positive net balance of proteins (Biolo, et at. 1995; Kumar, et al. 2009; Ogawa, et al. 2006; Tipton, Wolfe, 2001). If the net balance is in a negative state, protein breakdown exceeds protein synthesis, which leads to muscle loss (Biolo, et at. 1995; Kumar, et al. 2009; Tipton, Wolfe, 2001). The process of breaking down and building up protein structures is determined by protein turnover (Tipton, Wolfe, 2001). Resistance training elevates both mechanisms (Philips, et al. 1997; 2002), and studies have found that co-ingesting carbohydrates and protein supplements can blunt muscle protein breakdown (Beelen, et al. 2010; Børsheim, et al. 2004; Glynn et al., 2010 Kume, et al. 2020; Roy et al., 1997). There is also evidence to suggest different timing of ingestion in relation to exercise could affect the net balance in protein gain. Nutritional intake before resistance exercise resulted in lower protein breakdown than eating right after or staying fasted when measuring 3-MH excretion (Kume et al., 2020). Glucose intake increases insulin levels and addition to inhibiting protein breakdown trough Akt, and it has been hypothesized that insulin inhibits the activation of AMPK, which stimulates the expression of MuRF1 (Deng, et al. 2015). MuRF1 ligases are associated with skeletal muscle atrophy and is believed to be the main regulator of muscle mass through the FoxO families (Ogawa, et al. 2006; Peris-Moreno, et al. 2020). Therefore, this thesis will examine if the timing of glucose intake, either during resistance exercise or in the evening, cand reduce MuRF1 protein content after 5 training sessions. The hypothesis of this thesis is therefore stated as:

There is a significant reduction on protein markers of MuRF1 when ingesting glucose during resistance exercise.

3. Method

3.1 Subjects

Sixteen (male (n=10) and female (n=6)) moderately strength trained participants (20-45 years) were recruited to the study from Høgskolen in Innlandet, Lillehammer. Participant characteristics is presented as mean(Sd = standard deviation) in Table 1A on whole group and descriptive between left and right leg in table 1B. There was no significant difference between the legs (fat mass in kg, confidence interval ([CI: lower.CI, upper.CI]): Left [2.4, 4.16], Right [2.4, 4.3]. Lean mass in kg: Left [8.92, 10.5], Right [9.13, 10.7], total mass in kg: Left: [12.8, 14.6], Right [13, 14.9]).

Three participants were not able to finish the intervention because of injury unrelated to the study, or sickness during the intervention (n=1). The participants were non-smoking and moderately trained (having performed 2-8 resistance exercises per 14 days during the last six months leading up to the study). Exclusion criteria were previous injury or injury during intervention resulting in impaired strength, inability to perform resistance training, and self-reported medical history of metabolic disorder including hyperglycemia, disabling musculoskeletal disease, serious mental illness, or allergy to local anesthesia.

Table 1A: Partisipant descriptive

variable	
Age	23.7 (2.4)
Fatt mass (kg)	16 (6.2)
Fat free mass (kg)	59.7 (7.6)
Height (cm)	175.3 (5.8)
Lean mass (kg)	56.6 (7.3)
Weight (kg)	74.9 (7.2)

Table 1B: Leg descriptive

Leg	left	right
Fat (kg)	3.3 (1.4)	3.4 (1.4)
Lean (kg)	9.7 (1.4)	9.9 (1.4)
Total (kg)	13.7 (1.5)	14 (1.4)

Table 1: (A) Descriptive data on subjects showed in mean and standard deviation total group description. (B) Describes pr. Leg (left or right) in fat mass, lean mass and total mass on total group.

3.2 study overview

In this within participant interventional study, the participants completed a 3-week intervention. Participants performed in both conditions and their legs were randomly assigned to either glucose (GLU) or placebo (PLA) starting the training with either ingesting GLU or

PLA the first session. The legs performed on alternating days, but the days has been paired as illustrated in figure 1. The first week was the familiarization phase, containing 2x 1RM tests, body mass index, and isokinetic and isometric tests. Week 2 and week 3 was the interventional phase containing blood samples and biopsies (baseline (B), and 6), dietary program and resistance training (B-6), isokinetic and isometric tests (B, 2, 3, 4,5, 6) with ingestion of glucose (GLU), or placebo (PLAC) during resistance training. In addition to be randomized in either GLU or PLA, the participants was randomized to start the first training session with either the dominant, or non-dominant leg. Six training session were performed for each leg.

Figure 1: Intervention

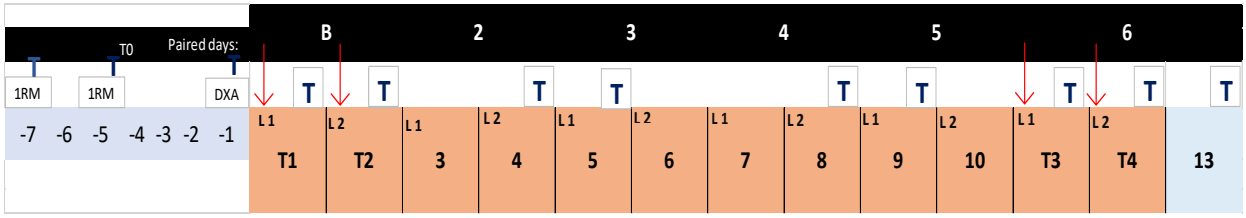

Figure 1 illustrates the training intervention. First week (T0) was the familiarization week with 1RM test, DXA and isokinetic and isometric tests (**T**). L1 illustrate the starting leg, L2 the second leg and how they trained on alternating days throughout the intervention. ↓ Illustrate blood samples and biopsies taken under the intervention.

3.3 Assesment of body composition

Body mass composition was measured using dual-energy x-ray absorptiometry (DXA, Lunar Prodigy, GE, Heathcare, Oslo, Norway) the last day in the familiarization phase (figur 1). The participant's weight and height were registered prior to measuring. Participants were asked to lay in the middle of the apparatus with minimal clothing. Arms and legs were placed inside marked positions on the bench with hands and feet pointing towards the ceiling. They were asked to lay still throughout the procedure, which took about 10 minutes. DXA was used to register the data on total weight, lean body mass, and fat mass and fat mass, lean mass and total mass on induvial legs (table 1B). Mean(sd) was analysed on the whole group.

3.4 Dietary protocol

Each participant consumed a total of 90grams of glucose (Glucosum monohydricum, Merck KGaA, Darmstadt, Germany) and 0.9 grams of stevia (Steviosa, Soma Nordic AS, Oslo, Norway), each day. Difference being the timing of ingestion. Drinks were mixed with Fun light juice (Orkla, Oslo, Norway). During training, participants ingested either glucose or placebo on three occasions, 300ml 30 minutes before exercise, 300ml right before, and 300ml right after exercise, they also consumed 900 ml of either glucose or placebo at the at 18:00 hours in the evening. The glucose drinks contained 30g of glucose pr. 300ml drink, or 0.3g pr. 300ml drink of stevia for placebo drinks. Before (2hrs) and after the training session the subjects consumed 25g (44 grams of protein each day) of Whey protein isolate (Proteinfabrikken, Stokke, Norway) mixed with 150ml H_2O. The data on group distribution was collected by a colleague not involved in the project, who kept data until all measurements were done to ensure true nonbiased randomization.

Participants were instructed to register their daily dietary intake throughout the study using an app-based data collection software (MyFitnessPal, Inc. Med). Participants were also asked to consume the same amount of macro nutrient each paired day to ensure non-biased nutrition status for the legs. The nutrition-supplements were calculated in the nutrition data for each participant after data collection. A blinded randomized drink taste test was done on last day after isokinetic test. The subject was handed four cups, numbered 1-4, where two contained fun light with glucose and two contained fun light with placebo. Before the test, the subject ingested one cup mixed with glucose and placebo to set taste. The content in the cups and the order to drink was randomized. Each cup contained 1 dl of fun light. The subject was ordered to drink one cup at a time, in his or her own time, and take notice of what they thought the cups contained. The data on blinded drink test showed that the participants could not detect taste differences.

3.5 Strength assessment

Maximum strength tests were assessed as one repetitions maximum (1RM) in the familiarization faze as a mean to adapt to exercises. After a standardized warm-up, the participants performed 1RM on one leg at a time on unilateral leg press and unilateral knee extension. Prior to the first test the participants were asked about their expected 1RM and this was used as the basis for test progression. The second test was to ensure the right 1RM

was found, based on the test conducted. The 1RM test was also the base for training intensity during the intervention.

Unilateral isokinetic knee extension torque at 60˚/sec and 240 ˚/sec and isometric knee extension force tests were performed using Humac Norm dynamometer (CSMi, Stoughton, Massachusetts, USA). Isokinetic and isometric test on day B was set as baseline to compare on tests day 2 and 3 (test 1), 4 and 5 (test 2) and mean of test 6 (test 3) as mean to conduct strength assessment throughout the intervention. To measure restitution effects, 4 tests was conducted on day 6: before exercise (baseline), 30 minutes after, 2 hours after and 23 hours after training session to conduct any differences between conditions. The participants were asked to sit on the so the knee joint was parallel with the rotary axis of the dynamometer. The thigh and the ankle were strapped to the seat. Participants were instructed to push as hard as they could in concentric phase, and to rest in the eccentric phase. Three repetitions per test session and angular velocity were performed in isokinetic tests, and one repetition in isometric test which lasted for 10 seconds or until peak failure. All data about participant position in the chair was recorded and used throughout all tests.

3.6 training protocol

Each training session consisted of 10 minutes warm up on an ergometric cycle, two specific warm up sets on unilateral leg-press, where first set was ca. 50% of 1RM, and the second ca. 70% 1RM, 10 repetition each. The main training consisted of three series of 10RM with 2 minutes rest between sets. The participants registered their session score using a 10-point scale fifteen minutes after the training session, where a score of 1 equals rest and 10 equals maximum effort. The morning after they register perceived rate of exertion (RPE) using a 9-point scale where 1 was very light and 9 was very heavy. Each participant had trained personnel supervising each training session during the whole intervention. The trained personnel followed the same participants throughout the intervention and there were no music playing to remove any potential confounding variables (Halperin, Pyne, Martin, 2015).

3.7 biopsies and blood samples

All biopsies were sampled in an overnight-sated fast at the same time of the day before training session for the individual subject (figure 1). The biopsies were sampled under antiseptic

conditions, and local anesthesia (Lidokain 10 mg ml-1, Mylan Hospital AS, Oslo Norway) using minimally invasive micro-biopsy technique, using 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).

Blood samples from finger stick were used to measure the blood glucose (Biosen C-line, lactat analysatior, EKF diagnostic GmbH, Barleben, Germany) levels on timepoint day 1, and several measurements at day 6. The samples at day one and the first sample at day 6 was set as baseline values. During day 6 blood samples were collected right before protein intake (0min), 45 min, 90 min, 120 min, 135 min, 150 min and 270 min after protein intake. This corresponds to training session as 30 min before warm-up session (90 minutes after protein, before first bolus of GLU/PLA drink), right after warm-up session (120 min after protein intake), in between exercises (135 minutes after protein intake), right after training session (150 minutes after protein intake) and 270 minutes after protein intake to look at glucose level in blood throughout training session. This means we should see elevated markers of glucose in blood during training.

3.8 Protein analysis

All steps were done on in room temperature if not otherwise stated. Muscle samples were homogenized in Eppendorf 1.5 ml tubes with 300ul Trizol using beads and a bead mill (Bullet blender, Next Advanced, Averill Park, NY, USA) 1 minute at speed 10 without thawing. After disruption of the muscle tissue, additional Trizol was added (total volume 1000ul), and run bullet blender (BB) at 1 min, at speed 3. Then the samples incubated for 5 min. After incubation 200ul of chloroform was added, shaken for 15 seconds, and incubated ~3 minutes. The tubes were placed in a centrifuge and spun at 12000g, 15 min at 4˚C for phase separation. After taken the aqueous phase, the remaining content in the tubes were stored at -20 ˚C until protein extraction.

To separate DNA and protein 300 ul ethanol 96% (EtoH 96%) was added to the tubes, inverted to mix. After 3 minutes incubations, 490 ul EtoH (96%) was added to phenol-ethanol supernatant and vortexed shortly and then added 100 ul bromochloropropane and vortexed shortly. For phase separation 450 ul ddH2O was added vortexed vigorously and centrifuged at 12000g, for 5 minutes. The upper phase was removed and 700 ul of EtOH (96%) was added. The tubes were vortexed vigorously and centrifuged at 12000g, for 5 minutes to form a pellet. The EtOH was then completely removed, and pellets set to air dry for 2-3 minutes, with lid

open. For re-suspension 40 ul of SDS-urea buffer with inhibitors (1.05 ml 1m tris pH 6.8, 2.075 ml 20% SDS, 1.25 ml glycerol, 207.5 ul 2-Mercaptoethanol 3.1 g urea, and added ddH_2O to final volume 10 ml, 1 crushed tablet of protease and phosphatase inhibitors each) was added after the pellets was dry, vigorously vortexed and incubated for 20 minutes or until all pellets was dissolved. After incubation, the samples were centrifuged at 10000g for 5 minutes. After dissolved sediment 30 ul was transferred to new tubes (b), and from there 4 ul was transferred to new tubes (c) that already contained 36 ul of ddH_2O, and both was stored at -20 ˚C. After all samples was extracted of proteins, they were ready for Bradford assay. In Bradford assay tray 250 ul of Bradford assay reagent was added with samples (c) mixed with ddH2O and run to determine the protein concentration. The samples protein concentration was standardized as average (avg) concentration times volume divided by 2 or 1.75 minus 26 ((avg x 26)/2, or 1.75)- 26.

To prepare for western blotting the samples were normalized to 1.5ug/ul protein and 20ug protein was loaded in each well on precast gels. 5 ul of ladder and 10 ul of samples was added to the gel, run on 250 v ~1 hour or until blue lines exits the gel in ice bath. To transfer protein the gels were placed in a cassette with PVDF membrane (Transfer membrane Immobilon-P PCDF) in a tank filled with chilled transfer buffer. The tanks containing magnetic stir bar were placed on a stir plate and run 400mA for 1hrs at 4 ˚C on ice bath. For protein visualization, MemCode (Thermo Fisher Scientific Inc. USA) was used. The membranes were rinsed with ddH_20 three times before adding sensitizer. After the membranes agitated for 2 minutes sensitizer was discarded and MemCode reversible stain was added. Stained proteins did then appear, and distain was used for 5 minutes to remove background colour. After rinsing distain with ddH_20 the membranes were photographed in G:BOX (G:BOX-CHEMI-XT4 gel documentation system, Rödermark, Germany) for membrane visualization. After cutting the membranes in desirable strips, eraser was used to remove stain. The membrane was never left to dry out under the whole protocol.

For protein identification with antibodies and enhanced chemiluminescence the membrane was blocked using blocking solution (10X TBS, 500 ul Tween, 25g non-fat dried milk powder and ddH2O) for 1 hour on a rotary platform. After decanted blocking solution, the membranes were incubating with primary antibodies MuRF1 (C-11, Santa Cruz Bioinformatics Dallas, Texas, U.S; sc-398608) (MuRF1, 1:100: diluted MuRF1 antibody in 1x TBS-T, 1 liter: 100mlX TBS, 1ml Tween and added ddH2O to final volume 1000 ml) overnight at 4˚C in a blot cycler (BlotCycler, Precision Biosystem, Mansfield, MA, USA). Luminol and peroxide was mixed 50/50 for electrochemiluminescence (Pierce, ECL Western Blotting substrate,

Rockford, USA) for or 5-7 minutes and added to the membrane. The membrane was then photographed in G:BOX on clear plastic sheet with black background. ImageJ (Reuden, et al 2017) was used to quantify the protein and measured as total protein background on grey area subtracted with whole well and between well values. The membrane was dried until completely dried and stored in -20˚C freezer.

4. Statistical analysis

Full dataset and script can be downloaded at: https://github.com/SaraChri/masterthesis

read.me is have an overview over all folders. All descriptive data is shown in mean and standard deviation (mean (sd)). All data was imported and analysed in R studio (R studio). To explore the difference between conditions, linear mixed model was used (LMM) and baseline set to dependent vairable. LMM take accountability for within subject variation with log metric calculation on the mean to take accountability to different baseline score. Analysis results are written as %change, 95% confidence interval (CI [Lower.CI, upper.CI]). The level of statistic significant was set to α=0.05.

5. Results

5.1 Nutrition status

All participants except for one delivered data on their daily nutrition intake illustrated in table 2. There was no difference between conditions on any macronutrient on any paired days or between conditions except for total calorie intake and protein/kg, which differed between each pair of days, but not between conditions.

Table 2: Nutrition intake

Time	Group	Calories	Carbohydrates	Fat	Protein	Pro/kg
2	G	2582.2 (569.8)	369.8 (74.7)	80.9 (41)	173.2 (33.9)	2.5 (0.4)
2	P	2587.4 (653.3)	367.8 (82.4)	66.6 (24.6)	170.6 (43.4)	2.4 (0.5)
3	G	2574.2 (532.6)	352.2 (50)	78.7 (31.6)	171.4 (41.1)	2.4 (0.5)
3	P	2522 (560)	354.8 (53.2)	80.2 (28.7)	172.4 (37.2)	2.5 (0.5)
4	G	2480.6 (573.4)	323.8 (91)	77.3 (34)	167 (32.4)	2.4 (0.4)
4	P	2444.8 (591.5)	316.2 (93.7)	79.3 (32.7)	163.6 (36.6)	2.3 (0.5)
5	G	2420.6 (724.6)	327.2 (92.1)	82.8 (44.3)	157.6 (34.2)	2.2 (0.4)
5	P	2488.2 (680)	344.4 (95.5)	87.9 (42.3)	164.2 (33)	2.3 (0.4)
6	G	2168 (535.5)	304 (70.4)	89.8 (32.4)	156 (24.6)	2.2 (0.2)
6	P	2257.5 (510.9)	302.5 (69.1)	82.3 (34.2)	153.8 (24.6)	2.2 (0.2)
B	G	2493.4 (539.2)	315 (86.9)	78.1 (23.9)	171 (40.8)	2.4 (0.5)
B	P	2349.2 (576.4)	315.6 (78.1)	73.5 (20.9)	168.6 (44.6)	2.4 (0.5)

Table 2: Nutrition intake pr. group each day in mean(SD). G= glucose, P = placebo. All data are in grams (g)

5.2 Training volume

There was no difference in total training volume between conditions on baseline values (PLA: 5351kg (1615), GLU: 5262kg (1799)). Total training volume on day 6 was 6438kg (2092) in PLA and 6319kg (2256) in GLU. The training intervention led to a 19% [0.1, 0.25]

increase in PLA and 18% [0.08, 0.24] increase in GLU in total volume after 6 training session compared with baseline values. There was a time effect on day 4-6 (figure 2) on total volume with no differences between conditions in relative increase in total training volume from baseline to any subsequent time-point.

Figure 2: Total Volume

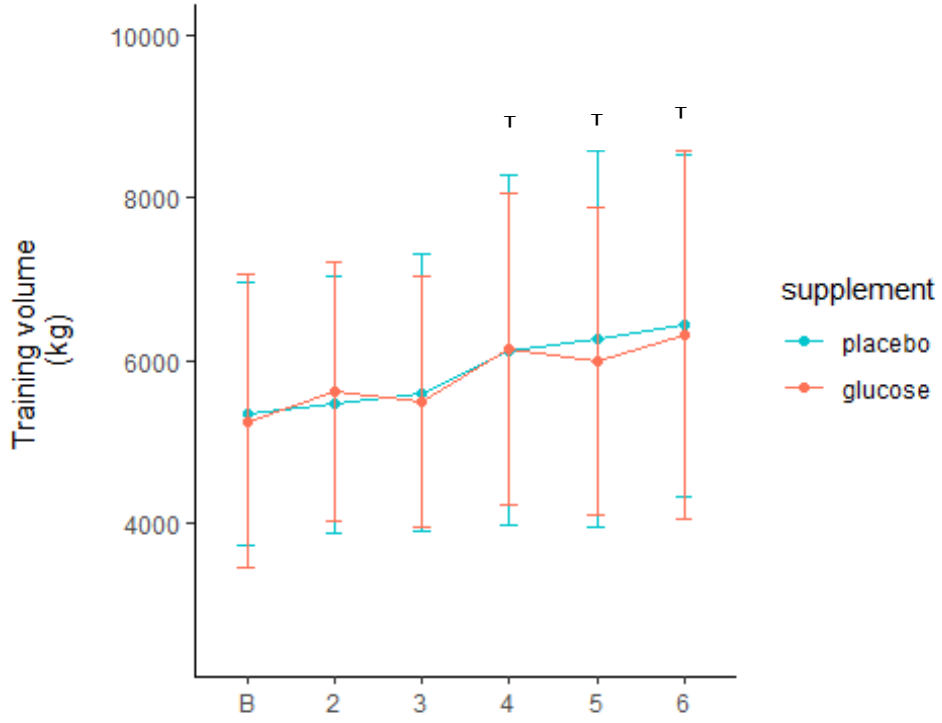

Figure 2 illustrates the total volume between conditions with timeline on x-axis and total volume in kg on y-axis. T = time effect.

5.3 Training measurements

Measurements of glucose in blood showed a clear difference on timepoints 120 min, 135 min, 150 min and 270 min after protein intake as shown in figure 3. GLU had 35% [0.31, 0.43], 22% [0.19, 0.31], 25% [0.22, 0.34] higher values, and decreased -7% [-0.18, -0.06] compared to PLA.

Figure 3: Blood glucose

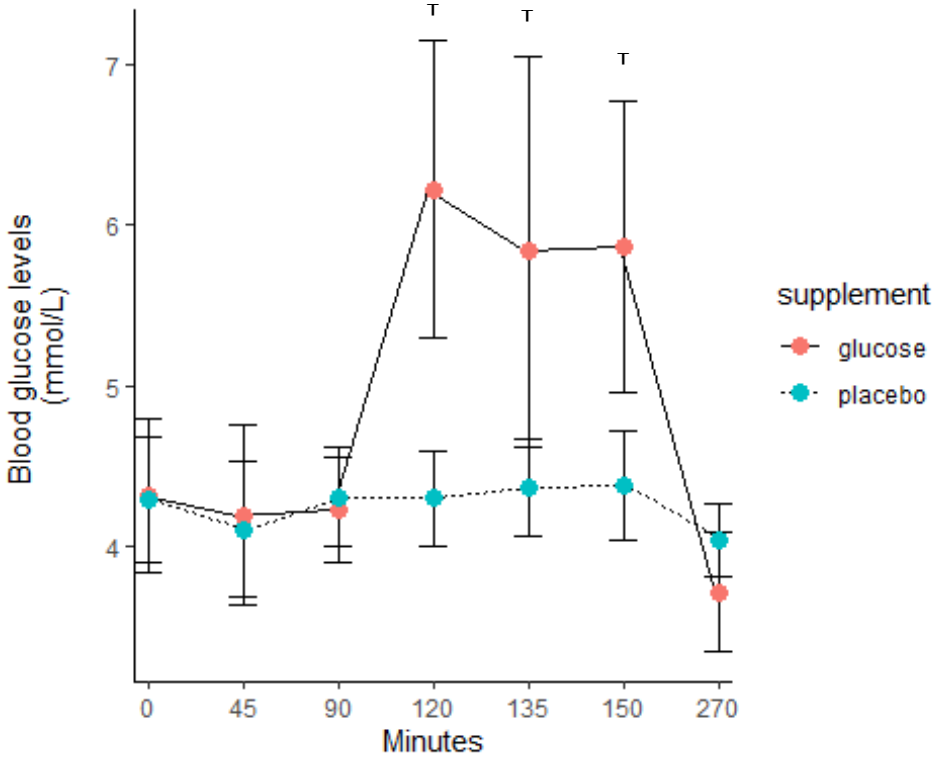

Figure 3 illustrates blood glucose levels as mmol/l after protein intake. 90 minutes marks the first GLU/PLA intake, and 120 minutes when training session starts. ᵀ == Time effect

There was no difference between conditions in isometric testing throughout the intervention compared with baseline values, but a significant time effect on test 2 (p=0.03) as shown in figure 4C (PLA 8% [0.007, 0.16], GLU 4% [-0.03, 0.12]). There was a decreased time effect on isokinetic 60˚sec on test 3 for both conditions (figure 4A, p= <0.001) with -18% [-0.25, -0.10] decrease in PLA compared with baseline values, and a significant difference between conditions with GLU 9% [-0.16, -0.01, (P=0.03)] higher values compared with PLA. There was a significant decreased time effect on isometric 240 ˚sec test 3 (PLA -7% [-0.14, -0.01], GLU -5.5% [-0.1, 0.009], p=0.02) compared with baseline values with no difference between conditions (figure 4B).

Figure 4 **Isokinetic and isometric time effect**

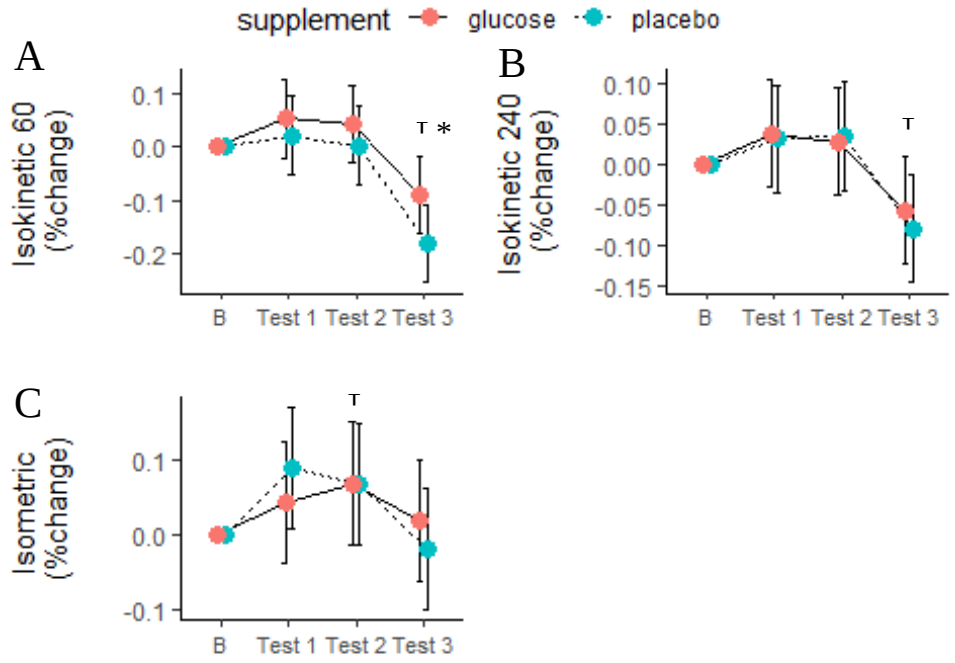

Figure 4 illustrates mean %fold change on each test throughout the intervention. (A) is for isokinetic 60 ˚sec, (B) isokinetic 240 ˚sec and (C) isometric test. B = test day 1, Test 2 = day 4 and 5, test 3 mean of all tests on day 6. ^{T} = time effect, * = significant difference between conditions.

Results on restitution time effect had no difference between conditions on isometric tests or isokinetic 240 ˚sec. There was a significant restitution time effect on isokinetic 60 ˚sec after 23 hours (p=0.02), with no difference between conditions. PLA increased by 10% [0.02,0.19] and GLU 4% [0.02,0.15] lower than PLA, compared with baseline values as shown in figure 5A.

Figure 5 **Isokinetic and isometric restitution tests**

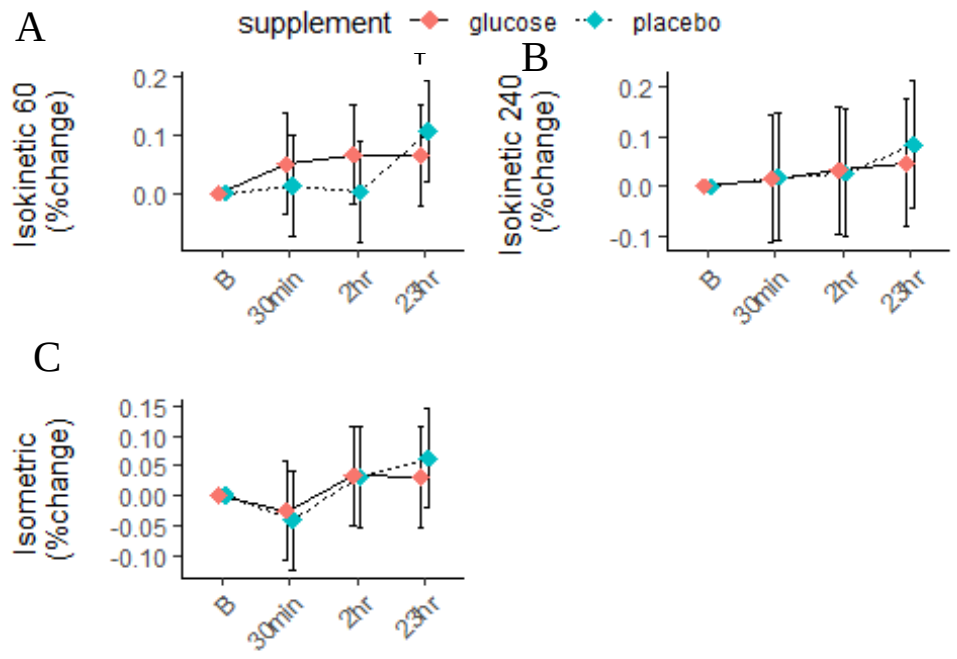

Figure 5 illustrates mean %fold change on each test taken 30min, 2 hours and 23 hours after training session to measure restitution effect between groups. (A) is for isokinetic 60 ˚sec, (B) isokinetic 240 ˚sec and (C) isometric tests. ^{T} = Time effect.

5.4 MuRF1

There was a significant difference between conditions in post values. MuRF1 content in GLU was 26% (p=0.02) lower compared with PLA (48%) in post (figure 6C). The total MuRF1 protein concentration in PLA was 1.03 [0.6, 1.4] in pre and 1.18 [0.8, 1.5] in post. In GLU the total protein concentration was 0.99 [0.6, 1.3] in pre and 1.01 [0.6, 1.4] in post as shown in figure 6A. The log fold change in PLA increased by 22% [-0.0, 0.4]. GLU had a -5.9% [-0.3, 0.2] less increase in total MuRF1 content compared by PLA, illustrated in figure 6B.

Figure 6: MuRF1

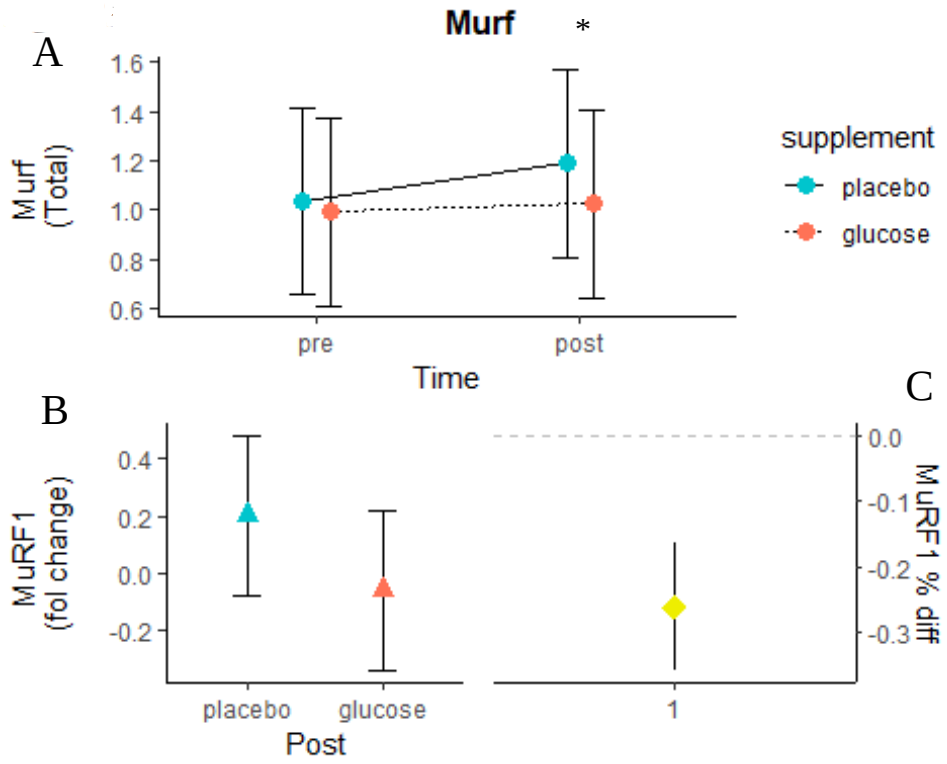

Figure 6 (A) illustrates the total MuRF1 conten in each group from pre to post. (B) illustrates fold change in post in each condition compared with PLU in pre. (C) illustrate the relative % difference between conditions in post.

* = significant difference between conditions.

6. Discussion

The goal of this study was to examine the effect on glucose intake during resistance training on protein expression on MuRF1 in vastus lateralis after five training sessions. The main findings were that accumulation of MuRF1 protein content was 26% lower in GLU after five training sessions.

Studies show that glucose uptake in active skeletal muscle increases more than 5 times or ~40% after glucose intake (Bird et al. 2006; Durham, et al. 2004). The participants in this study also trained with high glucose levels in blood, which should give optimal conditions to blunt protein expression on MuRF1 (O'Neill,et al. 2018; Peris-Moreno, et al.2020). Observation in this study shows an effect of the timing on glucose intake. 90 grams of glucose ingested during training exercise significantly influences MuRF1 in contrast to the same amount of glucose taken during the evening. Our observation finds that timing of glucose intake could be important in reducing protein breakdown, as it has been demonstrated in other studies that ingesting glucose after resistance exercise results in changes on protein breakdown (Beelen, et al. 2010; Børsheim, et al. 2004; Glynn et al., 2010 Kume, et al. 2020; Roy et al., 1997). An interesting observation from Durham et al. (2004) found no changes in muscle protein breakdown when measurement was taken right after training. This study measures the AV-balance ~45 minutes after resistance training, which might imply the importance of timing of data collection when measuring protein breakdown as this study took biopsy samples the day after training, and clearly found changes in markers on Murf1 expressions. As stated before, AC-balance does not measure protein expression on protein breakdown per se. but gives an indicator on protein turnover right after training (Pasiakos, Carbon, 2014).

It is tempting to conclude that ingesting glucose during resistance exercise attenuates protein breakdown, as there are several studies measuring protein turnover that finds these observation (Beelen, et al. 2010; Børsheim, et al. 2004; Glynn et al., 2010 Kume, et al. 2020; Roy et al., 1997). This thesis did not find any reduction in mRNA expression on MuRF1, which is a surprise but might be due to the participant's training status, as observation show that mRNA of MuRF1 is less expressed in trained (Churchley, et al. 2007), than in untrained participants (Yang, et al 2006).

The intervention led to a 19 % (PLA) and 18% (GLU) increase on total volume, with no significant difference between conditions. Studies and meta-analysis favours high loads (60-80%RM) compared with low loads (30% RM) when it comes to training protocols on maximizing muscular strength (Lopez, et al. 2020; Schoenfeld, et al 2016;2017). There was a

significant time effect on isometric tests on test 2 (day 4 and 5) (PLA 8% [0.007, 0.16], GLU 4% [-0.3, 0.12]) with no difference between conditions, which might imply strength adaptions for both conditions in the intervention (Wilson, Murphy, 1996). Although mean peak force on test 3 was almost identical to baseline values, the mean force is calculated on several tests for comparison on muscle fatigue between conditions. The tests indicate that glucose does not affect peak force compared with placebo. There was also no different observation between conditions at any time effects on isokinetic 60 ˚sec or 240 ˚sec throughout the intervention. We did however observe a mean higher peak torque on isokinetic 60 ˚sec for GLU on test 3, implying that glucose intake during resistance training could affect muscle fatigue in fast twitch muscle. After a prolonged restitution however, there was no difference between conditions. There was no difference between conditions on restitution effect in isometric or isokinetic 240 ˚sec tests, implying that glucose intake after intervention does not affect strength adaptions compared to placebo. Multiple sets have shown to lead to a greater increase in strength, which correlates in mass (Hammarström, et al. 2020). This thesis cannot say anything about measurements of muscle mass, and our observation on strength test show no difference between conditions, begging the question if blunting MuRF1 affects muscle adaption to training. However, studies have observed changes in biological markers for strength adaption after 2 weeks of resistance training, which predicted strength adaption for prolonged training (Hammarström, et al. 2020). It would be interesting to study if timing of glucose intake has a different effect on increased muscle mass with prolonged training, where glucose is used to blunt MuRF1.

In conclusion, observation in this study show that supplementing glucose during resistance training decreases protein expression MuRF1 in skeletal muscle, and that timing of glucose is an important factor.

7. Pros and cons

A within participant study design is a highly pros in this thesis, which removes biological effects, and we can conclude that glucose intake within subjects has an affect on the individual biological markers. This thesis had only 13 participants. While we should have at least 20 participants, this what not possible due to covid-19 restriction as stated before, making it harder to achieve size sample. Most of the studies used in this thesis has a small sample size. It would be interesting to use a bigger sample size to strengthen the results observed in this thesis. Another disadvantage was of antibody of MAFbx. We did measure protein expression of MAFbx but could not get the antibodies to work. We did also not get blood samples and deuterium oxides measurements in time and might have lost significant results. We did also not have any 1RM post test, or measurement of muscle mass that could give more information on glucose intake during resistance training.

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9. Attachments

Høgskolen i Innlandet

[03.06.2020]

Vil du delta i forskningsprosjektet

RIBOSE – EFFEKTER AV GLUKOSEINNTAK UNDER STYRKETRENING PÅ RIBOSOMAL BIOGENESE I SKJELETTMUSKEL?

9.1 formålet med prosjektet og hvorfor du blir spurt

Dette er et spørsmål til deg om å delta i et forskningsprosjekt hvor formålet er å skaffe ny kunnskap om hvorvidt karbohydratinntak før, under og etter styrketrening er positivt for treningsutbyttet. I dette skrivet gir vi deg informasjon om målene med prosjektet og hva deltakelse vil innebære for deg.

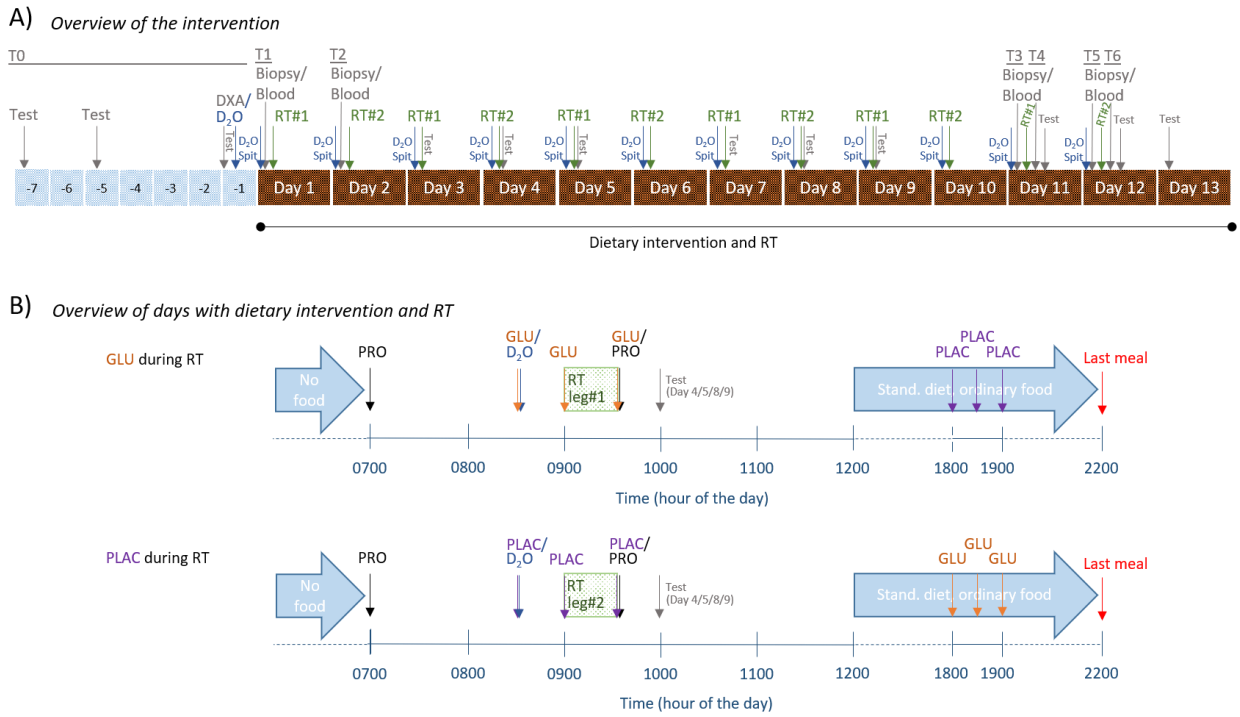
Bakgrunn. Styrketrening har en rekke positive effekter på menneskekroppens funksjoner og er et av de fremste virkemidlene for å fremme folkehelse. Ikke nok med at det forebygger livsstilssykdommer som for eksempel hjerte-karsykdom, respiratoriske sykdommer og metabolske sykdommer, det gir også styrke og utholdenhet til å beherske dagliglivets utfordringer. Mange av disse positive effektene er direkte knyttet til muskelvekst, som i sin tur er koordinert av en rekke muskelcellulære hendelser. Dessverre opplever mange av oss begrenset muskelvekst etter en periode med styrketrening (og dermed ei heller den foreskrevne helseeffekten). Det er derfor av stor interesse å finne nye måter å trene på som kan tilrettelegge for muskelvekst for alle. Dette må nødvendigvis involvere tiltak som kan endre på hendelsesforløpet i muskelcellene. En mulig strategi kan være nye kombinasjoner av trening og næringsinntak. Vi vet allerede at proteininntak i forbindelse med trening er et effektivt (og ofte nødvendig) tiltak for å oppnå muskelvekst. I denne studien ønsker vi å undersøke om dette også gjelder inntak av karbohydrater.

Formål. Denne studien skal primært undersøke effekten av fem styrketreningsøkter med og uten inntak av karbohydrat (heretter kalt hhv glukose/GLU og placebo/PLAC) på muskelcellers responser på

styrketrening. Studien skal gjennomføres som et randomisert dobbeltblindet kryssforsøk (se Figur 1 for oversikt over studien), hvori samtlige deltaker skal gjennomføre styrketrening med inntak av både GLU og PLAC: trening med inntak av GLU skal gjennomføres annenhver dag gjennom treningsperioden (på den ene foten), mens trening med inntak av PLAC skal gjennomføres på alternerende dager (på den andre foten). På alle treningsdager skal du også innta proteinsupplement før og etter trening (for å sikre optimale betingelser for treningstilpasninger i kroppen, i henhold til gjeldende anbefalinger). Halvparten av deltakerne starter treningsperioden med GLU, mens den andre halvparten starter treningsperioden med PLAC. Det vil være tilfeldig hvilket tilskudd du skal starte på. Alle treningsøkter vil bli ledet av en treningsveileder. Hverken du eller treningsveilederen vil vite hvilke dager du inntar GLU og hvilke dager du inntar PLAC.

I forkant av og underveis i treningsperioden skal vi ta en rekke prøver og gjennomføre en rekke tester, inklusive muskel-, spytt- og blodprøver, kroppsmassemåling og styrketester. Disse målingene skal gjøre det mulig å undersøke effekter av trening med og uten karbohydratinntak på muskelbiologiske trekk (som for eksempel cellers form og utseende, genuttrykk, og proteinforekomst og -funksjon), blodets sammensetning (som for eksempel hormoner, glukose og betennelsesprosesser), muskelstyrke og muskelmasse. Underveis i treningsperioden skal du også

2



Figur 1. A) Oversikt over treningsperioden. B) oversikt over treningsperioder med og uten karbohydratinntak (glukose/GLU vs. placebo/PLAC). Test, maksimale styrketester; RT#1, styrketrening bein #1; RT#2, styrketrening bein #2; D_2O, deuterium/tungtvann; DXA, kroppsmassemåling; biopsy, muskelprøvetaking; T0-T6, tidspunkter for gjennomføring av tester/prøvetaking.

drikke tungtvann (litt hver dag; deuterium, D_2O). Dette skal gjøre det mulig å måle nyproduksjon av ulike molekyler i muskelprøvene dine gjennom treningsperioden, som for eksempel protein- og RNA-produksjon.

Alle aktiviteter som krever oppmøte i laboratoriet vil bli gjennomført under tilsyn/veiledning. Næringssupplementene som inntas i prosjektperioden vil bli delt ut av testpersonell. Gjennomføring av studien vil involvere både master- og bachelorstudenter. Disse skal skrive oppgaver basert på resultatene. *Detaljert informasjon om studiens innhold og tidsforløp vil bli gitt i informasjonsmøte. Der vil det også være mulig å stille spørsmål*. For ytterligere informasjon, ta kontakt med prosjektansvarlig professor Stian Ellefsen.

9.2 Hva innebærer PROSJEKTET for deg?

For oversikt over aktivitetene i studien, se Figur 1.

Forberedelser. Tre dager før første oppmøte på laboratoriet (Dag -7, Figur 1) skal du gjennomføre en siste styrketreningsøkt før prosjektet settes i gang. Etter dette skal du ikke gjennomføre trening eller testing utover prosjektprotokollen (så lenge prosjektet varer).

Gjennomføring. Studien vil innebære 16 fysiske oppmøtedager på testlaboratoriet ved Høgskolen i Innlandet, campus Lillehammer (Figur 1). Forut for oppstart av treningsperioden skal du møte 3 ganger for gjennomføring av fysiske tester (maksimal muskelstyrke), kroppsmassemåling (Dual-energy X-ray absorptiometry, DXA) og inntak av tungtvann (moderat dose). Gjennom den 13 dager lange treningsperioden skal du møte daglig for gjennomføring av styrketrening (med inntak av GLU eller PLAC), inntak av tungtvann (små doser), og for gjennomføring av eventuelle fysiske tester (dag 3-5, dag 7-9 og 11-13) og muskel- og blodprøvetaking (dag 1-2 og dag 11-12). Det vil kun være aktuelt med ett oppmøte per dag, så sant dette lar seg gjøre for deg. Tidsomfanget vil variere fra dag til dag, nedad til 1 time på Dag 3, 6, 7 og 10, og oppad til ~5 timer på Dag 11 og 12. Treningsprogrammet skal gjennomføres som 3 sett med styrketrening i øvelsene kneekstensjon og beinpress, med 10 repetisjoner maksimum i hvert sett.

Under styrketreningsperioden skal du følge et bestemt kostholdsprogram. To timer forut for hver styrketreningsøkt skal du innta proteinshake (0.15 liter/25 g protein; Whey Protein Isolate, Proteinfabrikken): dvs proteinshake inntas kl 0700 dersom du skal trene kl 0900 (som eksemplifisert i Figur 1). Dette kan gjøres hjemme. Etter hver treningsøkt skal du gjenta inntak av proteinshake. 30 minutter forut for hver styrketreningsøkt skal du innta GLU eller PLAC (0.3 liter, Fun Light m/bringebærsmak tilsatt glukose eller kunstig søtningsstoff) og tungtvann. Dette gjøres på laboratoriet. Inntak av GLU eller PLAC gjentas to ganger i forbindelse med treningsøkten: umiddelbart før trening og umiddelbart etter trening. I tidsrommet mellom kl 2200 og kl 1200 skal du ikke innta annen mat og drikke enn proteinshake / GLU / PLAC / tungtvann (f.o.m. Dag -1 t.o.m. Dag 12). NB! Det angitte

tidsrommet er kun eksakt dersom din treningsøkt starter kl 0900. Det forskyves proporsjonalt med eventuelle justeringer av treningstidspunkt. I tidsrommet mellom kl 1200 og kl 2200 skal du innta et selvvalgt kosthold, med ernæringsmessig balansert sammensetning. Du vil få bistand til å utarbeide en balansert kostholdsplan. På parvise dager (eksempelvis Dag 1&2, Dag 3&4, etc) skal du ha identisk kosthold. Dette for å hindre at forskjeller i kosthold påvirker effektene av GLU og PLAC. Kosthold registrerer du selv ved hjelp av MyFitnessPal eller lignende applikasjoner. Du vil få veiledning i dette. I tidsrommet mellom kl 1800 and 1900 skal du gjenta inntak av GLU eller PLAC (3 doser; én dose per 20 min).

Punktvis oppstilling av aktiviteter/tester/prøvetaking (og dermed også opplysninger som vil bli lagret om deg):

- Daglig inntak av proteinshake (2 doser per dag): 2 timer forut for treningsøkt og umiddelbart etter avsluttet treningsøkt
- Daglig inntak av Fun Light med eller uten tilsatt glukose (GLU eller PLAC; 6 doser per dag): 3 doser i forbindelse med trening og 3 doser på kveldstid
- Daglig inntak av tungtvann (Dag -1, 5.25 ml · kg lean mass^{-1}; Dag 1-12, 0.53 ml · kg lean mass^{-1}). Inntas for å muliggjøre måling av nyproduksjon av byggemateriale i muskelceller (proteiner/RNA). Det er ingen kjente helsekonsekvenser knyttet til inntak av tungtvann i de doser som anvendes i studien. Lett svimmelhet kan forkomme. For å unngå svimmelhet, vil inntak av den første dosen (Dag -1; større enn inntak på øvrige dager) fordeles over to timer. Du vil få oppfølging av testpersonalet i perioden hvor svimmelhet kan inntreffe.
- Daglig gjennomføring av unilateral tung styrketrening i kneekstensjon og beinpress (3 x 10RM i hver øvelse). De to beina trenes på alternerende dager
- Måling av kroppssammensetning ved DXA. Denne testen gjennomføres forut for treningsperioden (Dag -1; fastende)
- Maksimale styrketester i beinpress og kneekstensjon før og etter treningsperioden (Dag -7, -4, 11 og 12)
- Statisk styrke i kneekstensjon (MVC) og isokinetiske tester før, underveis og etter treningsperioden (Dag -7, -4, 3, 4, 5, 7, 8, 9, 11, 12 og 13)
- Spyttprøver gjennom hele treningsperioden (Dag -1 t.o.m. 12; for å måle innhold av tungtvann i kroppen)
- Blodprøver på Dag 1, 2, 11 og 12 (fastende alle dager + forut for og i etterkant av treningsøkt på dag 11 og 12)
- Muskelvevsprøver på dag 1, 2, 11 og 12 (fastende alle dager; fire prøver per bein). Disse vevsprøvene tas med den skånsomme mikrobiopsimetoden. Noen synes vevsprøvetaking er ubehagelig. Man vil typisk bli litt støl i muskelen 1-2 dager i etterkant. I svært få tilfeller vil biopsitaking kunne føre til at følelsen i huden forsvinner for en lengre periode, eller gi tydelig arrdannelse. Biopsitaking er også forbundet med en viss infeksjonsfare. Risikoen for disse komplikasjonene er svært liten ved bruk av prosedyrene som benyttes i dette prosjektet. Du vil få klare instrukser om hvordan du skal behandle såret i etterkant av prøvetagningen.

9.3 Mulige fordeler og ulemper

Du vil lære mye om egen kropp og vil få ny kunnskap om styrketrening og effekter av styrketrening. Positive effekter av styrketrening inkluderer økt muskelstyrke og -mengde, bedre helse og sannsynligvis også velvære. Du vil få tilgang til tester som ikke normalt har tilgang til. Styrketrening vil kunne føre til skader. Risikoen for dette er liten i dette prosjektet, især fordi du vil få tett oppfølging

av treningskyndig personell under alle treningsøkter/tester. Det er ingen kjente helsekonsekvenser knyttet til inntak av tungtvann i de doser som anvendes i studien. Lett svimmelhet kan forkomme. For å unngå svimmelhet, vil inntak av den første (og største) dosen fordeles over to timer. Du vil få oppfølging av testpersonalet i perioden hvor svimmelhet kan inntreffe. Noen synes muskelbiopsier og blodprøver er ubehagelig. I etterkant av muskelbiopsier vil du typisk være støl i muskelen i 1-2 dager. I svært få tilfeller vil biopsitaking kunne føre til at følelsen i huden forsvinner for en lengre periode, eller gi tydelig arrdannelse. Biopsitaking er også forbundet med en viss infeksjonsfare. Risikoen for disse komplikasjonene er svært små ved bruk av prosedyrene som benyttes i dette prosjektet. Du vil få klare instrukser om hvordan du skal behandle såret i etterkant av prøvetagningen.

9.4 Frivillig deltakelse og mulighet for å trekke ditt 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. Det vil ikke ha noen negative konsekvenser for deg hvis du ikke vil delta eller senere velger å trekke deg. Dersom du trekker tilbake samtykket, vil det ikke forskes videre på dine helseopplysninger og ditt biologiske materiale. Du kan også kreve at dine opplysninger i prosjektet slettes eller utleveres innen 30 dager, og at det biologiske materialet destrueres. Adgangen til å kreve destruksjon, sletting eller utlevering gjelder ikke dersom materialet eller opplysningene er anonymisert. Denne adgangen kan også begrenses dersom opplysningene er inngått i utførte analyser, eller dersom materialet er bearbeidet og inngår i et annet biologisk produkt

Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte prosjektleder (se kontaktinformasjon på siste side).

9.5 Hva skjer med OPPLYSNINGENE om deg?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet under formålet med prosjektet, og planlegges brukt til 31.12.2023. I etterkant av dette vil biologisk material (og tilstøtende opplysninger) bli overført til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» (REK-ID: 2013/2041). Eventuelle utvidelser i bruk og oppbevaringstid kan kun skje etter godkjenning fra REK og andre relevante myndigheter. 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. Du kan klage på behandlingen av dine opplysninger til Datatilsynet og institusjonen sitt personvernombud.

Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger (=kodede opplysninger). En kode knytter deg til dine opplysninger gjennom en

navneliste. Det er kun prosjektleder Stina Ellefsen og Postdoktor Håvard Hamarsland som har tilgang til denne listen.

Opplysningene om deg vil bli oppbevart i fem år etter avslutning av den generelle biobanken (31.12.2038) prosjektslutt av kontrollhensyn.

9.6 deling av OPPLYSNINGER og overføring til UTLANdet

Ved å delta i prosjektet, samtykker du også til at kodet muskelbiopsimaterial kan overføres til utlandet (Danmark) som ledd i forskningssamarbeid og publisering. Koden som knytter deg til dine personidentifiserbare opplysninger vil ikke bli utlevert. Muskelbiopsimaterialet vil bli returnert til Høgskolen i Innlandet etter avsluttede analyser.

9.7 Hva skjer med prøver som blir tatt av deg?

Prøvene som tas av deg skal oppbevares i en forskningsbiobank tilknyttet prosjektet. I etterkant av prosjektslutt vil de bli overført til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» (REK-ID: 2013/2041), 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 og utseende, 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. 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. 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.

9.8 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 til 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).

9.9 Forsikring

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

9.10 Godkjenninger

Regional komité for medisinsk og helsefaglig forskningsetikk har gjort en forskningsetisk vurdering og godkjent prosjektet (søknadsid: 153628).

Høgskolen i Innlandet og prosjektleder Stian Ellefsen er ansvarlig for personvernet i prosjektet.

Vi behandler opplysningene basert på ditt samtykke.

9.11 kONTAKTOPPLYSNINGER

Dersom du har spørsmål til prosjektet eller ønsker å trekke deg fra deltakelse, kan du kontakte:

Prosjektleder: professor Stian Ellefsen (tlf: 61288103, epost: stian.ellefsen@inn.no), *eller*

Prosjektmedarbeider: postdoktor Håvard Hamarsland (tlf: 93445916, epost: havard.hamarsland@inn.no)

Dersom du har spørsmål om personvernet i prosjektet, kan du kontakte personvernombudet ved institusjonen: anne.lofthus@inn.no

Datatilsynets e-postadresse er personvernombudet@nsd.no

JEG SAMTYKKER TIL Å DELTA I PROSJEKTET OG TIL AT MINE PERSONOPPLYSNINGER OG MITT BIOLOGISKE MATERIALE BRUKES SLIK DET ER BESKREVET

Sted og dato	Deltakers signatur
	Deltakers navn med trykte bokstaver