Proteasome activity in human skeletal muscle – The role of glycogen availability

In healthy resistance-trained men and women

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Abstract

Aim: This thesis aimed to study how the molecular pathways that regulate protein degradation through the ubiquitin-proteasome system (UPS) are affected by glycogen availability and how that potentially affects training adaptations. The specific research questions to be answered were: **1.** Does low glycogen availability increase proteasome activity in human skeletal muscle following resistance exercise? **2.** Can ingestion of essential amino acids (EAA) modulate the response in proteasome activity induced by low glycogen availability? **Methods:** The project employed a randomized, cross-over design with a unilateral model for glycogen depletion. A total of twenty healthy participants were recruited, male (N=10) and female (N=10), who regularly performed resistance training. Each participant underwent two experimental trials where they performed resistance exercise (single leg, leg extension) with one glycogen-depleted leg and one glycogen-loaded leg after which they consumed a placebo drink in one trial, and an essential amino acid drink in the other trial. A total of 16 muscle biopsies were collected from each participant over the two experimental trials (4 muscle biopsies per leg x 2 legs x 2 trials = 16 muscle biopsies in total). UPS activity was measured using a fluorometric assay kit (Cat. # J4120, BP Bio, USA). Analyses were conducted on a Tecan Spark Multimode Microplate Reader with the onboard software SparkControl Magellan 3.1. **Results:** A three-way repeated measures ANOVA (Drink x Time x Leg) was performed which showed a significant main effect of Time (Pre, Post, 60 and 180 min, p=0.027) and Leg, the glycogen depleted leg had an upregulated UPS activity compared to the loaded leg (p<0.0001). EAA did not affect UPS activity (p=0.37). A Tukey's post-hoc test revealed significantly higher UPS activity in the depleted leg at, POST, 60min, and 180min (p<0.05), but no significance was found for the time PRE. There was a significant decrease in UPS activity in the loaded leg at 180 min compared to all other time points for both the loaded and depleted leg (p<0.01). **Conclusion:** Low glycogen availability has a significant effect on ubiquitin proteasome-mediated protein degradation and essential amino acids do not. Low glycogen availability increases UPS-meditated protein degradation within human skeletal muscle after resistance exercise. Increased protein degradation may negatively affect skeletal muscle mass which in turn may result in decreased performance and training adaptations in individuals with low glycogen availability.

Sammanfattning

Syfte och Frågeställning: Detta examensarbete syftade till att studera hur de molekylära vägarna som reglerar proteinnedbrytning via ubiquitin-proteasome systemet (UPS) påverkas av glykogentillgänglighet och hur detta potentiellt påverkar träningsanpassningar. De specifika frågeställningarna var: **1.** Ökar låg glykogentillgänglighet proteasome aktiviteten i human skelettmuskulatur efter styrketräning? **2.** Kan intag av essentiella aminosyror (EAA) påverka proteasome aktiviteten som induceras av låg glykogentillgänglighet? **Metod:** Studien använde en randomiserad, crossover-design med en unilateral modell för glykogentömning. Totalt rekryterades tjugo friska deltagare, män (N=10) och kvinnor (N=10), som regelbundet utförde styrketräning. Varje deltagare genomförde två enskilda försökstillfällen där de utförde styrketräning (enbens-extension) med ett glykogentömt ben och ett glykogenladdat ben, varefter de konsumerade en placebodryck i ett försök och en essentiell aminosyra-dryck i det andra försöket. Totalt samlades 16 muskelbiopsier in från varje deltagare över de två försökstillfällena (4 muskelbiopsier per ben x 2 ben x 2 försök = totalt 16 muskelbiopsier). UPS-aktivitet mättes med hjälp av ett fluorometriskt testkit (Katalog nr J4120, BP Bio, USA). Analyser utfördes på en Tecan Spark Multimode Microplate Reader med programvaran SparkControl Magellan 3.1. **Resultat:** En three-way repeated measures ANOVA (Dryck x Tid x Ben) utfördes, vilken visade en signifikant main effekt av Tid (Pre, Post, 60min och 180min, p=0,027) och Ben; det glykogentömda benet hade en uppreglerad UPS-aktivitet jämfört med det laddade benet (p<0,0001). EAA hade ingen effekt på UPS-aktiviteten (p=0,37). Tukey's post-hoc-test visade att UPS-aktivitet i det glykogentömda benet var signifikant högre för tidpunkterna Post, 60min och 180min (p<0,05), men inte vid tyngdpunkten Pre. Det fanns en signifikant minskning av UPS-aktiviteten i det laddade benet vid 180min jämfört med alla andra tidpunkter både för det laddade och tömda benet (p<0,01). **Slutsats:** Låg glykogentillgänglighet har en signifikant effekt på ubiquitin proteasom-medierad nedbrytning av proteiner, medan essentiella aminosyror inte har någon effekt. Låg glykogentillgänglighet ökar UPS-medierad proteinnedbrytning i human skelettmuskulatur efter styrketräning. Ökad nedbrytning av proteiner kan negativt påverka skelettmuskelmassan, vilket i sin tur kan resultera i minskad prestation och träningsanpassning hos individer med låg glykogentillgänglighet.

Table of Contents

1 Introduction 1
2 Aim & Research Question 4
2.1 Research Question 4
3 Methods 5
3.1 General Study Design 5
3.2 Participants 5
3.2.1 Estimated Power 6
3.3 Ethics 6
3.3.1 GDPR 6
3.4 Statistics 6
3.5 Pre-Testing 7
3.6 Experimental Trials 8
3.6.1 Muscle Biopsies 9
3.7 Analyses 10
3.7.1 Muscle Glycogen Analyses 10
3.7.2 Proteasome Activity Analyses 10
4 Results 13
4.1 Glycogen Analysis 13
4.2 Chymotrypsin-like (Suc-LLVY-AMC) 13
5 Discussion 15
References 19

Appendix 1 Literature search
Appendix 2 Informed consent
Appendix 3 Research participant information

1 Introduction

The role of nutritional strategies for training adaptations within sports is a widely accepted part of an elite athlete's training regime. Depending on the adaptation goals of the athlete, the strategies for nutrient intake may vary. Athletes need to meet the energetic and nutritional demands of training to promote the desired adaptation (Stellingwerff et al., 2019; Thomas et al., 2016). Jeukendrup (2017) discusses the current literature and recommendations regarding endurance athletes and training adaptations in relation to nutritional intake. Manipulation of glycogen availability (training low/compete high) is a common and well-researched practice by endurance athletes to potentially increase the oxidative capacity of the working muscles and more effectively oxidize free fatty acids as fuel during prolonged exercise or competition. However, in general, current guidelines recommend high carbohydrate intake to promote glycogen storage before exercise and to increase glycogen re-synthesis after exercise (Thomas et al., 2016). In contrast to endurance training, there is a substantial gap in the literature regarding adaptations to resistance exercise with low glycogen availability. Gaining strength and muscle mass, which are essential for most sports, is commonly achieved with a resistance exercise protocol. A low-carbohydrate diet combined with a resistance exercise protocol to retain muscle mass during weight loss is commonly used practice in Olympic weight-class sports such as boxing, wrestling, taekwondo, etc. (Reale et al., 2017). This approach will eventually result in low glycogen levels, which means that the athletes will perform resistance exercises with low or depleted glycogen storage. The approach might be detrimental to resistance exercise adaptions due to the increased energetic stress of low glycogen availability, which has been shown to increase protein breakdown through the autophagy system (Jeukendrup, 2017; Thomson, 2018; Tong et al., 2009). There is a substantial gap in the literature within the field of resistance exercise and how glycogen availability affects exercise response, and expanding this research topic is therefore highly relevant.

Nishimura et al (2021) discuss that physical exercise triggers the repair and remodeling of skeletal muscle which leads to beneficial adaptations and improved health. Exercise is an acknowledged approach for preventing and treating metabolic diseases, but there is a substantial gap in the literature regarding the underlying mechanisms. From a cellular perspective, muscle protein turnover is key for modulating muscle mass and protein quality. Protein turnover is the combined reaction of protein synthesis and degradation (Nishimura et al., 2021; Tipton et al., 2018). Protein degradation is primarily managed by the autophagy

pathway and the ubiquitin-proteasome systems (UPS) (Nishimura et al., 2021; Russell et al., 2014).

The ubiquitin-proteasome system (UPS) is a highly regulated cellular process responsible for the degradation of damaged, misfolded, or unnecessary proteins. The UPS was first discovered by Fred Goldberg in 1972 when he was researching the degradation of abnormal proteins in Escherichia coli bacteria. Three main components make up the UPS; ubiquitin, ubiquitin ligases, and the proteasome (Murton et al., 2008; Nishimura et al., 2021; Ohtake, 2022; Raffeiner et al., 2023; Russell et al., 2014). Ubiquitin is a protein that is made up of 76 amino acids and is classified as a globular protein. Ubiquitin's most widely known and researched function is to tag proteins for proteasome-mediated degradation. Ubiquitin attaches to its target protein's lysine residue through the conjugation of its C-terminal glycine. Ubiquitin is attached to cellular proteins through a process known as ubiquitination. The ubiquitination process involves three classes of enzymes; ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). Of these three enzymes classes, the E3 enzymes are the main factor that determines target specificities, and which substrate is put through the ubiquitination process, see Figure 1 (Khalil, 2018; Komander & Rape, 2012; Ohtake, 2022; Singh et al., 2022). The E1 enzymes activate the ubiquitin

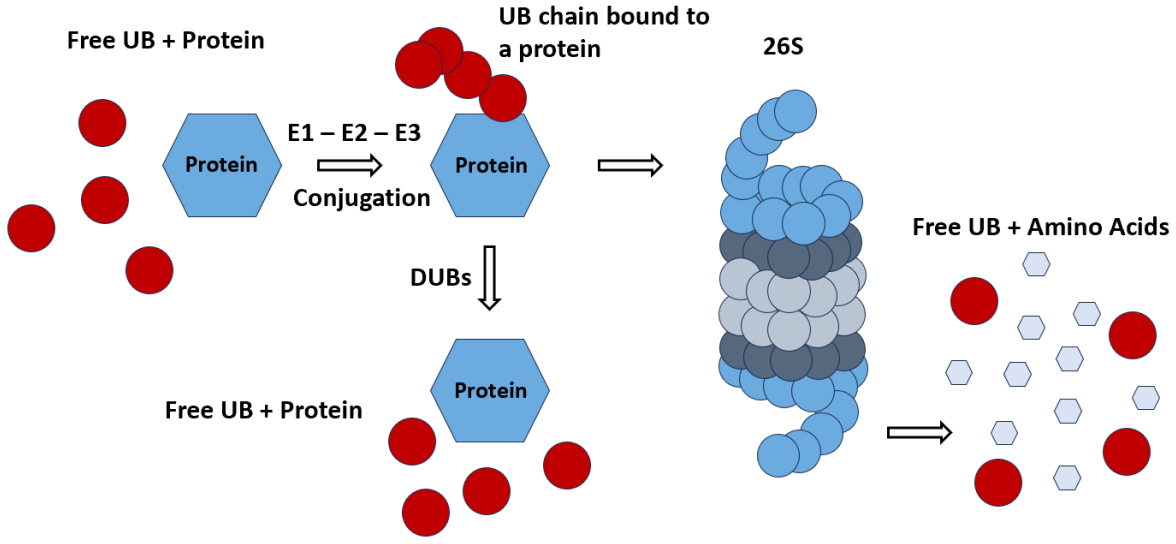

Figure 1– A schematic illustration of the ubiquitin-proteasome system (UPS). Free ubiquitin (UB) is bound to a protein by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3), afterward its transported to the 26S proteasome for degradation and is released into the cytosol as free UB and free amino acids. Unless ubiquitination is reversed by deubiquitylating enzymes (DUBs), then it's released back to the cytosol as free UB and free protein.

molecule by grabbing one ATP molecule and catalyzing the acylation of the ubiquitin C-terminus, and releasing one AMP molecule in the process. Afterward, ubiquitin is transferred to the conjugating enzyme E2 via a process called transthioesterification. In the last step, the activated ubiquitin is transferred via an E3 ligase to a lysine group on the target protein. Now the protein is tagged for degradation and can be transferred to the 26S proteasome (Tipon et al., 2018). Ubiquitination can be reversed by deubiquitylating enzymes (DUBs) (see Figure 1), which means that proteins marked for degradation can be saved (Ohtake, 2022). Once a protein has been tagged with ubiquitin and been guided to a 26S proteasome for breakdown, the 19S protein complex binds to the ubiquitinated protein and unfolds it before translocating it to the 20S core protein complex of the 26S proteasome (see Figure 2). The 20S core contains multiple protease active sites that cleave the bindings of the protein into free peptides that will be released to the cytosol for recycling or further breakdown. The three active peptidases that cleave the amino-acid bindings in the proteasome are chymotrypsin-like, trypsin-like, and caspase-like (Nishimura et al., 2021; Raffeiner et al., 2023).

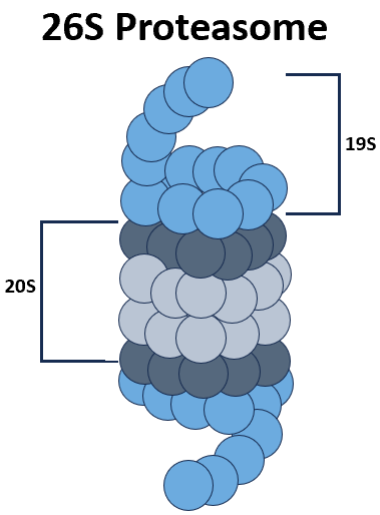

Figure 2– An illustration of 26S proteasome. And its subunits 19S and 20S core.

AMP-activated protein kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1) coordinate the phosphorylation of ULK1 which activates the autophagy system and thereby regulates protein degradation (Klionsky et al.,2016; Russell et al., 2014). During glucose starvation, the activity of AMPK will be upregulated which directly activates ULK1 by phosphorylation, which will promote autophagy activity. On the contrary, high mTORC1 activity can be seen under conditions of nutrient sufficiency. Increased mTORC1 activity will disrupt the interaction between AMPK and ULK1 and thereby down-regulate the autophagy system (Klionsky et al.,2016). AMPK activation in cell culture models has been shown to increase the expression of muscle-specific E3-ligases. This could potentially increase the ubiquitination of proteins and therefore increase protein degradation through the UPS (Murton et al., 2008; Tong et al., 2009). Furthermore, it has been shown that intense acute exercise and functional overload upregulate proteasome activity in both human and rodent skeletal muscles. This indicates that UPS has an essential role in muscle remodeling, protein quality control, and adaptation to exercise (Nishimura et al., 2021; Tipton et al., 2018).

Low glycogen availability has been shown to increase phosphorylation of the protein AMPK which, as mentioned earlier, activates the autophagy system and may indirectly activate UPS, resulting in protein degradation within the cell (Jeukendrup, 2017; Thomson, 2018; Tong et al., 2009). This is supported by preliminary data (Apró, unpublished) that indicates a significant upregulation of autophagy due to low glycogen availability. In theory, combined upregulated autophagy and UPS activities due to low glycogen availability could result in a negative protein balance and thereby have a negative effect on strength training adaptations. The ingestion of essential amino acids (EAA) after resistance training could potentially decrease autophagy and UPS signaling, due to its potential to activate mTORC1 (Edman et al., 2019; Klionsky et al.,2016). This could theoretically also inhibit or down-regulate UPS, and potentially reduce the negative effects of training with low glycogen availability. Autophagy is emerging as a key factor in skeletal muscle remodeling and exercise adaptations. Meanwhile, only a small number of studies have been conducted on the UPS in human skeletal muscles, and at this time no studies have been conducted on the relationship between glycogen availability and UPS activity. The aim of this project is therefore to investigate how the molecular pathways that regulate protein degradation through the UPS are affected by glycogen availability and how that potentially affects training adaptations.

2 Aim & Research Question

This thesis project is part of an ongoing study within William Aprós research group at the Swedish School of Sports and Health Sciences (GIH), Åstrand laboratory in Stockholm, Sweden. William Apró's research group aims to study the role of glycogen availability on multiple molecular pathways involved in regulating muscle mass and training adaptations. This thesis aims to study how the molecular pathways that regulate protein degradation through the UPS are affected by glycogen availability and how that potentially affects training adaptations.

2.1 Research Question

1. Does low glycogen availability increase proteasome activity in human skeletal muscle following resistance exercise?

H_1 – Low glycogen availability causes a significant increase in proteasome activity following resistance exercise.

H_0 – Low glycogen availability does not cause a significant increase in proteasome activity following resistance exercise.

2. Can ingestion of essential amino acids (EAA) modulate the response in proteasome activity induced by low glycogen availability?

H_1 – Ingestion of essential amino acids (EAA) causes a significant decrease in proteasome activity following resistance exercise.
H_0 – Ingestion of essential amino acids (EAA) does not cause a significant decrease in proteasome activity following resistance exercise.

3 Methods

Before the start of this thesis, Willam Apro´ and colleagues conducted all the experimental trials and collected all the muscle biopsies. Therefore, this paper will focus on parts of the research groups' planned analyses of the collected muscle biopsies.

3.1 General Study Design

The project employed a randomized, cross-over design with a unilateral model for glycogen depletion. Recruited participants performed two experimental trials where they performed resistance exercise with one glycogen-depleted leg and one glycogen-loaded leg after which they consumed a placebo drink in one trial, and an essential amino acid drink (EAA) in the other. Both intervention groups (Placebo or EAA) and which leg that was depleted were randomized for the first trial and then switched for the second trial.

3.2 Participants

A total of twenty participants were recruited, male (N=10) and female (N=10), all were healthy and performed resistance training regularly, which was defined as 2 to 3 sessions per week over the previous 6-month period. Male participants had a VO_{2max} over 45 ml/min/kg and female over 40 ml/min/kg. To account for the menstrual cycle in the female participants and for standardization purposes, the two trials were separated by one month in all participants. Participants were recruited via advertising on The Swedish School of Sport and Health Sciences (GIH) website, through the university’s social media accounts, and posted notices around the GIH campus as well as posted notices in local gyms around Stockholm, Sweden. See Table 1 for participant characteristics.

Table 1 – Participants characteristics for gender and combined (Male & Female) as Mean ± SD.

Gender	Age (years)	Mass (kg)	Height (cm)	Body fat %	VO_2max (ml/min/kg)	Wmax (W)	N
Male	29,5 ± 5,5	82 ± 8,3	179,5 ± 6,1	13,8 ± 1,8	49,6 ± 4,5	350,5 ± 34	10
Female	26,2 ± 4,3	62,7 ± 4,6	164,6 ± 5	22,9 ± 3	43,5 ± 2,1	228,2 ± 19	10
Male & Female	27,8 ± 5,1	72,4 ± 11,9	172 ± 9,4	18,3 ± 5,3	46,6 ± 4,7	289,4 ± 68,2	20

3.2.1 Estimated Power

In the current literature, there were no published data assessing the autophagy- and ubiquitin-proteasome system's response to variations in glycogen availability. Thus, the power calculations were done on pre-existing unpublished data. Based on the unpublished data set, a significant difference in autophagy and UPS signaling in resistance-trained men can be detected with an N of 8. To account for potential sex differences an N of 10 was chosen for both men and women.

3.3 Ethics

The study has received ethical approval from the Swedish Ethical Review Authority (DNR: 2019-0038 1). The project's primary ethical concern was the biopsy sampling because it is an invasive procedure with some concerns of infection and discomfort. Nevertheless, when the correct sterile procedure is utilized and participants are given comprehensive instructions for good hygiene after biopsy collection, the risks associated with biopsy sampling are thought to be minor. In line with Vetenskapsrådet's (2017) rules and guidelines, all participants were informed both verbally and in writing about the potential health risk, their rights as volunteers, and the purpose of the study before they provided written consent for participation (Appendix 2). If the participants provided written consent, they completed a health questionnaire to ensure they did not have any underlying health issues that could cause complications during testing.

3.3.1 GDPR

To ensure the safety of and protect the privacy and integrity of all participants all data samples are coded upon collection and handled according to GDPR and in line with Vetenskapsrådet (2017) rules and guidelines. Collected muscle biopsies were stored in a registered biobank at the Swedish School of Sport and Health Sciences.

3.4 Statistics

GraphPad Prism 10.0.1 (GraphPad., Boston, MA, USA) was used for all analyses and Microsoft Excel (Version 16529.20182) was used to sort all data sets after exporting them

from the plate reader software. All data are presented as mean ± standard deviation (SD) unless otherwise noted. The normal distribution of variables was analyzed with the Shapiro-Wilks test of normality and visually with a Q-Q plot and it was concluded that all data were acceptable for parametrical statistical tests. A three-way repeated measures analysis of variance (ANOVA) for intervention (Placebo/EAA) x time x leg (Loaded/Depleted) was conducted. Significance levels were set at $p<0.05$. If an interaction or main effect was found, a Tukey's post-hoc test was performed to investigate where the effects were located with the data set. Significance levels were set at $p<0.05$.

3.5 Pre-Testing

Five weeks before the first experimental trial two separate pre-testing sessions were conducted where VO_{2max} and leg strength were examined. On the first day, VO_{2max} was determined by incremental bicycle test (SRM Ergometer, Germany) and expired gas was measured and analyzed with an Oxycon Pro (Erich Jaegar, Hoechberg, Germany). VO_{2max} was defined as the average of the four highest measurement points in a row expressed in ml/kg/min and Watt peak (W) was defined as the highest intensity participants could complete before reaching failure. The protocol started with a warm-up of 8 minutes on 100W for men, and 70W for women. Trials started at 2-3W/kg body weight with the cadence set to 90rpm. Every 15 seconds the load was increased with 5W for men, and 3W for women. The test was completed when participants reached volitional failure and this was defined as when participants could not keep the cadence over 80rpm. On the second pre-testing session, the participants arrived at the Åstrand-laboratory at 07:00 after an overnight fast and 48h of rest to measure body composition with bio-impedance using a Quadscan 4000, Bodystat. Afterward, leg strength was tested. Leg strength was defined as the maximum weight participants could perform 10 repetitions with a full range of motion on a Cybex Eagle, leg extension machine. The range of motion was supervised visually by the supervisor on site. To minimize the training effect between the two experimental trials the participants went through two familiarization sessions in which they performed the pre-determined experimental protocol. The familiarization session were conducted 7 days prior to the experimental trials. If needed, the weight was adjusted during the familiarization sessions to find the optimal load for the experimental trials.

3.6 Experimental Trials

Each participant performed the experimental trials on two separate occasions to enable the cross-over design; once with essential amino acids and once with a calorie-free placebo beverage. Preparation for every participant started three days prior to the experimental trials in the Åstrand-laboratory, with a carbohydrate loading protocol to raise glycogen levels in both legs. On day one at ≈ 07:00 participants arrived at the Åstrand Laboratory after an overnight fast for the first glycogen depletion session. The purpose of the two-legged "pre-depletion" session was to increase glycogen synthase activity (Wojtaszewski et al., 2003) an enzyme that's involved in glycogen synthesis (Burke et al., 2017). The session was conducted on a Monark 839E, (Vansbro, Sweden). The depletion protocol started with a 5-minute warm-up at 50% of watt (W) peak (% of workload was calculated from the VO_{2max} test). After the warm-up was completed, the participants began the interval depletion sessions which consisted of 2 minutes at 90% of W-peak keeping a steady cadence of 70rpm, and a 1-minute active rest at 50% of W-peak. This was repeated until failure, when the participants failed to keep a steady cadence, the workload was decreased to 80%-, 70%, - and 60% of W-peak. After completing the two-legged depletion session the participants were supplied with prepacked and weighed portions of food and supplements from the Åstrand Laboratory with the instruction to only eat the supplied food (water was not supplied). Participants were also instructed to not consume any dairy products and to rest (no exercise). Furthermore, participants kept a dietary log that was collected by the test leaders after the glycogen loading was completed. Participants were instructed to consume 10 g of carbohydrates per kg body weight per day. For days one and two the carbohydrates were divided between meals and supplements (65% of carbohydrates were provided from food and 35% from the supplement Vitagro Carbloader, the flavor was either orange or summer fruits). Meals consisted of slices of white bread for breakfast (Jättefranska, Pågen), and lunch and dinner consisted of pasta (Strozzapreti, Zeta) with pre-prepared pasta sauces (Zeta). The Vitagro supplementation was ingested three times during the day between meals. On the third day, the participants had the same breakfast as the previous days, but only half the amount of carbohydrates for lunch. The final meal on day three was consumed at 15:00 and consisted of two protein bars (Star Nutrition) which contained 20 g of protein, 13-15 g of carbohydrates, and 7.0-8.3 g of fat. Participants arrived at the Åstrand Laboratory at 18:00 for the second glycogen depletion session. A unilateral model was applied to achieve the desired difference in glycogen content between the legs, meaning that each participant needed to complete a glycogen depletion

session with only one leg the day before the experimental trials. The glycogen depletion sessions were performed on a one-legged bicycle (Monark RC6, Vansbro, Sweden). The glycogen depletion protocol was the same as mentioned earlier in this paper, with the adjustment of workloads being divided by two and rounded to the closest W setting of the bicycle. After the intervals for the unilateral depletion session were completed, the participants completed an arm cranking protocol consisting of 10 two-minute intervals on a Monark Egromedic 891E (Vansbro, Sweden) at 70rpm. With a workload of approximately 105 W for men and 70 W for women, with two minutes of sitting rest in between. This was done to minimize glycogen restoration during the night. From the depletion session to the experimental trials, the participants fasted to ensure the difference in glycogen availability between legs. After the unilateral glycogen depletion and a night of fasting, participants arrived again at the Åstrand Laboratory at ≈ 06:00. After 150 minutes of resting in a supine position, a resting muscle biopsy was taken from both legs. After completing the biopsy, the resistance unilateral training protocol began, and it consisted of a warm-up of 3 sets of 10 repetitions on 30%, 60%, and 80% of the chosen load, with two minutes of rest between sets. Participants rested for three minutes before starting the 10 sets at 10 RM (repetition max) protocol. The resistance was set to the determined weight from the pre-testing session. To eliminate any difference in performance between the legs due to glycogen availability, the depleted leg did the first bout of exercise followed by the loaded leg, which performed the same number of repetitions against the same amount of resistance. The participants rested for 90 seconds between legs and a total of 3 minutes before the next set for the first leg. If the participants failed to complete seven or more repetitions with full range of motion the weight was decreased. This protocol was chosen because the current literature has shown that it can stimulate signaling pathways that both activate and inhibit autophagy in human muscle (Apró et al. 2013; Apró et al. 2015a). After the resistance exercise protocol was completed another muscle biopsy was taken in each leg, this was then followed by the consumption of 240mg of EAA per kg of body weight (Fight pharm, Apple flavor) or a calorie-free placebo. Two more muscle biopsies were sampled in each leg one and three hours after completing the resistance exercise protocol.

3.6.1 Muscle Biopsies

Under local anesthesia (Lidocaine 20 mg/ml Astra Zeneca, Sweden), muscle biopsies were obtained from m. vastus lateralis separated with approximately two to three centimeters between each insertion. Four muscle biopsies were obtained from each leg (a total of 8)

during each trial, using a 5 mm Bergström needle modified for suction (Ekblom, 2017). The process was repeated during the second trial and a total of 16 samples were collected from each participant (4 muscle biopsies per leg x 2 legs x 2 trials = 16 muscle biopsies in total). Biopsies were directly cleaned free from blood and frozen with liquid nitrogen for later analyses. In the next step, the freeze-dried muscle biopsies were prepared for further analysis by cleaning them from fat, connective tissue, and the remaining blood.

3.7 Analyses

All muscle biopsy analyses for this project were conducted at Åstrand Laboratory (GIH) in Stockholm, Sweden.

3.7.1 Muscle Glycogen Analyses

The muscle glycogen analyses have been performed by William Apró and colleagues and the protocol for the analyses was performed according to Leighton et al (1989).

3.7.2 Proteasome Activity Analyses

Muscle Homogenization – The muscle samples were thawed on silica gel for 30min before approximately 2mg of muscle were weighed up on a Mettler Toledo XA105 Dual Range Scale (d = 0.01mg/0.1mg) for all 320 biopsies. After this process, the samples were refrozen at -80° Celsius and stored. Before the homogenization, the weighed muscle samples were thawed on silica gel for 30 minutes, and ATP and $MgCl_2$ were thawed on ice to slow down the thawing process. During this time, the homogenization buffer containing 40mM Tris, pH 7.2, 50mM NaCl, 2mM βME, 2mM ATP, 5mM $MgCI_2$, and 10% glycerol was prepared. After the muscle was thawed, zirconium oxides beads (ø 0.5mm) were added to the samples to aid the breakdown of the muscle tissue during homogenization. From here on, all samples including the homogenization buffer were kept on ice to slow down the biochemical processes constantly occurring within each sample. 250µl of homogenization buffer was added to each muscle sample using an Eppendorf E3x Multipette. Directly after the homogenization buffer was added, the samples were dissolved in a Bullet Blender – Storm Pro (USA) using the settings of 60s on level 8 for 5 times with a 60s pause between each bout. Afterward, the samples were shaken for 5 minutes on an IKA-Vibrax-VXR at maximum speed. To remove the supernatant from the zirconium oxide beads all samples were centrifuged for 3s at 4° Celsius (Eppendorf, Centrifuge 5414 R) before pipetting samples to a new test tube. Then the samples were centrifuged once again for 20 min at a speed of 16.000

x g at 4° Celsius (Eppendorf, Centrifuge 5414 R) and pipetted to a new set of test tubes to remove any solid materials from the muscle homogenates. In the next step, 40µl of distilled H_2O s was added to a new set of test tubes and then 10µl of muscle homogenate was added to each test tube. All samples were centrifuged for 3s (Labnet Mini Centrifuge, Korea) and then vortexed. The resulting supernatant will contain the proteasome components from the muscle sample. To determine the protein content of each sample, 10µl of all samples and pre-diluted protein assay standards (Thermo Scientific, USA) were pipetted as triplicates to a Microtest Plate 96 well (Sarstedt, Ref: 82.1581001) using reverse pipetting. An Eppendorf E3x Multipette was used to add 150µl of Pierce 660nm protein assay reagent (Thermo Scientific, USA) to the plate. The plate was incubated for 5 minutes on a Stuart Microtitre plate shaker SSM5 (setting 750). When incubation was completed, the measurements were conducted on a Tecan Spark, Multimode Microplate Reader using the software SparkControl Magellan 3.1. Protein centration was calculated and all samples were diluted to the same protein concentration and divided into three sets of test tubes (100µl, 70µl, 70µl) to prepare for the proteasome activity measurements. After the homogenization and dilution process was completed, the samples were frozen at -80° Celsius and stored until further analysis.

Proteasome Activity measurements – Proteasome Activity Fluorometric Assay Kit II (Cat. # J4120, BP Bio, USA) was used to assess the activity of the ubiquitin-proteasome system within each sample. The underlying principle of the assay is that upon cleavage of the different substrates by the proteasome system, the fluorescent probe AMC is released from the substrate which in turn will yield a fluorescent signal. The amount of fluorescence will therefore be proportional to proteasome activity and are presented as fluorescent units per min or FU/min. To measure proteasome-specific substrate cleavage, the proteasome inhibitor MG132 was added to a subset of samples to create a negative control. This kit contains three separate substrates that allow the monitoring of the chymotrypsin-like (Suc-LLVY-AMC), trypsin-like (Boc-LRR-AMC), and, caspase-like (Z-LLE-AMC) activities of the proteasome system. Assay measurements were performed on a Tecan Spark, Multimode Microplate

Reader using the software SparkControl Magellan 3.1. The proteasome activity protocol starts with pre-heating the Multimode Microplate Reader, HLC heating plate (model: HBT-2 132), and the incubator (Bibby Scientific Limited) to 37° Celsius. For subsequent heating of the Proteasome Assay Buffer. Afterward, the muscle samples were thawed on ice and kept on ice during the preparation process to slow down the biochemical processes constantly occurring within each sample. The assay buffer (AB) and substrate (SUB) with (+) and without (-) inhibitor (MG132) were prepared and kept on the heating plate at 37° Celsius. (AB+: 1688µl Assay buffer mix + 11.3µl MG132, AB-: 1699µl Assay buffer mix, SUB+: 4,2µl 1000xSuc-LLVY-AMC + 2085.3µl Assay buffer mix + 10,5µl MG132, SUB-: 4,2µl 1000xSuc-LLVY-AMC + 2095.8 µl Assay buffer mix). For these analyses, a non-binding, black 96-well microplate with a flat bottom (Greiner Bio-One, Germany) was used. The microplate was loaded with 10µl of sample in double triplicates on the same row to enable measurement with and without inhibitor MG132 (see Figure 3). In the next step, AB+ and AB- were added to the microplate which was then placed in the incubator on a Stuart Microtitre plate shaker SSM5 (setting 750) for 30 minutes. When the incubation time was completed, 40µl of SUB+ and SUB- were added to the microplate before starting the analysis. Each assay had a total running time of 120 minutes with a measurement taken every 5 minutes for each triplicate.

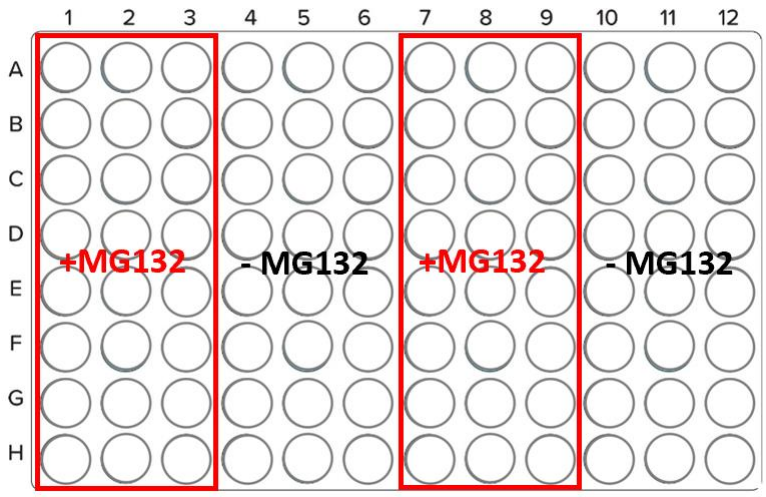

Figure 3 – A schematic illustration of how the inhibitor was loaded on the microplates. +MG132 marks the wells where the inhibitor was added with a red rectangle.

4 Results

Due to methodological issues regarding the linear stability of the substrate's trypsin-like (Boc-LRR-AMC) and caspase-like (Z-LLE-AMC), we were only able to analyze the activity of the chymotrypsin-like (Suc-LLVY-AMC) within the given timeframe of this project.

4.1 Glycogen Analysis

The muscle glycogen analyses have been performed according to Leighton et al (1989) by William Apró and colleagues. The groups (Placebo and EAA) were combined when measuring glycogen content. This was done because the placebo or EAA beverage would not affect the muscle glycogen content within the given timeframe. A main effect was found for depleted- vs loaded leg for all combined time points (p<0.0001). Mean ± SD for depleted leg 133.9 ± 59 (n = 160), loaded leg 566.6 ± 115.9 (n =160) mmol · kg^{-1} dry weight of muscle. Tukey's post-hoc test showed a significant difference between the depleted and loaded leg for all the time points (p<0.001) see Figure 4.

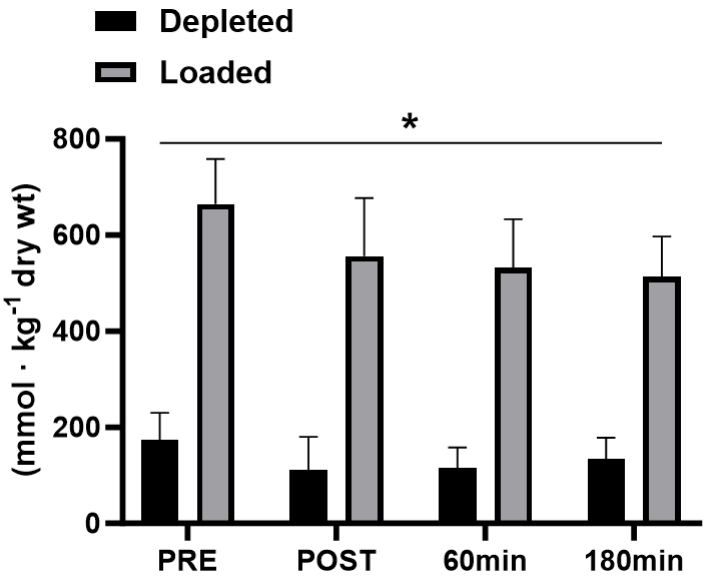

Figure 4 – Data described as mean ± SD. Y-axis (mmol · kg^{-1} dry wt), X-axis time points. ***P*<0.01 significant difference between Depleted & Loaded leg.

4.2 Chymotrypsin-like (Suc-LLVY-AMC)

All data sets for protein breakdown mediated by the enzyme chymotrypsin-like were combined to investigate where potential interactions or main effects would be detected. A significant main effect over time for all combined data sets was found (p=0.027) see Figure 5. There was no significant main effect between the intervention trials (Placebo vs EAA) (p=0.3705). And no significant difference over time for the intervention trials (Time x Placebo vs EAA) (p=0.906). A significant main effect was found between the glycogen-loaded and glycogen-depleted leg (p<0.0001) see Figure 5. And a significant difference over time between the glycogen-loaded and glycogen-depleted leg (Time x Depleted vs Loaded) (p=0.0001). Further examination of the significant results with Tukey's post-hoc test showed

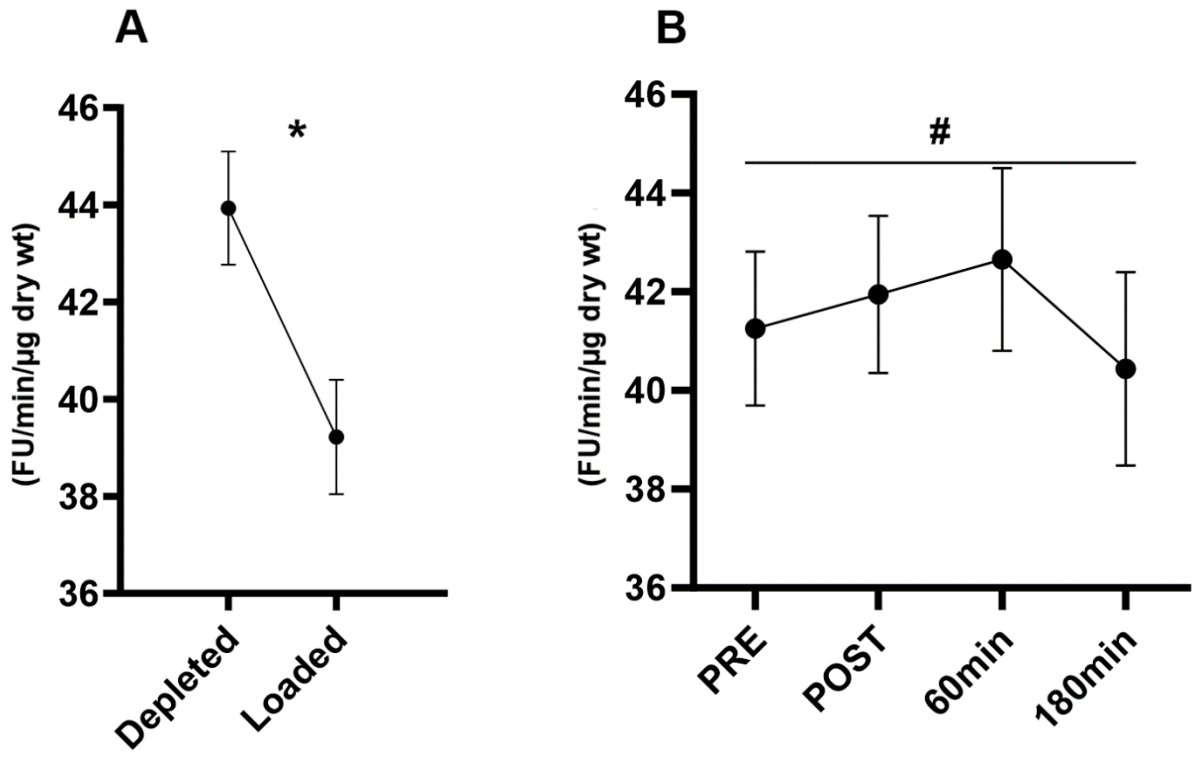

Figure 5 – Results of Main Effects, data described as mean with 95% CI. A) Y-axis (FU/min/µg dry wt), X-axis Leg (Depleted & Loaded) ***p*<0.0001 depleted vs loaded leg. B) Y-axis (FU/min/µg dry wt), X-axis time #*p*<0.05 between time points.

a significant difference between the depleted and loaded leg for the times, POST, 60min, and 180min ($p<0.05$), but no significance was found for the time PRE (see Figure 6). A significant difference between 180min loaded and the times, PRE, POST, 60min, and 180min depleted were also found ($p<0.01$). This indicates that protein breakdown mediated by the enzyme chymotrypsin-like within the proteasome decreased in the loaded leg at 180min, see Figure 6. Mean (FU/min/µg dry wt) ± SD for time per leg: Depleted leg, PRE 42.1 ± 7.3 (N = 40), POST 43.7 ± 6.9 (N = 40), 60min 45.1 ± 7.9 (N = 40), 180min 44.8 ± 7.7 (N = 40).

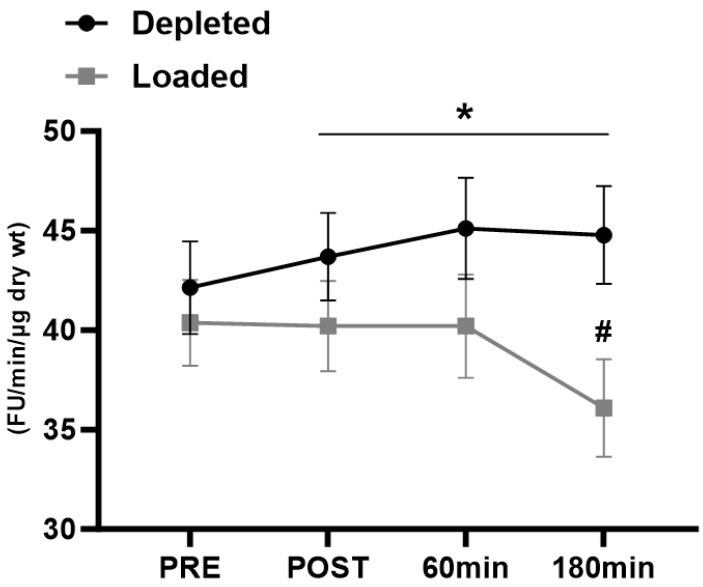

Figure 6 – Data described as mean with 95% CI. Y-axis (FU/min/µg dry wt), X-axis time. ***p*<0.05 depleted vs loaded leg within the same time point. #*p*<0.01 180min vs all time points (depleted and loaded leg).

Loaded leg, PRE 40.4 ± 6.8 (N = 40), POST 40.2 ± 7.1 (N = 40), 60min 40.2 ± 8.1 (N = 40), 180min 36.1 ± 7.7 (N = 40).

5 Discussion

To this day, few studies have been conducted on how human skeletal muscle protein degradation is affected by glycogen availability, this is true for all combined pathways of degradation. No studies were found by the author of this thesis regarding UPS activity in relation to glycogen availability in human skeletal muscle. Therefore, the aim of this thesis was to study how the molecular pathways that regulate protein degradation through the UPS are affected by glycogen availability and how that potentially affects training adaptations. The research questions were framed as follows 1) Does low glycogen availability increase proteasome activity in human skeletal muscle following resistance exercise? and 2) Can ingestion of essential amino acids (EAA) modulate the response in proteasome activity induced by low glycogen availability?

The result of this study shows that low glycogen availability upregulates UPS activity within human skeletal muscle, results of the intervention (Placebo vs EAA) showed that EAA had no effect on UPS-mediated protein degradation regardless of time or glycogen availability, and UPS is minimally affected by acute exercise. Interestingly, the UPS activity was lower in the glycogen-loaded leg after 180min. The underlying biochemical process responsible for this decrease at 180min remains unclear, but a possible hypothesis could be that sufficient energy availability decreases the need for free amino acids to sustain the energy needed for recovery. Since the results state that low-glycogen availability increases the activity of UPS after resistance exercise compared to high-glycogen availability we can accept our initial hypothesis. That “low glycogen availability causes a significant increase in proteasome activity following resistance exercise”. The reason for the increased UPS activity could be explained by an increased need for free amino acids to substitute the energetic demands within the cell during low glycogen availability. As discussed in the introduction of this thesis low glycogen availability has been shown to increase phosphorylation of the protein AMPK that activates the autophagy system, resulting in protein degradation within the cell (Jeukendrup, 2017; Thomson, 2018; Tong et al., 2009). If both UPS and autophagy activity remain upregulated to sustain the energy demand, total protein turnover will increase, and this might result in a negative net protein balance. Similar results have been found by Howarth et al (2010) when measuring whole-body protein kinetics during prolonged exercise. They found

that low glycogen availability resulted in increased protein degradation as well as a decrease in protein resynthesis late in exercise. This led to a greater total protein degradation compared to the high glycogen counterpart. Camera et al (2012) found that low glycogen availability does not affect the anabolic response within human skeletal muscle 4h after resistance exercise. This means that the increased degradation might not negatively affect training adaptations over time if nutritional demands are being met after resistance exercise. When analyzing Figure 6 we can see a non-significant pattern for the depleted leg, which shows a tendency towards elevated proteasome activity that increases over time compared to the loaded leg. If protein degradation through UPS follows this pattern under low glycogen conditions, it might be detrimental to training adaptations if nutritional and energetic demands are not met. We did not measure protein synthesis within the framework of this thesis, and the increased degradation needs to be but in relation to the rate of protein synthesis post-exercise, to enable a greater understanding of the possible adaptations to the given protocol of this study. As this is non-significant, we can't draw any conclusions, merely speculate that an increased number of participants could potentially increase the power and detect a significant difference.

The results of the intervention (Placebo vs EAA) showed that EAA did not affect UPS-mediated protein degradation regardless of time or glycogen availability. We had to reject our hypothesis that EAA would downregulate UPS activity, it seems that EAA has no acute effect on UPS-mediated protein breakdown. The hypothesis was based on EAA´s potential to upregulate mTORC1(Edman et al., 2019; Klionsky et al., 2016), and thereby disrupt the interaction between AMPK and ULK1 which has been shown to downregulate the autophagy activity in human skeletal muscle (Klionsky et al., 2016). Hence the exact regulation of UPS remains unclear, we aimed to investigate if ingestion of EAA would impair UPS activity in a similar manner. Dickinson et al (2017) have shown that ingestion of EAA's post-resistance exercise down-regulates the activity of autophagy in older men, similar to our result they did not detect a significant downregulation of the UPS which further strengthens the rejection of our hypothesis. Biolo et al (1995) found that both muscle protein degradation and synthesis increased post-resistance exercises, and this resulted in a net protein turnover balance closer to zero. Baehr et al (2014) had similar results when exposing mice to functional overload, this increased both protein synthesis and degradation through the UPS. Tipon et al. (2018) discuss that measured protein degradation resulting from exercise to some degree represents the degradation of damaged proteins, which likely are an important part of the remodelling

process of muscle protein's adaptation response to exercise. An upregulated protein turnover (muscle protein synthesis – degradation) that breaks even or has a positive net balance seems to be crucial for muscle protein adaptations to exercise and thereby training adaptations for health or sports performance. Interestingly within the works of this study there is no increase in protein degradation through the UPS in the glycogen loaded leg, only in the depleted leg. It would be interesting to further investigate if there is a difference in proteasome protein expression and content between the legs. A higher proteasome protein content might result in a greater total amount of protein degraded with the same activity rate, due to a greater number of active cites for degradation. It's also possible that the proteasome protein expression was upregulated in the depleted leg due to the increased energetic stress caused by the glycogen depletion protocol. This could potentially lead to a higher total protein degradation after the resistance protocol. Energy deficiency has been shown to increase the expression of ubiquitin ligases MuRF1 and atrogin-1, and thereby potentially upregulate total UPS activity (Carbone et al., 2013). In contrary to the reflection just made, our data did not show a significant difference between the depleted and loaded leg for the time point PRE. This means that the depleted leg didn't have an upregulated UPS activity compared to the depleted when the experimental protocol started. When reflecting on the experimental design, both legs do the same amount of absolute work with different energetic conditions (glycogen depleted vs loaded). This means that the depleted leg will work closer to its maximum capacity compered to its loaded counterpart. Resulting in a higher metabolic stress response within the depleted leg, this could be one factor that upregulated UPS activity in the depleted leg. EAA has been shown to upregulate protein synthesis and the expression of mTORC1 (Edman et al., 2019; Klionsky et al., 2016; Walker et al., 2011). This together with EAA's ability to down-regulate autophagy activity (Edman et al., 2019; Klionsky et al., 2016) might cancel out the upregulated UPS activity caused by low glycogen availability found in the present study. This means that EAA might have a positive effect on the anabolic process when training with low glycogen availability, despite its inability to decrease UPS activity. To maximize the potential adaptations of resistance exercise, the author of this thesis would not recommend training with low glycogen availability. Low glycogen availability can decrease both training volume and intensity and at the same time increase muscle protein degradation. This may negatively affect skeletal muscle mass which in turn may result in decreased performance and training adaptations in individuals with low glycogen availability. Instead focus should be on meeting the nutritional and energetic demands of the given exercise protocol and adaptation goal.

Potential limitations within this paper are the chosen post-hoc test (Tukey's) and that comparisons were not adjusted for false discovery rate (FDR), the threshold for significance for the post-hoc test was only set to $p<0.05$. For statistical validity, Bonferroni's post-hoc test would have been preferable over Tukey's since it's a more rigours comparison of the three-way ANOVA findings. The argument for choosing Tukey's over Bonferroni was that we are not interested in all comparisons that will be made, only Placebo VS EAA and Loaded VS Depleted leg for each time. The Bonferroni comparison will be more conservative than needed, due to the small sample size (for statistical analysis), to choose Bonferroni would have increased the risk of type II error (Sangseok & Dong, 2018). Taking this into consideration, lowering the threshold for detecting significant results decreases the risk of type II errors, but at the same time increases the risk of type I errors. To adjust for this problem more studies and-/or greater sample sizes need to be conducted to enable greater statistical power and clear significant results with less risk of type I and II error. Another limitation of this paper, as just mentioned above is that the estimated power of an N of 20 (men N = 10, women N = 10) resulted in a weaker statistical power than expected. An N of 20 participants is still a large sample size when you consider the rigorous trial protocol with multiple long sessions and the hard invasive nature of 2 x 8 biopsies per participant. And that each participant must complete two entire protocols to be included in the present study. On the other hand, the cross-over design strengthens the study compared to a parallel group design, as just mentioned above it makes the study harder to complete but reduces physiological variations between groups compared to a parallel group design.

In conclusion, glycogen availability has a significant effect on ubiquitin proteasome-mediated protein degradation and essential amino acids do not. Low glycogen availability increases UPS-meditated protein degradation within human skeletal muscle after resistance exercise. Further research will need to investigate if the UPS substrate's trypsin-like and caspase-like are upregulated during low glycogen availability as chymotrypsin-like was in the present study. Low glycogen availability can negatively affect both training volume and intensity and at the same time increase muscle protein degradation. This may negatively affect skeletal muscle mass which in turn may result in decreased performance and training adaptations in individuals with low glycogen availability. Instead focus should be on meeting the nutritional and energetic demands of the given exercise protocol and adaptation goal.

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Appendix 1

Litteratursökning

Syfte och frågeställningar:

Aim: This thesis aims to study how the molecular pathways that regulate protein degradation through the UPS are affected by glycogen availability and how that potentially affects training adaptations.

Research Questions:

1. Does low glycogen availability increase proteasome activity in human skeletal muscle following resistance exercise?

2. Can ingestion of essential amino acids (EAA) modulate the response in proteasome activity induced by low glycogen availability?

Vilka sökord har du använt?

Ämnesord och synonymer svenska	Ämnesord och synonymer engelska
Proteasome, ubiquitin, muskel, kod, fluoruserandet, protein, omsättning, degradation, glykogen	*Proteasome, ubiquitin, muscle, code, fluorescent, protein, turnover, degradation, glycogen*

Var och hur har du sökt?

Databaser och andra källor	Sökkombination
PubMED	((proteasome) AND (ubiquitin)) AND (muscle) ”The ubiquitin code”
Discovery	“making weight for combat sports” ((ubiquitin proteasome system) AND (fluorescent) ((protein turnover) AND (glycogen)

Kommentarer

Articles were also obtained by reading reviews and investigating their references. Articles were also recommended by my supervisor.

Appendix 2

Stockholm 2018-12-20

SAMTYCKE

Projekttitel: Inverkan av glykogentillgänglighet på autofagisignalering och träningsanpassning hos män och kvinnor

Jag har muntligen informerats och därtill tagit del av skriftlig information, fått tillfälle att ställa frågor, fått dem besvarade och samtycker till deltagande i studien samt till behandling av personuppgifter och lagring av prover i biobank (efter medgivande).

…….	…….	……
Datum	Namnteckning	Namnförtydligande

Appendix 3

Reviderad Bilaga 4a Forskningspersoninformation
Stockholm 2019-03-21

INFORMATION TILL FORSKNINGSPERSONER

Projekttitel: Inverkan av glykogentillgänglighet på autofagisignalering och träningsanpassning hos män och kvinnor.

Bakgrund och syfte De molekylära processerna som reglerar muskeluppbyggnad och muskelnedbrytning påverkas av energi- och näringstillgänglighet inuti musklerna. Vid god tillgång på energi och näringsämnen stimuleras uppbyggande (anabola) processer som medför en ökad muskeluppbyggnad medan vid energi- och näringsbrist stimuleras istället nedbrytande (katabola) processer som därmed resulterar i en ökad muskelnedbrytning. Energibrist kan uppstå i skelettmuskeln i samband med högintensivt muskelarbete men även till följd av låga glykogennivåer i muskeln. Tidigare forskning har visat att låga glykogennivåer i sig kan stimulera muskelns nedbrytande processer, och det är därför möjligt att träning som utförs med låga glykogennivåer ger en förstärkt aktivering av muskelnedbrytning jämfört med träning som utförs med höga glykogennivåer. Annan forskning har visat att intag av protein eller aminosyror (proteinets beståndsdelar) hämmar de muskelnedbrytande processerna och istället stimulerar de muskeluppbyggande processerna. Det är därför möjligt att en ökad muskelnedbrytning efter träning med låga glykogennivåer kan motverkas genom intag av aminosyror. Detta är dock i dagsläget helt ostuderat. Syftet med studien är därför att undersöka om styrketräning med låga glykogennivåer ger upphov till kraftigare aktivering av de nedbrytande processerna i muskeln, samt om denna kraftigare aktivering kan motverkas genom intag av aminosyror efter styrketräningen. För att undersöka detta kommer du som studiedeltagare att få styrketräna med ett ben som är glykogentömt kvällen innan, samt ett ben som är glykogenfyllt och därefter få inta en dryck innehållandes enbart smaksatt vatten (placebo) eller aminosyror.

Försöken kommer att genomföras vid Åstrandlaboratoriet på Gymnastik- och idrottshögskolan (GIH) i Stockholm och forskningshuvudman är Gymnastik- och idrottshögskolan.

Hur går studien till?

Förtester
Fyra typer av förtester kommer att genomföras. Vid det första kommer du att få fylla i en hälsodeklaration vars syfte är att säkerställa att du är frisk och kan delta i studien. Du kommer även att genomgå en kroppssammansättningsmätning samt utföra ett enbens-styrketest i benspark. Vid det andra kommer du att få genomföra ett maximalt syreupptagningstest på cykel. Vid det tredje ska du få genomgå ett glykogentömningspass genom att cykla med ett ben. Vid det fjärde kommer du att genomföra det riktiga försöksprotokollet som innefattar styrketräning med ett ben i taget (se nedan). Förtesterna 3 och 4 kommer du att få upprepa inför det andra huvudförsöket (se nedan). Total tidsåtgång för varje förtest beräknas till ca 1-2 timmar. Förtesterna sker med ca 3-5 dagars mellanrum.
Reviderad Bilaga 4a

Inför huvudförsöken Studien innefattar två huvudförsök (se nedan). Båda huvudförsöken kommer att föregås av en 3-dagars förberedelseperiod. På morgonen, tre dagar innan respektive huvudförsök, kommer du att få genomföra ett glykogentömningspass på cykel med två ben. Detta tömningspass kommer medföra att båda benen töms på glykogen. Du kommer därefter att få konsumera en högkolhydratkost under tre dagar vilket medför att båda benens glykogenlager fylls upp maximalt till en nivå som är högre än det var innan glykogentömningen. Detta brukar i regel kallas för att man glykogenladdar. På kvällen (ca 18.00) under den tredje dagen av glykogenladdning så kommer du återigen att få utföra ett glykogentömmande pass på cykel, fast denna gång endast med ett ben. Efter glykogentömningen får du inte äta någon mat utan endast dricka vatten. Detta är extremt viktigt för studieresultatens skull.

Respektive 3-dagars förberedelseperiod kommer att resultera i att du kommer ha ett glykogenfyllt ben samt ett glykogentömt ben inför huvudförsöken som sker dagen efter glykogentömningen.

Under respektive 3-dagars förberedelseperiod skall du avstå ifrån all fysisk träning utöver den som ingår i studien. Under dessa dagar skall du även avstå från alkohol samt från att äta värkmediciner av typen Magnecyl, Ipren, Alvedon etc. eftersom dessa läkemedel kan påverka studieresultaten negativt. Du får heller inte äta någon form av kosttillskott (exempelvis kreatin, koffeintabletter, pre-workout-produkter etc.).

Huvudförsöken
Dagen efter glykogentömningen (se ovan) anländer du till laboratoriet kl. 7.00 på morgonen i ett fastande tillstånd. Det innebär att du inte får äta efter glykogentömningen kvällen innan och du får inte äta eller dricka något annat än vatten på huvudförsökets morgon. Det innebär även att du inte får dricka kaffe, te eller energidryck eller ta någon form av kosttillskott som exempelvis koffeintabletter. Detta är väldigt viktigt eftersom intag av mat eller dryck (annat än vatten) kommer förstöra studieresultaten. Det är också viktigt att du inte stressar till laboratoriet. Skulle du vara sen så är det viktigt att du hör av dig och tar det lugnt. Du skall inte springa eller cykla hit eftersom denna typ av fysisk aktivitet kommer förstöra studieresultaten. Det är därför viktigt att du antingen går, åker bil eller åker kommunalt när du kommer till laboratoriet.

Respektive huvudförsök inleds med att en kateter läggs i en ven vid båda armvecken för upprepad blodprovstagning innan, under och efter träningspasset samt för kontinuerlig infusion av en stabil isotopinmärkt aminosyra (fenylalanin). Det är en naturligt förekommande, icke-radioaktiv och helt ofarlig aminosyra som du redan har i kroppen. Genom att tillföra denna aminosyra i väldigt små mängder under huvudförsöket kan vi mäta din proteinsyntes innan, under och efter träningspasset.

Efter att infusionen startats får du vila i 2.5 timmar varefter en biopsi (se nedan) tas från lårets framsida (vastus lateralis) i respektive ben. Därefter inleds styrketräningen som innebär att du genomför 3 uppvärmnings-set samt 10 arbets-set med ett ben i taget med 3 minuters vila mellan varje set. Belastningen är densamma som du tränat på under förtest 4. Direkt efter styrketräningen tas ytterligare en muskelbiopsi i respektive ben. Direkt efter biopsierna får du inta en dryck innehållandes antingen essentiella aminosyror eller smaksatt vatten (placebo). Därefter får du vila i 60 minuter varefter det tas en muskelbiopsi från vartdera benet. Därefter får du vila i ytterligare 120 minuter varefter de sista två biopsierna tas (en i varje ben). Under hela försökets gång tas blodprover vid upprepade tillfällen. Totalt tas 180 ml blod vilket motsvarar ca 3.5 % av din totala blodvolym vilket motsvarar ungefär en tredjedel av vad som

normalt ges vid en bloddonation. Huvudförsöket tar ca 7,5 h och avslutas ca 14.30. Efter att försöket avslutats får du duscha och äta en måltid på plats.

Vilka är riskerna?

Biopsiprovtagning Biopsiprovtagning utförs av utbildad personal under uppsikt av läkare. Biopsin tas från yttre delen av lårets framsida (vastus lateralis). Inför varje biopsitagning ges lokalbedövning i huden (2-3 ml) varefter det läggs ett ca 0,5 cm snitt i huden. Genom detta snitt tas sedan ett litet muskelprov ut. Muskelbiopsin väger ca 50-100 mg vilket motsvarar storleken av en ärta. Under själva biopsitagningen kan du uppleva en kortvarig obehagskänsla. När bedövningen sedan släpper kan du känna en ömhet under de närmaste dagarna efter biopsitagningen. Efter varje snitt i huden kommer det att bildas ett litet ärr ungefär som vid ett vanligt sår. Under respektive huvudförsök tas sammanlagt 8 muskelbiopsier vilket innebär att det under studien tas totalt 16 biopsier.

Riskerna med biopsitagning är små och det är väldigt ovanligt med komplikationer. Under de två dagarna efter biopsitagningen skall du inte bada för att minimera risken för infektion. Om du duschar, täck över biopsiställena med ett plastskydd.

Vilka är fördelarna? Genom ditt medverkande i studien kommer du att få genomföra fysiologiska tester (styrketest och syreupptagningstest) som ger dig värdefull information om din styrka och kondition som du kan utnyttja i din egen träning och dessa tester är kostsamma om de skulle utföras privat. Som studiedeltagare kommer du även att förses med en stor mängd mat inför perioderna med glykogenladdning samt få tillgång till dina unika data om hur dina muskler reagerar på träning med olika mängd glykogen samt med och utan aminosyror. Denna information är helt omöjligt att få tillgång till utan att medverka i en vetenskaplig studie som denna.

Vad händer med mina prover? Det är vår avsikt att delar av muskelbiopsierna kommer att lagras i en biobank för framtida analyser. Avdelningen vid GIH har en biobank registrerad vid Socialstyrelsens Biobanksregister (org. Nr. 56). Dessa biopsier kan komma att analyseras med hjälp av nya analysmetoder som är under utveckling. Detta kräver ditt medgivande annars förstörs proverna efter det att analyserna i samband med denna studie genomförts. Du har möjlighet att ångra ditt samtycke till att dina prover sparas i biobank. I så fall kommer proverna att kastas eller avidentifieras. Om du ångrar ditt samtycke måste du meddela detta till ansvarig forskare William Apró (se nedan för kontaktuppgifter). Proverna får bara användas på det sätt som du har gett samtycke till. Om det tillkommer forskning som ännu inte är planerad, kommer etikprövningsmyndigheten att besluta om du skall tillfrågas på nytt.

Alla rutiner, protokoll och data kommer att förvaras säkert på filer i en särskild laboratoriemapp och kodade datafiler.

Reviderad Bilaga 4a Forskningspersoninformation

Alla personuppgifter kodas och lagras konfidentiellt och anonymt. Gymnastik- och idrottshögskolan ansvarar för dina personuppgifter. Enligt EU:s dataskyddsförordning har du rätt att kostnadsfritt få ta del av de uppgifter om dig som hanteras i studien, och vid behov få eventuella fel rättade. Du kan också begära att uppgifter om dig raderas samt att behandlingen av dina personuppgifter begränsas. Om du vill ta del av uppgifterna ska du kontakta ansvarig forskare (se nedan). GIHs dataskyddsombud Birgitta Edenius nås på birgitta.edenius@gih.se och 08-120 537 42. Om du är missnöjd med hur dina personuppgifter behandlas har du rätt att ge in klagomål till Datainspektionen, som är tillsynsmyndighet.

Hur får jag information om studieresultaten? Du kan ta del av dina egna resultat om så önskas genom att kontakta ansvarig forskare. Resultaten kommer att publiceras i vetenskapliga tidskrifter utan möjlighet att urskilja dina individuella resultat.

Försäkring och ersättning Under försöken är du täckt av Patientförsäkringen samt av projektspecifik försäkring tecknad hos Kammarkollegiet. Efter helt fullföljt studiedeltagande utgår ekonomisk ersättning om 5000 kr skattefritt. Om du väljer att avbryta ditt studiedeltagande efter det första huvudförsöket utgår ekonomisk ersättning om 750 kr skattefritt. Om du väljer att avbryta ditt deltagande innan det första huvudförsöket utgår ingen ekonomisk ersättning.

Ditt deltagande är frivilligt Om du känner att du inte vill fortsätta eller av någon annan anledning inte kan genomföra försöket har du rätt att avbryta försöket när som helst utan någon som helst påföljd och utan att du behöver ange orsak. Det kommer heller inte att påverka din framtida vård eller behandling.

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