**Forside 1s**

Ord forkortelser

MPB – Muscle protein brakdown

MPS – muscle protein synthesis

Forkortelser:

CHO – Carbohydrates

EAA – essential amino acid

MPS – muscle protein syntheses

MPB – musle protein breakdown

**Sammendrag/abtract <300 ord**

**Teori 2000-3000 ord**

**Resistance exercise**

**Resistance exercises is**

[**https://link.springer.com/article/10.2165/00007256-200838070-00001**](https://link.springer.com/article/10.2165/00007256-200838070-00001)

[**https://link.springer.com/article/10.2165/00007256-200434100-00004**](https://link.springer.com/article/10.2165/00007256-200434100-00004)

[**https://link.springer.com/article/10.2165/00007256-200434100-00004**](https://link.springer.com/article/10.2165/00007256-200434100-00004)

[**https://asset-pdf.scinapse.io/prod/2097198989/2097198989.pdf**](https://asset-pdf.scinapse.io/prod/2097198989/2097198989.pdf)

[**https://www.tandfonline.com/doi/full/10.1080/02640414.2021.1898094?src=**](https://www.tandfonline.com/doi/full/10.1080/02640414.2021.1898094?src=)

**R**

[**https://rkabacoff.github.io/datavis/Models.html#Corrplot**](https://rkabacoff.github.io/datavis/Models.html#Corrplot)

[**https://bookdown.org/kdonovan125/ibis\_data\_analysis\_r4/documenting-your-results-with-r-markdown.html#creating-tables-with-r-markdown**](https://bookdown.org/kdonovan125/ibis_data_analysis_r4/documenting-your-results-with-r-markdown.html#creating-tables-with-r-markdown)

**mixed model**

[**https://m-clark.github.io/mixed-models-with-R/random\_intercepts.html#the-mixed-model**](https://m-clark.github.io/mixed-models-with-R/random_intercepts.html#the-mixed-model)

**The\_Effect\_of\_Different\_Rest\_Intervals\_Between**

[**https://journals.lww.com/nsca-jscr/Fulltext/2008/01000/The\_Effect\_of\_Different\_Rest\_Intervals\_Between.22.aspx**](https://journals.lww.com/nsca-jscr/Fulltext/2008/01000/The_Effect_of_Different_Rest_Intervals_Between.22.aspx)

1.1 Resistance exercise and proteins

Muskel biologiske.

Skrive om sett på andre protein protein også men ikke tatt med i studien

Metabolsk stress, muskelsvelling og muskel skade

High load exercise can lead to muscular fatigue and micro muscle damage.

### 1.1 Proteins

Our knowledge about muscle growth is widely studied and protein supplementations have a lot of positive properties for muscle adaption (Tipton, Ferrando, Phillips, Doule Jr, Wolfe, 1999). Resistance exercise alone can lead to muscle hypertrophy and greater post-exercise MPS and the human body always control the proteins content, and the cells can make new or degrade proteins (Schoenfeld, Ogborn, Krieger, 2017, Levers, K., Vargo, K., 2015, Silverthorn, 2019). Proteins are essential for the human body and can be specialized to function in a specific way, speed up chemical reaction and give signals to the cells (Silverthorn, 2019). Proteins influence the cells mechanical properties and structure, and have the ability to compliance, elastance, strength, flexibility and fluidity (Silverthorn, 2019). When ingested, free amino acids are taken up by the liver to create structural or functional proteins structures (Silverthorn, 2019). It was believed that timing of protein ingestion was crucial for preventing protein breakdown, and to get the most out of nutrition, but when the total amount of free amino acids exceeds the body’s ability for protein syntheses, the excess amino acids are either stores as fat, or used as an energy source (Levers, K., Vargo, K., 2015, Silverthorne, 2019). The purpose of degrading proteins are the mechanism for breaking down proteins (Silverthorn, 2019) The regulation of protein concentration is important for physiological adaption, and muscle protein syntheses (MPS) and muscle protein breakdown (MPB) is affected by each other (Silverthorn, 2019). If the total amount of free amino acids in the human body exceeds MPB, accumulation of proteins accrue and MPB is less needed to make new proteins. If the total amount of free amino acids is less than the rate of MPS, the breakdown of proteins are needed and MPS rate exceeds MPS (Silverthorne, 2019). So how does this happen?

Hypertrophy is the increase of strength and lean mass in skeletal muscle (Levers, K., Vargo, K., 2015). Resistance exercise alone can lead to muscle hypertrophy and impact both MPS and MPB (Levers, K., Vargo, K., 2015). Insulin is important for free amino acids transport to skeletal muscle and can minimizing the breakdown of muscle protein (Levers, K., Vargo, K., 2015). Ingestion of glucose surrounding an exercise session will increase plasma insulin levels and the transport rate of free amino acids, and prevent muscle protein breakdown (Levers, K., Vargo, K., 2015). It is also important to clarify that MPB can only be influenced by carbohydrates when it is ingested with proteins to maintain a positive net protein balance (Levers, K., Vargo, K., 2015). Although ingestion of carbohydrate supplement with protein supplement does not improve MPS further than ingestion of protein supplements alone (Levers, K., Vargo, K., 2015 + flere kider). The main role of carbohydrate surrounding resistance exercise is replenish glycogen stores, prevent MPB and increase insulin levels (Levers, K., Vargo, K., 2015).

Our knowledge about skeletal muscle adaption is widely observed and we do know that protein supplements have a lot of positive properties for muscle adaption (Tipton, Ferrando, Phillips, Doule Jr, Wolfe, 1999). Resistance exercise alone can lead to muscle hypertrophy and greater postexercise MPS (Schoenfeld, Ogborn, Krieger, 2017, Levers, K., Vargo, K., 2015). Proteins are essential for the human body and can be specialized to function in a specific way, speed up chemical reaction and give signals to the cells (Silverthorn, 2019). Proteins influence the cells mechanical properties and structure, have the ability to compliance, elastance, strength, flexibility and fluidity (Silverthorn, 2019). When ingested, free amino acids are taken up by the liver to create structural or functional proteins structures (Silverthorn, 2019). There has been believed that timing of nutrition har been crucial for preventing protein breakdown, especially ingesting proteins right after a resistance exercise, but when the total amount of free amino acids exceeds the bodies ability for protein syntheses, the excess amino acids are either stores as fat, or used as an energy source (Levers, K., Vargo, K., 2015, Silverthorne, 2019).

Hypertrophy is the increase of lean mass (kilde). Resistance exercise can impact both MPS and MPB. Insulin is important for amino acids to incorporation into skeletal muscle as well as minimizing the breakdown of muscle protein. Ingestion of carbohydrate surrounding an exercise session will increase plasma insulin levels, but a increase will also be seen in response to the infusion of free amino acids. Reacent studies has shown a significant insulin response to the ingestion of a carbohydrate, whole protein and free amino acid mixture when compared to only CHO at rest and postexercise in trained athletes. Therefor consumpten of cho and pro surrounding exercise is important cus synthesis. Consuming both carbohydrate and protein before and after resistance traning attunates protein breakdown, and accelerated syntheisis. May help muscle hypertrophy. Primarly role of CHO is replenish glycogen store, prevent muscle protein breakdown and increase insulin levels. Levers, K., Vargo, K., 2015

Muscle hypertrophy and strength correlatets. Myofibrillar hypertrophy is the result of muscle protein balance. Resistance exercise may simulate MPD. Amino acid transport increases after resistance exercise. The goal is to influence net protein balance by the ability to maximize FSR and minimize MPD thus maximizing the rate and amount of muscle hypertrophy. Studie have shown that ingestion of 1g/kg of carbohydrate immediately and 1~hour after resistance exerice significantly decrease myofibrillar protein breakdown, with slight increase of FSR, resulting in a positive protein balance. Although as little as 30g ingestion of carbohydrate is shown the same results (kilde). Coneumption of glucose after resistance exercise increase insulin concentration and result in higher rate of protein transport though blood. (Roy, Tanopolsky, Macdougall, Fawles, Yarasheski, 1997)

Resistance exercise stimulates MPS within 1-4 h postexercise, and can remain elevated for 24-48 h. https://journals.physiology.org/doi/full/10.1152/japplphysiol.90395.2008

The human body always control the proteins content at all times, and the cells can make new or degrade proteins (Alberts, et al. 2019; Silverthorn, 2019). The purpose of degrading proteins is the mechanism for breaking down proteins into amino acids (Alberts, et al. 2019; Silverthorn, 2019) The regulation of protein concentration is important for physiological adaption, and muscle protein syntheses (MPS) and muscle protein breakdown (MPB) is affected by each other (silverthorn, 2019). If the total amount of free amino acids in the human body exceeds MPB, accumulation of proteins accrues and MPB is less needed to make new proteins, and the total amount of free amino acids is less than the rate of MPS, the breakdown of proteins is needed to make new protein complexes and MPS rate exceeds MPS (Silverthorne, 2019). So how does this happen?

Proteins are essential for protein synthesis and are well studied (kilder). What is lesser studies is protein degrading surrounding strength exercise (kilde). There are a few studies on protein degrading and little is known about co-ingestion of nutrition during exercises.

### 2. Mechanisme of protein degrading

### 2.1 Skeletal muscle protein degradation

Proteolysis is the regulation of total protein content in a living organism (Alberts, et al. 2019; MacArdle, Katch, Katch, 2015; Silverthorn, 2019). In science, proteolysis is often overlooked, but is a fundamental biological process that provides amino acids for synthesis to vital organs, tissue, repairing and remodeling (Alberts, et al. 2019; Pasiakos, Carbone, 2014). Proteases is the 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). Proteasome is the protein complex that degrades proteins (Alberts, et al. 2019). Proteasome is in the cytosol and nucleus within the cell, in a cylindric form made from proteases (Alberts, et al. 2019). The ends of the cylinder unfold proteins complexes marked for degradation by ubiquitin, transports them into the inner chamber where proteases cut them into short peptides and shoots them out of either sides of the proteasome (Alberts, et al. 2019). The process of breaking down proteins can take from seconds and minutes, or months and years, depending on where the proteins are contributed (Alberts, et al. 2019; McArdle, Katch Katch, 2015).

Protein net content balance the forholdet between MPS and MPB. Resistance exercise with ingestion of EAA has a greater increase in MPS than resistance training, with or without ingestion of EAA alone.

mTORC1 – mammalian target of rapamycin complex 1 correspond in the increase in MPS after resistance exercise or after ingestion of eaa or cho+eaa. The combination of eating eaa+cho elicit greater mps and signaling through the mTORC1 pathway to either stimulus alone. Additional ingestion of cho does not affet MPS in combination of eaa.

Proteins degrades as fast as--- in skeletal muscle.

290 (Alberts, et al. 2019)

### 2.2 Ubiquination

**UPS**

Skeletal muscle protein breakdown is regulated by primary by four proteolytic systems; autophagy-lysosomal, calcium dependent calpains, the cysteine protease caspase enzyme and ubiquitin-proteasome pathway (UPS) (KILDE). The UPS is the major pathway of selective protein degradation in skeletal muscle (KILDE) Ubiguition function as a marker that target cytosolic and nuclear proteins for rapid protelyse (KILDE). Proteins marked with ubiquitin is attached to the side chain of a lysine residue (kilde). Ubiquitin are then added to form a multiubiquitin chain (kilde). Polybiquinated proteins are then recognized and degraded by a large, multisubunit protease complex called proteasome (kilde). Ubiquitin is released in the process so it can be reused (kilde). Ubiquination is a multistep process, where ubiquitin is activated by being attached to the ubiquitin-activating enzyme E1. The ubiquitin is then transferred to a second enzyme, ubiquitin-conjugating enzyme E2, transfred to the tardet protein and mediated by a third enzyme, ubiquitin ligase (E3), wich is responsible for the selective recognition of apporpirate substrate proteins. In some cases, ubiquitin is transferred from E2 to E3, then the target protein, or directially from E2.

Ubiquitin is a target protein, attaching itself by covalently bonding to an active site of a protein that is to be destroyed (McArdle, Katch, Katch, 2015). When the proteins are tagged, the ubiquinated protein enters the proteasome, degrades it into smaller peptide units, before expelling it with the ubiquitin tag (McArdle, Katch, Katch, 2015). Proteolysis and proteasomes are important for the human body, to get rid of misfolded, misformed, oxidized or denatured proteins. (chapter 33, McArdle, Katch, Katch, 2015)

Ups is ATP dependent. 26s proteasome, is assembled by the association of the 19s regulatory particle with the 20s proteolytic core particle in an atp dependent manner. ATP within a level of lw millimolar range has shown to suppress proteasome pepridase activities in vitro. This study speculates that altering ATP levels in the cell can manipulate proteasome function <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2996470/>

ubiquitin 153, 257, 528, 617, 618F, 634F Myung, Kim, Crews, 2001

<https://jps.biomedcentral.com/articles/10.1186/s12576-020-00768-9#Sec17>

<https://onlinelibrary.wiley.com/doi/epdf/10.1002/med.1009>

Skeletal muscle protein degrading is regulated by primary by the ubiquitin proteasome pathway (UPS) (Myung, Kim, Crews, 2001; Pasiakos, S., M., Carbone, J., W. 2014). The UPS is a target protein and the major pathway of selective protein degradation in skeletal muscle, it is known to break down bulks of protein, but also myosin and actin in skeletal muscle (McArdle, Katch, Katch, 2015; Myung, Kim, Crews, 2001; Wagenmakers, 2012 p. 437). The ubiquitin function as a marker that target cytosolic and nuclear proteins for rapid protelysis (KILDE). When a protein is tagged with ubiquitin, the ubiquinated protein enters protasome and degrades into smaller peptide units (McArdle, Katch, Katch, 2015). When a protein is set for degrading, the ubiquitin conjugating cascade (carboxyl group of Gly-76) is activated by ubiquitin-activating enzyme E1 (Myung, Kim, Crews, 2001). The activated ubiquitin is 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).

Ubiquitin are added to for a multiubiquitin chain and the polyubiquinated proteins are then recognized and degraded by proteasome, a mulisubunit rotease complex (KILDE) The ubiquitin is released in the process so it can be reused (KILDE).

The role of Murf and maf

]. When knocked out, MAFbx−/− and MuRF-1 −/− mice appeared to be resistant to the effects of denervation-induced muscle atrophy, with a 56% and 36% respective sparing of muscle loss, compared to controls [16].

Rom, O., & Reznick, A. Z. (2016). The role of E3 ubiquitin-ligases MuRF-1 and MAFbx in loss of skeletal muscle mass. Free Radical Biology and Medicine, 98, 218–230. doi:10.1016/j.freeradbiomed.2015.12.031

url to share this paper:  
[sci-hub.do/10.1016/j.freeradbiomed.2015.12.031](https://sci-hub.do/10.1016/j.freeradbiomed.2015.12.031)

Muscle RINg finger 1 (MuRF1) and muscle atrophy F-box/atrogin-1 (MAFbx) were identiefied more than 15 years ago as two muscle-specific E3 ubiquition ligases. They are used as markers of muscle atrophy.

### 3. Nutrition supplement and exercise

Dietary supplements are sold and consumed all over the world. Protein supplements can enhance MPS (Kilde).

Protein synthesis remain unchanged when co-ingested CHO and EAA before strength exercise, compared with group in fasted state (Fujita, et al. 2009). Co-ingestion of CHO and EAA raises the plasma insulin concentration, but it does not further MPS during recovery (Koopman, et al. 2007). Muscle FSR does increase immediately following eaa+cho ingestion, return to basal during exercise and remained unchanged at 1h postexercise, after 2h postexercise FSR increased by ~50% in both groups (Koopman, et al. 2007).

Co-ingestion of proteins supplement with carbohydrates improves whole body protein synthesis and lowers the rate of protein breakdown rates by 8.4%, compared with ingestion of carbohydrates only (Beelen, et al. 2008). In Beelen, et al (2008) study, the subject did not train in a fasted state, where a standardized meal was ingested the evening before training.

Previous studies on protein degradation with ingestion of carbohydrate with strength exercise.

Ingestoin of glucose and eaa during exercise does not alter traningadaptions

There are not many studies on protein degradation with ingestion of carbohydrate before, during or after strength exercise. https://journals.physiology.org/doi/full/10.1152/ajpendo.00135.2007).

Data indicates when ingested larger quantity of leucine in older men (72+-2yr) following RE may firther reduce postexercise sleketal muscle autophagy, but does not appear to influence the acurte postexercise elevation in markers of the ubiquitin proteasome system or the breakdown of intact proteins.

When consumed normal protein ingestion through daily nutrition, further timed protein supplement ingestion before and after resistance training do not further enhance skeletal muscle mass or strength in elderly men

Elderly

On untrained men, ingestion of liquid carbohydrate (CHO) and essential amino acids (EAA) has been shown to attenuate protein degradation in myofibrillas, when ingested between exercise sets (Bird, S. P., Tarpenning, K. M., Marino, F. E., 2006). Strength exercise has also proven to give higher rate of AA transport during strength exercise when co-ingestion of CHO and EAA (Biolo, G., Williams, B. D., Fleming, R. Y., Wolfe, R. R., 1999).

# The impact of postexercise essential amino acid ingestion on the ubiquitin proteasome and autophagosomal-lysosomal systems in skeletal muscle of older men [Jared M. Dickinson](https://journals.physiology.org/doi/full/10.1152/japplphysiol.00632.2016), [Paul T. Reidy](https://journals.physiology.org/doi/full/10.1152/japplphysiol.00632.2016), [David M. Gundermann](https://journals.physiology.org/doi/full/10.1152/japplphysiol.00632.2016), Michael S. Borack, [Dillon K. Walker](https://journals.physiology.org/doi/full/10.1152/japplphysiol.00632.2016), Andrew C. D’Lugos, [Elena Volpi](https://journals.physiology.org/doi/full/10.1152/japplphysiol.00632.2016), [Blake B. Rasmussen](https://journals.physiology.org/doi/full/10.1152/japplphysiol.00632.2016)

06 MAR 2017<https://doi.org/10.1152/japplphysiol.00632.2016>

. Verdijk, L. B., Jonkers, R. A., Gleeson, B. G., Beelen, M., Meijer, K., Savelberg, H. H., … van Loon, L. J. (2008). Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy after resistance training in elderly men. The American Journal of Clinical Nutrition, 89(2), 608–616. doi:10.3945/ajcn.2008.26626

Welle, S., Thornton, C., & Statt, M. (1995). *Myofibrillar protein synthesis in young and old human subjects after three months of resistance training. American Journal of Physiology-Endocrinology and Metabolism, 268(3), E422–E427.* doi:10.1152/ajpendo.1995.268.3.e422

Co ingestion on synthesis

In conclusion, coingestion of carbohydrate does not further augment muscle protein synthesis rates during recovery from resistance-type exercise under conditions where ample protein is ingested.

During endurance exercise leucine oxidation higher rate after training https://journals.physiology.org/doi/full/10.1152/ajpendo.00543.2003

Health and protein degradation

In elder there is a higher rate of muscle breakdown. Ingestion of EAA has health benefits of enhancing post exercise protein synthesis, but ingestion of nutrition on protein degradation is lesser understood (KILDE). Dickinson, Reidy, Gundermann, Borack and Walker (2017) reported that ingestion of larger quantity (3.5 g) of leucine after resistance training may lead to further reduction in postexercise skeletal muscle autophagy. Although this was a small study (n = 15 older healthy men)

Therefore this study is going to examine ingestion of glucose during strength exercise and how it affect protein degradation. 1. Hypothesis is that glucose attunate muscle protein degradation with tendensis to lower the rate of Murf and Maf

Amino acids rate and insulin

**Introduksjon <1000 ord**

Resistance exercise (is a growing community) developing all over the world and are more and more looked into with its health and performance enhancements for all different kinds of groups, from elderly, young, sickness and atheltes. To enhance the effects of resistance exercise many studies has been done to see how resistanace exercise can be enhanced to benefits for said group.

There is an understanding that muscle protein snytheisis (MPS) and muscle protein breakdown (MPB) henger I hop. MPS is well established, but MPB and degradation of proteins is often overlooked (KILDE). Ubiquitin-proteasome pathway is the major pathway for protein degradation in skeletal muscle. Studies examining human skeletal muscle protein turnover has primarly focused on protein synthesis, and MPB is often overlooked. Protelysis is a fundamental biological process determined of net protein balance, provide amino acids as precursos for protein synthesis in vital organs, tissue and repair and remodelling (assessment of skeletal proteolysis…). In eledery and sickness there is a higher rate of MPB, and resistance exercise alone have seen as beneficial to blunt MPB and enhance MPS. Studies have found that co ingestion of carbohydrates and EAA surrounding resistne exercise have a tendensees to blunt MPB without affecting MPS. Therefor I am going to study muscle protein breakdown after 2 weeks of strength exercise while consuming carbohydrates during the session. One hypothesis is that MPB will be lower in post exercise than in pre.

The purpose of this study was to examine MPB after 2 weeks of strength exercise using a withing individual intervention design.

**Metode –**

**Data innsamling**

Study overview.

In this random pre-post within subject interventional study, the subjects completed a 12 days resistance exercise intervention, consisted of strength training protocol, strength tests, body mass index, dietary program, biopsies, and blood samples. This study had two groups with the same training protocol and dietary plan every day. The subjects performed in both groups on alternating days with ingestion of glucose (GLU) and/or placebo (PLAC) in connection with training, accompanied by ingestion of protein to ensure muscle growth. The subjects performed the training protocol on one leg alternating days, with Day 1 involved training of the firs leg (RT#1) and Day 2 on the second leg (RT#2), and Day 3 involving training on RT#1 and so on. Six training session was performed for each leg. The dietary plan was ingested with the training of one leg, and ensured within-subject comparisons, and removing biological diversity. The subject was randomized into GLU or plac, where half (n=10) performed with GLU on the first day, and the other half with PLAC. In each group, the subject where randomly distributed (n=5) starting on the dominant leg, and the other half with the non-dominant leg.

Et bilde som inneholder innendørs, overvåke, skjermbilde, skjerm

Automatisk generert beskrivelse

**Subjects**

16 male (n=10) and female (n=6) moderate strength trained participtants (20-45 years) was recruited to the study from høgskolen in innlandet, Lillehammer. 3 subject was not able to finish the internvention because of injury under resistance training and sicknes. They was non-smoking and moderately trained (having performed 2-8 resistance training session per 14 days for the last six months). The exclusion criteria are previous injury resulting in impaired strength, inability to perform resistance training and symptoms, and medical record of metabolic disorders including hyperglycemia, i.e. fasting venous plasma glucose >- 6.1 mmol/L and/or 2-hour glucose tolerance >- 7.8mmol/L, and/or HbA1c>42mmol/mol. Not able to understand Norwegian, unstable cardiovascular disease, illness or injury contradicting heavy strength training, disabling musculoskeletal disease, serious mental illness, allergy to local anaesthesia, impaired glucose tolerance

**Et bilde som inneholder skjermbilde

Automatisk generert beskrivelse**

**Design**

This study is a randomized interventional within subject study.

**1RM protocoll**

On test day -7 and -5 1RM test was executed. Each part

ipants had 10 minutes of warm up on ergometer cycling. Deltakerne gjennomførte først 1RM test på ett ben benpress og så ettbens kneekstensjon. Det var to oppvarmingssett for å fjerne mest mulig teknisk progresjon. Under den første testen på -7 ble forsøkspersonene spurt om hva de trodde var sin 1RM som instruktørene tok utgangspunkt fra. Forsøkspersnene fikk 5 forsøk, eller arbeid til failure. 1RM test på -5 var for å sikre at riktig 1RM var funnet. There were to warm up set consisted with low volume to ensure technical progression.

1RM testene fungerte også som treningstilpasning gjennom intervensjonen.

**Training protocol**

The training protocol consisted of unilateral one-legged press and unilateral one-legged knee extension performed as three sets of 10RM. Each subject was randomly assigned to train one leg each day (dominant or non-dominant) and trained each leg on alternating days, associated with either GLU or PLAC ingestion. Half the subject started with GLU on their dominant leg, and the other half started on their non-dominated leg. The same was for the group starting with PLAC. Each training session consisted 10 minutes of warm up on ergometric cycle, two warm up set where the first set was 50% of 1RM, and the second 70% 1RM. The main training was 10 repetition of 10RM with 3 series. Each subject had 6 training sessions on each leg within the 12 days of intervention. 15 minutes after the training session, the subjects registered their perceived rate of exertion using a 10-point scale. Trained personnel monitored all training sessions, end every training sessions was logged.

Isokintesisk test.

iso

**Assessments of body composition.**

Body mass composition was measured using duel-energy x-ray absorptiometry (DXA) prior to the internvention (Day -1). DXA was used to register data on lean body mass, fat mass, and bone mineral density. Data on lean body mass was used to enable proper dosage of deuterium ingestion (D2O, heavy water).

**D2O**

På day -1 fikk deltakerne den største dosen () 1 del D2O på to deler vann. Gjennom intervensjonen fikk de påfyllings mengden i tre deler, blandet sammen med saft.

Spit

Forsøkspersonene fikk spyttube som inneholdt to kamre hver dag (Day -1 – Day 12). Det øverste kammeret innehold bommulsdelen som de hadde i munn i ett minutt. Det nederste kammeret ble spytte sentrifugert på 10 … i 2 minutter. Derettet pipeterte vi ut spyttet til egne prøverør som ble fryst i -80 grader. Spyttprøvene skal bli sendt til danmark for undersøke D2O innhold gjennom intervensjonen.

Biopsi

Sampling of muscle tissue, blood and analysis.

Muscle biopsies was sampled from m. vastus lateralis at 4 timepoints; T1, T2, T3 and T4 during the intervention: 1) before the intervention from the performing leg on Day 1 (T1, pre RT#1), 2) before the intervention on the performing leg on Day 2 (T2, pre RT#2), 3) before the sixth training session on RT#1 on Day 11 (T3, post RT#1), and 4) before the sixth training session on RT#2 on Day 12 (T4, RT#2). All biopsies were sampled in an overnight-sated state at the same time of the day (feks 0545, 0645, 0745 depending on the daily timing of dietary intervention-onset, corresponding to 0600, 0700, and 0800 hrs, respectively). The biopsies was sampled under antiseptic conditions, and local anaesthesia (Lidokain 10 mg ml-1, Mylan Hospital AS, Oslo Norway) using minimally invasive micro-biopsy technique (kilde), 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).

**Blind drink test**

A blinded randomized drink test was done on D13 after last isokinetic test. The subjects got four cups, numbelitred 1-4, where two contained fun light with glucose or fun light with placebo. Before the test, the subject consumed 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 1dl of fun light glucose or placebo. 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.

**Protein extraction protocol:**

After taken all aqueous phase taken out.

Added 300ul EtOH (100%) and inverted to mix. The tubes then incubated for 3 minutes. Thereafter I transferred (1,75ug/(tissue weight/950)=x to a new tube (a). 490ul Etoh (100%) was added to phenol-ethanol supernatant and vortexed. 100ul BCP(navn) was added and vortexed then added 450ul ddH2O(navn) for phase separation and vortexed vigorously. Thereafter the samples was centrifuged 12000g at room temperature (@RT) for 5 minutes. I removed and discarded the upper phase and added 700ul EtOH (100%) and vortexed vigorously. The samples were then centrifuged 12000g @RT for 5 minutes, and a pellet of protein formed. I removed the supernatant, and the pellets was set to dry @RT for 2-3 minutes, or until dry. I checked the samples often, to ensure the pellets did not over dry. 40ul od SDS-urea buffer with inhibitors was added, vortexed vigorously and incubated for 20 minutes @RT. After the incubation, the samples were centrifuged 10000g @RT for 5 minutes, or until sediment undissolved protein. I transferred 30ul to new tubes(b). Thereafter I transferred 4ul to new tubes that already contained 36ul ddH2O(c). The samples were frozen -20(grader)C and was now ready for bradford assay. The Bradford assay was used to determined protein concentration in samples, and thereafter corrigated to standardize concentration in sample (b)

**Bradford Assay:**

**To get braford assay reagent I used /4**

SDS-Urea buffer:

4,2ml (1,05ml) 1m tris (ph 6.8)

8,3ml (2,075ml) 20% SDS

5ml (1,25ml) glycerol

830 ul (207,5 ul) beta-merc

12,4g urea (3,1 g) (whole box)

ddH2O for final volume (41,5ul)

Take out 10ml and add proteinase and phosphatase inhibitors. Mixed.

Crush the pills before adding to ensure miksing.

**Data management and biobank**

The data management plan is in line with the FAIR-principle. The project will be integrated into the Norwegian Services for sensitive data (TSD), allowing collection, storage, sharing and analyses of sensitive research data in a secure environment. Data will remain coded until after completion of cleaning of primary outcome data. After finalization of the project (31.12.2023), biological samples will be transferred from the project-specific biobank to the general biobank “«The TrainOME – humane cellers tilpasning til trening og miljø» (REK-ID: 2013/2041). Muscle biopsy material will be transferred to Denmark for analyses of rates of RNA and/or protein synthesis (in a coded state, remaining material will be returned to Norway/Lillehammer).

**Study financing**

The study will be financed by the Inland Norway University of Applied Sciences. The project consortium has no conflicts of interest to declare.

**Dissemination**

The results will be published in peer-reviewed international biomedical and biological journals, preferably open access. In addition, the results will be presented at scientific conferences (national and international) and will be communicated to the general public through mass media, social media, web blogs and podcasts.

Statistisk analyse

Subject

Table descriptive

1. about subject between gender
2. about each leg

nutrition status

1. between groups

training status

1. total volume
2. glucose during T3, T4
3. humac

protein analyse

1. on murf from pre to post

Ta hensyn til pre verdiene er likt

<https://cran.r-project.org/web/packages/dabestr/vignettes/using-dabestr.html> - paird and undpaired plot with devation between groups.

Cellulært stress I begge grupper - høyere i placebo – høyere cellulær skader i placebo. Knytter ubf og murf sammen.

En tidslinje på styrke ,målingene, volum, ernæring, glucose,

Parre dagene

Placebo, 1, 2, 3, 4, 5, 6

Glucose, 1, 2, 3, 4, 5, 6,

For treningsverdiene, lag en tabell fra hver dag som viser økning gjennom interventsjonen, dette er for å vise at intervensjonen fungerte. Da trens styrkemålingene og ernæringsdatene + insulin måling. må sammenligne dagene i forhold til placebo/glukose,

1|2|3|4|5|6|7|8|9|10|11|12|(13)|

**Reusltater -**

**Diskusjon <2500 ord**

**Referanseliste -**

Diskusjon/klonklusjon

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Github.com

* Plass man kan lagre data
* Make it

Git add -A

Git commit -m «messange»

Git push

Git pull

Liten g

Western blotting

1. Wash gel with ddh20
2. Place precast gel with running buffer
3. Load gel with ladder and samples
4. Load tray with runningbuffer until fill
5. Run gel at 250v ~1 hour on icebath in kjløerrom (?)

Protein tranfer on pvdf membrane

1. Gjør klar 5 bokser

* 1 Assembly tray – kasette og filter
* 1 boks ddh2o for svamper
* 1 boks transfer for gel
* 1 boks transfer for membrane
* 1 boks med meOH for membrane

1. Legg casett I assembly trai og fyll tra med transfer
2. Legg filterpapir I assembly tray med transfer
3. Sett membrane 5 min I meOH
4. Deretter 5 min I transfer
5. Fyll ddh2o for å fjerne luftbobler i svart svamp
6. åpne gel på begge sider med pinsett
7. legg hele gel med cast i transfer i 45 sek- sørg for å få transfer inni gellen
8. legg svart svamp i kasett
9. ta ett filterpapir på gel, legg filter papir neders
10. legg membran
11. legg filter papir
12. legg svamp
13. sett sammen kasetten
14. legg i boks med 50% fylt transfer
15. fyll på transfer til fill
16. run@ 400 mA i 1 time @4 celsius

Protein vizualization using memcode

keep the membrane side facing the gel up at all times, do not let the membrane dry!

Preparation

1. destain with meOH 1:1
2. eraser with meOH 1:1

Stain

1. place mebrane In conctainer and rinse with ddh2o – rock 3 times and quickly decant
2. add memcode sensitizer -agitate @ RT 2 min on rotary platform, moderate speed. Decant solution
3. add memcode reversible stain, agitate @RT 1 min on rotary platform, moderate speed. Decant solution. Protein bands should be blue.

Destaine

1. add ddh2o Rock three times, decant. Repeate 2 times (3 full)
2. add destain/meOH, agitate @RT, 5 min on rotary platform, moderate speed. Decant solution.
3. Add ddh2o, rock 3 times quicle decant. Do 4 additional times.
4. Take photograph with g:box on plastic sheet
5. Cut in desirable strips

Erase

1. Add erase/meOH, agitate @ RT 10 min on rotary platform, moderate speed.
2. Can be up t0 20 min
3. Decant solution, rinse with ddh2o, rock 3 times and quickly decant. Do additional 4 times.
4. (leave in ddh20)

Protein identification.

1. Block pdvf membrane for 1 hour in 1x tbst-t / 5% nonfat dried milk

* Blocking solution
* 50 ml 10x tbs
* 500 ul tween
* 25g nonfat dried milj powder
* Ddh2o to total volume 500 ml

Incubate in antibodies

Murf

* () dilute antibodies in 1x tbs-t/5% nonfat dried milk -

Maf

* () dilute antibodies in 1xtbs-t/5% nonfat dried milk

Ubf

* () dilute antibodies in 1x tbs-t/5% nonfat dried milk