Ingestion of liquid glucose during exercise prevents protein degradation in vastus lateralis on healthy trained adults.

### Title

Ingestion of liquid glucose during exercise prevents protein degradation in vastus lateralis on healthy trained adults.

### Forord

“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

Strength is performed in many ways, from beauty competitions, athlete’s performance and rehabilitations. 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 university of Innlandet 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 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 motivating we are in learning and teaching.

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. Thank you for being there for each other. 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 on physical exercise in the human body.

Just like any other exercise and training, this pandemic has been feeling like going to steps forward, and one step behind. Never knowing if life will get back to normal, but a year in training is a short time of progress, and even though time feels like standing still, the workers in the field of science has done a lot of remarkable and unbelievable progress in a very short time.

### Abstract

Purpose: The purpose of this study was to see the effects of ingesting fluid glucose during resistance exercise on protein degradation after 6 training sessions. Ingestion of glucose surrounding a training session has been seen to blunt protein degradation when co-ingested with proteins. This study was to determine the effect on murf (and maf) when co-ingestion of glucose during strength exercise.

Method: thirteen healthy trained individuals was included in this within subject study and randomized in to two groups, (PLAC or GLU). The subjects concluded three weeks intervention consistent of adaptions training week 1 (3 sessions) with 1 rm tests, body composition test. In the two weeks of intervention, resistance exercise performed where each leg trained on alterned days ingestion either glucose or placebo, isokinetic and isometric test and biopsies.

Results:

Conclusion: After 6 training session with ingestion of glucose did blunt murf, but with no difference in training volume.

### Content

### 1. Theory

Resistance training is performed with the purpose to increase performance in sporting competitions or to improve health or 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,well as a mean to achieve health improvements through blood pressure, improve glucose tolerance, insulin sensitivity (Folland, Williams, 2007; Kraemer, Raramess, French, 2002). Much of the beneficial effects of resistance training 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 syntheses which lead to muscle loss (Biolo, et at. 1995; Kumar, et al. 2009; Tipton, Wolfe, 2001). This process of breakdown and synthesis of protein structures is determined by protein turnover. Protein turnover is affected by catabolic and anabolic stimuli which respectively controls the rate of breakdown and synthesis. Resistance exercise elevates both mechanism of protein synthesis and protein breakdown and it is the fractional rate between those two that determine an increase or reduction in muscle mass (Philips, et al. 1997; 2002). Ingestion of protein and carbohydrate increases the rate of syntheses and blunt protein breakdown (Biolo, et al. 1995). Co-ingestion of carbohydrates and amino acids surrounding resistance training can blunt protein breakdown (Beelan, et al, 2008; Roy, et al. 1997). MuRF1 is an E3 ligase in the ubiquitin proteasome pathway that is myofibrillar specific and high levels of insulin can blunt E3 ligases through phosphorylation by Akt/PKB.

## Hypertrophy and protein turnover

On a cellular level, actin and myosin are the primary proteins that increases in size and results in muscle hypertrophy (Levers, et al. 2015). To induce this response there are two major signaling pathways, insulin-like growth factor 1 (IGF-1), which upregulate 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 upregulation of protein breakdown (Peris-Moreno, et al. 2020; Schiaffino, et al. 2013). Protein turnover is an important factor that plays a role in maximizing adaptions to training and muscular growth can only occur through a positive net balance of amino acids (Biolo, et at. 1995; Kumar, et al. 2009; Tipton, Wolfe, 2001). Studies shows that protein synthesis is elevated up to 48 hours as an effect of resistance exercise, while protein breakdown is only elevated for up to 24 hours before returning to baseline values (Philips et al. 1997;2002; Yang, et al. 2006) Proteins supplements is often used to provide sufficient amino acids to ensure this positive net balance (Macnaughton, et al. 2014; Tipton, et al. 1999), but chronic inactivity leads to elevated markers of protein breakdown, even with a normal dietary intake (Ogawa, et al. 2006). This fact demonstrates that nutrition alone cannot improve protein turnover to a positive state. This way, resistance exercise may contribute to protein turnover and nutrition status will determine if the net balance is in favor for synthesis or breakdown (Macnaughton, et al. 2014; Philips, et al. 1997). There has been question if inhibiting protein breakdown during resistance exercise can further increase the net gain of proteins. This might be due to increased insulin levels which acutely activates IGF-1 pathway, upregulate Akt and mTOR, which in turn upregulates protein synthesis but also downregulates pathways for muscle protein breakdown (Peris-Moreno, et al. 2020; Yoon, 2017).

## Protein degradation and ubiquitination

Proteolysis is a fundamental biological process providing amino acids for synthesis to vital organs, tissue, repairing and remodeling (Alberts, et al. 2019; Pasiakos, Carbone, 2014). Proteases are enzymes that control proteolysis, which hydrolyze peptide bonds, splitting them into smaller chains and then into individual amino acids (Alberts, et al. 2019; McArdle, Katch, Katch, 2015). Proteasomes are protein complexes that degrades proteins (Alberts, et al. 2019). Proteasomes resides in the cytosol and nucleus within the cell, in a cylindric 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 degrades 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 tagged with ubiquitin, the ubiquitinated protein enters the proteasome and degrades into smaller peptide units (McArdle, Katch, Katch, 2015). 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). It is 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 is ligases associate with skeletal muscle atrophy and is believed to be the main regulator for 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 insulin signalling Akt, concluding that MuRF1 is highly important for 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 downregulates 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). This is an important note, when studies have found 1:1 rna-protein.To establish the participants changes in MuRF1 expression in this study, western blotting for protein content in vastus lateralis has been used.

## Analysis of protein breakdown.

There are several methods used to measure protein breakdown in the human body. Arteriovenous balance (AV-balance) requires trained personnel and equipment that is not easily obtainable, and do not measure protein breakdown per se., but can measure protein breakdown, synthesis, and net balance by blood flow across the muscle (Pasiakos, Carbon, 2014). To measure protein breakdown urinary 3-methylhistidine (3-MH) is often used in combination or without AV-balance (Pasiakos, Carbone, 2014). It is a valid method to measure muscle breakdown as 3-MH are residues derived from actin and myosin degradation and requires less equipment than AV-balance (Pasiakos, Carbone, 2014). 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, when 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 analyze 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 arteriovenous (AV) blood flow to gather evidence of protein breakdown (Tipton et al., 2018). While those method cannot represent cumulative changes in the muscle, they will give a snapshot of the intramuscular milieu at a specific point in time (Pasiakos, Carbone, 2014). When using a within-participants study design with western blotting for protein markers, the design will remove any biological differences and hopefully give strong evidence on between conditions (Hammarström et al., 2019). This study also found that changes in biological markers after only two weeks were indicative of future training response (Hammarström et al., 2019).

## Supplement

Ingestion of amino acids through a protein rich meal or supplement stimulates muscle protein synthesis (Miller, 2007) and ingesting 40 grams (g) of essential amino acids after resistance exercise is sufficient to accelerate protein synthesis (Macnaughton, et al. 2014). Carbohydrates improves the protein net balance, mainly through reduction of protein breakdown when not ingested with amino acids (Børsheim, et al, 2004). Ingesting 100 grams of carbohydrates after resistance training can improve net protein balance but did not reach positive net balance for synthesis (Børsheim, et al, 2004). With a normal nutrition intake evening before resistance exercise, ingesting carbohydrate and protein supplements during resistance exercise improves whole body protein synthesis (Beelen, et al. 2010). The timing of ingesting carbohydrates may be an important factor to minimize markers for protein breakdown. When co-ingesting supplements after resistance exercise, markers for protein breakdown are only modestly reduced (Glynn, et al. 2010) but ingesting a mixture of amino acids and carbohydrates after resistance exercise decreases the rate of protein breakdown (Beelen, et al. 2008; Borsheim, et al. 2004; Kume, et al. 2020; Roy, et al. 1997). A study examining supplements right before and after training has shown to decrease markers for protein breakdown in urinary samples stating that timing of supplements is important to maximize protein turnover by increasing protein net balance with amino acids and minimizing protein breakdown (Kume, et al. 2020). 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% in (Bird, S. P., Tarpenning, K. M., Marino, F. E., 2006). Therefor this thesis will examine if timing on glucose intake during resistance or in the evening reduces MuRF1 protein content after 5 training sessions. The hypothesis of this thesis is therefore: There is a significant reduction on protein markers of MuRF1 when ingesting glucose during resistance exercise.

## Introduction

## Method

## 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. Participants 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 different 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 not related to the study before or during intervention (n=2) 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: Participants descriptive

|  |  |
| --- | --- |
| variable | stat |
| 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 | 74.9 (7.2) |

Table 1B: Leg descriptive

|  |  |  |
| --- | --- | --- |
| variable | left | right |
| fatmass | 3.3 (1.4) | 3.4 (1.4) |
| leanmass | 9.7 (1.4) | 9.9 (1.4) |
| totalmasskg | 13.7 (1.5) | 14 (1.4) |

## Study overview

In this within participant interventional study, the participants completed a 3-week intervention. The first week was the familiarization phase, containing 1RM tests (Day -7, -5 and -1), body mass index, and isokinetic and isometric tests (Day -1). Week 2 and week 3 was the interventional phase containing blood samples and biopsies (T1-T2, T3 -T4), dietary program (Day 1-12), resistance training (Day 1 – 12), isokinetic and isometric tests (Day -1, T1-T2, Day 4-5, Day 8-9, T3-T4 and day 13), with ingestion of glucose (GLU), or placebo (PLAC) during resistance training. Participants performed in both conditions and their legs were randomly assigned to either glucose (GLU) or placebo (PLA). The participants performed the same training protocol and dietary plan each day, where only ingestion of glucose or placebo during training differed, for participant comparison and to remove biological diversity. The participants started the training protocol on either the dominant or non-dominant leg alternating days, with day 1 (T1) involved training of the first leg (RT#1) and Day 2 (T2) on the second leg (RT#2), where Day 3 involved training the first leg (RT#1) and so on. A total of six training sessions was performed for each leg. Six training sessions was performed for each leg. Before 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 H2O to ensure muscle growth . After the subject was placed in a group, each leg started the intervention randomly on either the dominant or non-dominant leg.

Figur 1: Intervention

## Assessments of body composition

Body mass composition was measured using dual-energy x-ray absorptiometry (DXA, Lunar Prodigy, GE, Heathcare, Oslo, Norway) last day in the familiarization phase (figur 1). The participants weight and height were registered prior to the measurements. The participants were asked to lay in the middle of the bench with minimal clothing. The participants arms and legs were placed inside marked position on the bench with hands and feet pointing upwards, towards the ceiling. They were asked to lay still throughout the measurements which took about 10 minutes. DXA was used to register data on total weight, lean body mass, and fat mass and mean(sd) was analyzed on total group

## 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, the only differed being the timing of ingestion (during training session or in the evening). Det drinks was mixed with Fun light juice (Orkla, Oslo, Norway). During training, the subject 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 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 H2O to ensure muscle growth. The data on group distrubution was conducted day by a colleague not involved in the project, who kept data until all measurements were collected, 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). The participants were asked to consume same amount of macro nutrition on paired days (T1-T2, day 3 – 4, and so on) to ensure non-biased nutrition status for the legs. The nutrition supplements was calcualted in the nutrition data for each participants after data colection. A blinded randomized drink taste test was done on Day 13 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 their own time, and take notice of what they thought the cups contained.

## Strength assessment

Maximum strength test was assessed as one repetitions maximum (1RM) on day -7 and -5. 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 on -7 the participants were asked about their expected 1RM and used as the base for test progression. The test on -5 was to ensure the right 1RM was found, based on the test conducted on -7. The subject got 5 tries and 1RM was set on the set before failure trial. The 1RM test was also the base for training intensity during the intervention. Unilateral isokinetic knee extension torque at 60 degres/second and 240 degrees/second, and isometric knee extension force tests were performed at Day -1 to set baseline, Day 4-5, 8-9, T3, T4, and Day 13 using Humac Norm dynamometer (CSMi, Stoughton, Massachusetts, USA). Isokinteic and isometric test on test day T3, T4 and day 13, was conducted at 4 timepoints; before exercise (baseline), 30 minutes after, 2 hours after and 24 hours after as restitution effects after training intervention to see any different between conditions. The participants were asked to sit on the chair which was set so the knee joint was parallel with the rotary axis of the dynamometer. The thigh and the ankle were strapped to the seat. Range of motion was set to 90 degrees of flexion. The participants were instructed to push as hard as they could in concentric phase, and to rest in the eccentric phase. The isometric test lasted for 10 seconds, or until peak failure. Three repetitions per test session and angular velocity were performed in isokinetic tests and one repetition in isometric test. The mean peak tourque was used for measurments. All data about participant position in the chair was recorded and used throughout all tests.

## Training protocol

Each training session consisted of 10 minutes of warm up on 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 was three series of 10RM with 2 minutes rest between. The participants registered their session score using a 10-point scale, fifteen minutes after the training session, where 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 very heavy. Each participant had trained personnel under each training session during the whole intervention. The trained personnel were following the same participants throughout the intervention and there were no music playing to remove any potential confounding variables (Haplerin, Pyne, Martin, 2015).

##Biopsies and blood samples

Muscle biopsies was sampled from m. vastus lateralis at 4 timepoints during the intervention: 1) before training session from the performing leg on Day 1 (T1, pre RT#1), 2) before training session on the performing leg on Day 2 (T2, pre RT#2), 3) before the sixth training session on the performing leg on Day 11 (T3, post RT#1), and 4) before the sixth training session on the performing leg on Day 12 (T4, RT#2). All biopsies were sampled in an overnight-sated fast at the same time of the day for the individual subject, depending on the daily timing of dietary intervention. 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 12–14-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 T1, and at several timepoint on T3 and T4. The samples from T1 and the first at T3 was set as baseline measures. During T3 and T4 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 on glucose in blood during training session.

## Muscle tissue analyses

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 in BB at 1 min, @ 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 @ 12000g, 15 min @ 4grader Celsius for phase separation. 450ul of the aqueous phase was placed in a new tube for RNA extraction. The rest of the aqueous phase was taken out, discarded, and the remaining content in the tubes were stored at -20 Celsius 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 @12000g, for 5 minutes. The upper phase was removed and 700 ul of EtOH (96%) was added. The tubes were vortexed vigorously and centrifuged @ 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 ddH2O 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 @RT, or until all pellets was dissolved. After incubation, the samples were centrifuged @ 10000 g @RT 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 ddH2O, and both was stored at -20 Celsius. 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.

##SDS-page and Western blottning

The samples were normalized to 1.5ug/ul protein and 20ug protein was loaded in each well on precast gels. 5 ul of ladder (name) 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 PFVS membrane 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 4grader on ice bath. For protein visualization, MemCode (Fabric name here) was used. The membranes were rinsed with ddH20 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 color. After rinsing distain with ddH20 the membranes were photographed in G:BOX (fabric name here) 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.

## 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) (MuRF, 1:100: diluted MuRF antibody in 1x TBS-T, 1 liter: 100mlX TBS, 1ml Tween and added ddH2O to final volume 1000 ml) overnight @4 Celsius 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. The membrane was dried @RT until completely dried and stored in -20 freezer.

## Statistic analysis

Fullstendig datasett og skript kan lastes ned her; http…………. It is of interest to read read.me file for an overview over all folders. All data was imported and analysed in R studio (R studio…) All descriptive data is shown in mean and standard deviation (mean (sd)). To explore the difference between conditions, linear mixed model was used (LMM). LMM take accountability for within subject variation with logmetric 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 apla=0.05.

## Results

## nutrition status

All participants except for one delivered data on their daily nutrition intake. There was no difference between condition on any macronutrient on any paired days or between conditions except for total calorie intake and protein/kg, which differed each paired days, but not differed between conditions. The participant could not detect different taste in blind drink test.

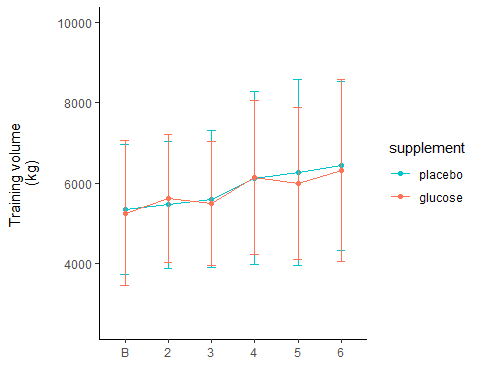
Table 2: Nutrition intake

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| timepoint | group | calories | carbohydrates | fat | protein | proprkg |
| 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)

## Training Volume

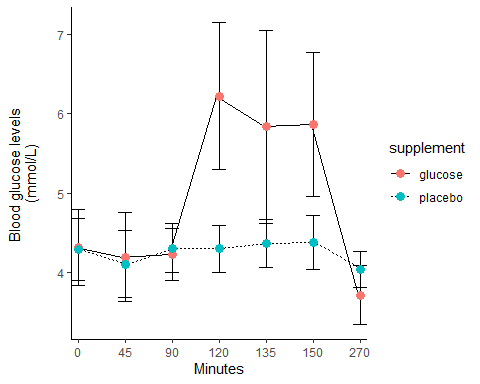
There was no different on total training volume between condition on baseline values (PLA: 5351kg(1615), GLU: 5262kg(1799)) Total training volume on day 6 was 6438(2092)kg in PLA and 6319(2256)kg in GLU. The training intervention led to a 19% [0.1, 0.25] increase in PLA and 18% [0.08, 0.24] increase in GLU in total volume after 6 training session compared with baseline values. There was a time effect on day 4-6 on total volume with no differences between conditions in relative increase in total training volume from baseline to any subsequent time-point (Figure 2)

Figure 2: Total volume change  Figure 2: The total training volume between conditions shown in kg.

## Training measurments

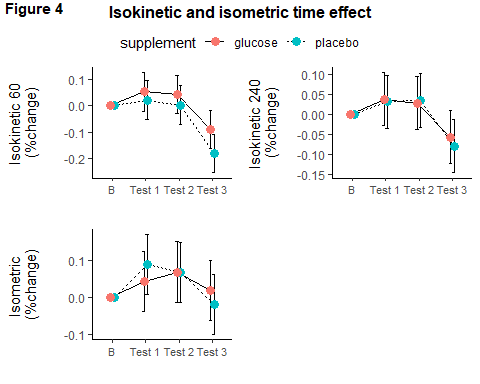
Measurements of glucose in blood showed a clear difference on timepoints 120 min, 135 min, 150 min and 270 min after protein intake as shown in figure 3. GLU had 35% [0.31, 0.43], 22% [0.19, 0.31], 25% [0.22, 0.34] higher values, and decreased -7% [-0.18, -0.06] compared to PLA.

Figure 3: Blood glucose

 Figure 3: Blood glucose levels shown as mmol/l minutes after protein intake. 90 minutes is first GLU/PLA drink intake.

There was no difference between condition on isometric test 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,v0.16], GLU 4% [-0.03, 0.12]. there was no time effect in isocinetik 60 or 240 in or bewteen condition during the intervention. There was a decreased time effect on isokinetic 60 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 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 4C).

Figure 4

 Figur 4 show mean %change on each test taken throughout the intervension where A is for isokinetic 60, B isokinetic 240 and C isometric tests.

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

Figure 5

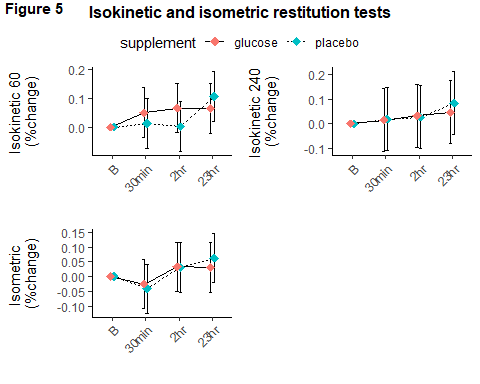


Figure 5 show mean %change on each test taken 30min, 2 hours and 23 hours after training session to measure restitution effect between groups.A is for isokinetic 60, B isokinetic 240 and C isometric tests.

## MuRF1

There was a significant difference between condition i 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.

Figrue 6

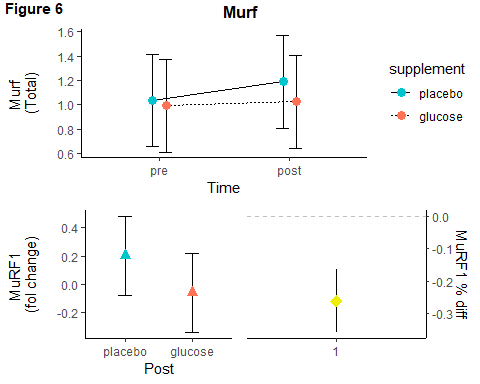


Figure 6: A illustrates the totalt MuRF1 conten in each group from pre to post. B illustrtes fold change in each group compared to PLA in pre. C illustrates the relative % differenec between groups in post.

## Discussion:

The goal of this study was to examine the effect of glucose intake during resistance exercise on MuRF1 protein content in vastus lateralis after five training sessions. The main finding of accumulations of MuRF1 protein content was 26% lower in the GLU group after five training sessions. Studies on glucose uptake in active skeletal muscle increases more than 5 times or ~40% after intake, which is consistent with our observation and the participants trained with high blood glucose (Bird et al. 2006; Durham, et al 2004) which should give optimal conditions for blunt protein expression on MuRF1 (O’Neill, et al. 2018; Peris-moreno et al. 2020). These observation shows an effect of timing on intake of 90 grams of glucose influences MuRF1 in contrast to glucose intake during the evening. Our observation on MuRF1 is consistent with other studies in the field, with lower levels of MuRF1 concentration after glucose intake during resistance training (Kume et al. 2020). Our observation finds that timing of glucose intake is important to reduce muscle protein break down, when ingesting glucose after training session give only modestly changes (Glynn et al.). An interesting observation from Durham et al (2004) found no changes in muscle protein breakdown when measurement was taken right after training. This study measured the AV-balance ~45 minutes after training, which might imply the important for timing of measurements taking, when our thesis took biopsy samples the day after training. As stated before, AV-balance does not measure protein expression on protein breakdown pre se. but, gives an indicator for protein turnover right after training (Pasiakos, Carbon 2014) It is tempting to conclude that ingesting glucose during resistance training attenuates protein breakdown, when studies measuring protein turnover finds these observation (Beelen et al. 2008; Borsheim et al. 2004; Kume et al. 2020; Roy et al. 1997). This thesis did not find any reduction in mRNA expression of MuRF1, which is a surprise, but might be due to the participant training status when observation on mRNA expression of MuRF1 is less expressed in trained (Churchley et al 2007), than in untrained participants (Yang, et al).

The intervention led to a 19% (PLA) and 18% (GLU) increase on total volume, with no significant difference between conditions. Studies and meta-analysis favor high loads (60-80%RM) compared to low loads (30%RM) when it comes to training protocols on maximizing muscular strength (Fink, et al, 2016; Lopez et al. 2020; Schoenfeld, et al 2015;2017). There was a significant time effect on isometric test on test 2 (day 4 and 5) (PLA 8% [0.007, 0.16], GLU 4% [-0.03, 0.12]) with no difference between condition, which imply strength adaption for both condition from 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 condition. The tests indicates that glucose intake does not affect peak force compared with PLA. We did not observe any time effect on isok.60 or isok 240 throughout the intervention. We did observe a mean higher peak torque value on isok.60 for GLU on test 3, implying that glucose intake during resistance exercise could affect muscle fatigue on 60grad in fast twitch muscle, but after prolonged restitution there was no difference between condition. We did not observe any difference between condition on isometric or isokinetic 240 test, implying that glucose intake after intervention does not affect strength adaption compared to placebo. Multiple sets has shown to lead to greater increases in strength which correlates with increases in mass (Hammarström, et al 2020). This thesis cannot say anything about measurements of muscle mass, and our observation on strength test might imply not a difference between condition on muscle mass, setting the question if blunting MuRF1 affects muscle adaption to training. Studies has observed changes in biological markers for strength adaption after 2 weeks of resistance exercise, which predicted strength adaptions in prolonged exercise (Hammarström, et al 2020). Therefore it would be interesting to study if glucose timing has different effect on increasing muscle mass with in or if prolonged training with glucose blunting MuRF1 for a longer period of time.  
In conclusion, our observations finds that glucose supplement during resistance training decreases protein content of MuRF1 in skeletal muscle, and timing of glucose is an important factor to minimize protein breakdown after resistance training.

## Litterature

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