Resistance exercise in the glycogen depleted state increases autophagy signalling

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Abstract

Autophagy is a catabolic pathway responsible for the degradation och muscle proteins and organelles regulated by the energy sensing kinase 5`-adenosine monophosphate activated protein kinase (AMPK) which is sensitive to glycogen availability. **Aim** The primary aim of this study was to evaluate the influence of glycogen availability on autophagy signalling post resistance exercise. A secondary aim was to evaluate the effect of supplementation with essential amino acids (EAA) post resistance exercise on autophagy signalling. **Method** A randomized controlled trial with crossover design was employed. 10 subjects glycogen loaded for two and a half days (10 g carbohydrates/kg/day) and subsequently depleted one leg by interval cycling. The next day, subjects performed 10 sets of 10 repetitions leg extension with one glycogen loaded and one glycogen depleted leg and immediately after the exercise, received either a placebo-drink or a drink containing 240 mg/kg body weight EAA in a randomized order. Muscle biopsies were obtained at baseline, post exercise, 60-and 180 minutes post drink. Muscle glycogen content was evaluated spectrophotometrically. Phosphorylation status of $AMPK^{thr172}$, Unc-51-like kinase 1 $(ULK1)^{ser555}$, acetyl coA carboxylase $(ACC)^{ser79}$ and the total amount of LC3b-II and the LC3b-II/I ratio was analysed with Western blot to evaluate autophagy. A three-way ANOVA was used to analyze for interactions between supplement x glycogen content x time. In case there was no significant interaction effect for the whole model, a two-way ANOVA of glycogen content x time was employed. Statistical significance was set at $p <0,05$. **Results** The glycogen loading- and depletion protocol generated significant differences between legs in muscle glycogen at all timepoints. There were no differences in phosphorylation status at baseline between legs or trials. Post resistance exercise there was significantly higher phosphorylation of $AMPK^{thr172}$, acetyl coA carboxylase $(ACC)^{ser79}$ and $ULK1^{Ser555}$ in the depleted leg compared to the loaded leg, which remained elevated at 60- and 180 minutes post drink during the three hours of recovery. Moreover, there was a significant decrease in levels of LC3b-II and the LC3b-II/I ratio over time in both legs, significantly more so in the depleted leg at 180 minutes post drink. There was no significant differences between trials indicating no effect of EAA-supplementation on autophagy. **Conclusions** Resistance exercise in the glycogen depleted state increases autophagy signaling compared to the glycogen loaded state and this was associated with a decrease in LC3b-II/I ratio. EAA supplementation had no attenuating effect on autophagy. Resistance exercise with low glycogen content could potentially have a negative impact on muscle mass due to increased catabolic signaling.

Sammanfattning

Autofagi är en katabol process som bryter ned muskelproteiner och organeller och denna process styrs av den energikännande kinasen AMPK, vilken i sin tur styrs av glykogentillgänglighet i muskeln. **Syfte** Det primära syftet med denna studie var att utvärdera effekten av glykogentillgänglighet på autofagisignalering efter styrketräning. Ett sekundärt syfte vara att utvärdera effekten av supplementering med essentiella aminosyror (EAA) på autofagisignalering efter styrketräning. **Metod** En randomiserad kontrollerad studie med överkorsningsdesign. 10 deltagare kolhydratsladdade under två och en halv dag (10 g kolhydrater/kg/dag) och tömde sedan ett ben genom intervallcykling. Dagen efter genomförde deltagarna 10 set av 10 repetitioner benspark med ett glykogenladdat och ett glykogentömt ben vilket omedelbart följdes av supplementering med antingen en placebodryck eller en dryck med 240 mg/kg kroppsvikt av essentiella aminosyror i en randomiserad ordning. Muskelbiopsier erhölls i vila, efter träning, samt 60- och 180 minuter efter dryck. Muskelglykogen utvärderades med spektrofotometri. Fosforylering av $AMPK^{thr172}$, $ULK1^{ser555}$, ACC^{ser79} och total mängd LC3b-II samt LC3b-II/I kvot analyserades med Western blot för utvärdering av autofagi. En trevägs-ANOVA användes för analys av interaktioner mellan supplementering x glykogeninnehåll x tid. Vid ingen effekt av hela modellen, analyserades istället glykogeninnehåll x tid med en två-vägs ANOVA. Statistisk signifikans sattes till $p < 0,05$. **Resultat** Glykogenladdnings- och tömningsprotokollet genererade signifikanta skillnader mellan benen i muskelglykogen vid samtliga tidpunkter. Det var ingen skillnad i proteinfosforylering mellan ben och försök. Efter styrketräning var det signifikant högre fosforylering av $AMPK^{thr172}$, acetyl coA carboxylase $(ACC)^{ser79}$ och $ULK1^{Ser555}$ i det tömda benet jämfört med det laddade vilket kvarstod vid 60- och 180 minuter efter dryck. Dessutom var det en signifikant minskning av LC3b-II och LC3b- II/I kvot över tid i båda benen, och signifikant större minskning i det tömda benet vid 180 minuter efter dryck. Det var inga interaktionseffekter av supplementering vilket indikerar ingen effekt av EAA-supplementering på autofagi. **Konklusion** Styrketräning i glykogentömt tillstånd ökar autofagisignalering jämfört med i glykogenladdat tillstånd och detta var associerat med en minskad LC3b-II/I kvot. EAA supplementering hade ingen dämpande effekt på autofagi. Styrketräning med lågt muskelglykogen kan potentiellt ha en negativ effekt på muskelmassa på grund av ökad katabol signalering.

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1 Introduction

The accretion of muscle mass depends on the rate of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), and the balance between the two, referred to as the net balance (NBAL) (Tipton, Hamilton, & Gallagher, 2018; Francaux & Deldicque, 2019). A positive net balance occurs when protein synthesis exceeds MPB (Burd, Tang, Moore & Philips, 2009). It may therefore be beneficial to maximize muscle protein synthesis and minimize muscle protein breakdown for the purpose of gaining muscle mass. MPS increases from resistance exercise (RE) (Wackerhage et al., 2019), and from protein supplementation (Morton et al., 2018) in a dose dependent manner through activation of the mechanistic target of rapamycin complex 1 (mTORC1)-pathway and its downstream targets eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1) (Smiles et al., 2016). Supplementation with approximately 20 g of protein is sufficient to stimulate MPS maximally after a single bout of resistance exercise (approximately 8,6 g Essential amino acids) Moore et al., 2009). Neither resistance exercise alone, nor protein supplementation, alters the NBAL to the positive since RE also increases MPB (Burd et al., 2009; Biolo et al., 1995). Both exercise and nutrition are therefore required for a positive net balance. While EAA supplementation increases anabolic signalling (Apró et al., 2015a), low intramuscular glycogen content has been seen to increase MPB (Howarth et al, 2010).

Anabolism and catabolism are ongoing processes regulated by energy availability, with anabolism dominating in energy rich conditions, and catabolism during energy deficiency (Russel et al., 2014). MPS and its pathways are well characterized (Tipton et al., 2018) and have greater variability in magnitude, with approximately two-fold increases in muscle protein fractional synthetic rates after a single bout of resistance exercise (Biolo et al., 1995). MPB has not been studied to the same extent (Tipton et al., 2018), however has been shown to increase with up to 50% in fractional breakdown rates after exercise (Biolo et al., 1995).

Muscle protein breakdown is comprised of three different systems: the ubiquitin proteasome pathway, calpain Ca^{2+}- dependent cysteine proteases and autophagy (Tipton et al, 2018). Proteasomal degradation, through the E3 ubiquitin ligases muscle specific ring finger protein 1 (MuRF1) and muscle atrophy F-box (MAFbx) (Bell et al., 2016), stands for a large part of total protein break down, however it cannot degrade myofibrillar proteins alone (Tipton et al,

2018). The calpain system seem to target myofibrillar proteins, making them available for proteasomal break down (Tipton et al, 2018). While more is known about ubiquitin-proteasome degradation in human muscle, autophagy has been less studied. Autophagy is a catabolic pathway characterized by the formation of double membrane vesicles known as autophagosomes (Bell et al., 2016). Autophagosomes engulf damaged cellular proteins and organelles, and fuses with the lysosome for subsequent breakdown of the autophagosome and its content (Bell et al., 2016). Resulting amino acids from the degradation process may be re-used for energy or other anabolic processes (Tipton et al., 2018; Vainshtein & Hood, 2016). Autophagy has been seen to be particularly active in muscle cells (Francaux & Deldicque, 2019), targeting the receptors, ion- channels and membranes (Tipton et al., 2018). Basal autophagy is required for normal muscular function as blocking of autophagic genes in muscle (ATG7), has been shown to decrease muscle mass and force production in mice compared to controls (Masiero et al., 2009). Thus, autophagy seems to be necessary for maintaining muscle mass and for quality control of organelles in muscle cells.

1.1 Regulation of autophagy

Autophagy stands under the regulation of both catabolic and anabolic kinases (i.e. enzymes that phosphorylate other proteins) increasing or decreasing autophagy, respectively. Autophagy induction is regulated by the enzyme 5`-adenosine monophosphate activated protein kinase (AMPK) whereas mTORC1 inhibits the autophagic process. AMPK is known as an energy sensing enzyme reacting to intracellular energy availability (Thomson, 2018). During stressful situations, such as limited energy supply or intense exercise, the ATP-AMP ratio decreases which activates AMPK (Thomson, 2018) through thr172 phosphorylation by liver kinase B1 (LKB1) (Janzen et al., 2018). AMPK exists in complexes, consisting of α, β and γ subunits existing in different combinations (Janzen et al., 2018). There are two different α-subunits, two different β and three γ (Thomson, 2018). The activating phosphorylation site thr172 and the catalytic subunits are located within the α-domain (Thomson, 2018), AMPKα2 being most active in human skeletal muscle in response to glycogen content (Wojtaszewski et al., 2003). The β-subunit contains a glycogen binding domain and the γ-subunit has a regulatory role as it senses the levels of nucleotides (ATP-ADP-AMP) (Thomson, 2018). The activity of AMPKα2 has been seen to increase due to several energy consuming activities such as continuous endurance exercise at both high (70 % VO_2peak) and low (55 % VO_2peak)

intensities (Schwalm et al., 2015), ultra-marathon running (Jamart et al., 2012) and high intensity interval cycling (Apró et al, 2015b).

Energy stress occurs when demands are higher than supply. It was shown in rodents that super-compensated glycogen levels (100-250% of normal) decreases basal AMPKα2 activity as opposed to slightly lowered stores (80% of normal) (Wojtaszewski et al., 2002). In humans, low intramuscular glycogen stores (163 mmol/kg dry weight) increases AMPKα2 activity, both at rest and after endurance exercise compared to super compensated glycogen stores (909 mmol/kg dry weight) (Wojtaszewski et al., 2003).

One of the targets of AMPKα is Unc-51-like kinase 1 (ULK1) which is phosphorylated at Ser555 (Russel et al., 2014; Fritzen et al., 2016) or Ser317 (Schwalm et al., 2015) and initiates the formation of autophagosomes (Russel et al., 2014). Besides its role in autophagy induction, AMPK has been shown to decrease activity of mTORC1 through phosphorylation of tuberous sclerosis complex 2 (TSC2), a protein complex upstream of mTORC1 which acts to block mTORC1 activity (Inoki et al., 2003). Furthermore, activation of AMPK with 5-aminoimidazole-4-carboxamide ribonucleoside ((AICAR) an AMPK activating drug which does not change energy availability), has also been shown to increase AMPK activity towards the mTORC1 subunit raptor leading to decreased activity towards to mTORC1´s downstream targets (Gwinn et al., 2008). Thus, AMPK activity may affect the NBAL through autophagy induction via ULK1 phosphorylation and simultaneously by the inhibition of anabolic processes through inhibition of mTORC1 activity.

ULK1 is a crucial protein in autophagy regulation as both anabolic (mTORC1) and catabolic (AMPK) kinases may phosphorylate the protein at different sites decreasing or increasing autophagy signalling respectively. As described, the nutritional status seems to be decisive for the outcome. In nutrient balance, mTORC1 phosphorylates ULK1 at ser757 (Russel et al., 2014), thus blocking ULK1´s downstream signalling for autophagy. Long term fasting (i.e energy stress) has been shown to decrease this inhibitory phosphorylation of ULK1 at Ser757 compared to the post absorptive state (Vendelbo et al., 2014). On the other hand, insulin injections have been seen to increase mTORC1's inhibitory phosphorylation of ULK1 at ser757 (Vendelbo et al., 2014; Fritzen et al., 2016) confirming that anabolic pathways regulate ULK1. Furthermore, it was shown by Creer et al (2005) that resistance exercise with

high glycogen availability (591 mmol/kg dry wt) increases Akt-phosphorylation upstream of mTORC1 post RE compared to low glycogen (174 mmol/kg/dry wt).

Although high glycogen content has been seen to increase Akt phosphorylation upstream of mTORC1, glycogen availability does not seem to affect MPS post RE (Creer et al., 2005). Furthermore, Camera et al (2012) compared RE with one depleted and one glycogen loaded leg with no effect on $AMPK^{thr172}$ phosphorylation and no difference in MPS between legs. Similar results were found by Apró et al (2015b), who investigated the effects of concurrent exercise on MPS. It was shown that MPS was not attenuated post resistance exercise despite prior AMPKα2 phosphorylation from interval cycling.

1.2 Evaluation of autophagy

The whole autophagic process from induction to degradation in the lysosome, is commonly assessed by the ratio between microtubule-associated protein- 1b light chain 3 (LC3b) II/I. As mentioned, AMPKα and ULK1 are involved in the induction process and through a cascade of signals, autophagosome formation increases. LC3b is a protein involved in the lipidation and membrane formation of the phagophore (precursor to the autophagosome) (Vainshtein & Hood, 2016). LC3b exists in two versions, LC3b-I exists in the cytosol and is converted to LC3b-II in the process of membrane formation of the phagophore (Klionsky et al., 2016). LC3b-II is located at the membrane of the autophagosome and interacts with p62 and ubiquitin for recognition and engulfment of damaged proteins (Vainshtein & Hood, 2016). The autophagosome subsequently fuses with the lysosome for degradation of itself and its cargo (Vainshtein & Hood, 2016). The amount of LC3b-II is considered to be a reliable marker of the number of autophagosomes present in the sample at a specific time (Francaux & Deldicque, 2019; Klionsky et al., 2016; Martin- Rincon., 2018) and the ratio between LC3b-II and LC3b-I is often used as a marker of autophagosome synthesis and autophagic degradation since LC3b-II is degraded in the process along with the autophagosome (Klionsky et al, 2016; Martin-Rincon et al., 2018). Thus, an increased amount of LC3b-II indicates higher autophagosome presence in the sample which might be due to either an increased autophagosome formation, or a decreased degradation rate (Martin- Rincon., 2018). On the other hand, the LC3b- II/I ratio may decrease despite an increased autophagosome formation due to increased speed of lysosomal degradation (Klionsky et al, 2016). Because of

the complexity of interpretation of the amount of LC3b-II and LC3b- II/I ratio, it is important to consider other markers in the autophagic pathway (AMPK, ULK1) in the analysis for a comprehensive view.

Previous research on autophagy in response to exercise show conflicting results. After high intensity endurance exercise, the LC3b-II/I ratio has been shown to decrease along with decreased levels of p62 (Schwalm., et al, 2015). Contrary to this, the LC3b-II/I ratio has been shown to increase in response to exercise and even more so in the fasted state in mice indicating a possible difference between species (Jamart et al., 2013). Furthermore, levels of LC3b-II have been shown to increase after ultra-marathon running, indicating autophagosome accumulation, however LC3b-II/I ratio was not investigated (Jamart et al., 2012).

Only a few studies have investigated autophagy post resistance exercise. Fry et al (2013) showed decreased LC3b-ratio 3, 6 and 24 hours after leg extension exercise in both young and old adults, but no significant alterations in AMPK phosphorylation. Glynn et al (2010) showed increased AMPKα phosphorylation 1- hour post exercise as compared to baseline which was accompanied with a decreased LC3b-II/I ratio at 2 hours post exercise. Furthermore, Ato et al (2017) showed increased AMPKα phosphorylation as well as increased ULK1 ser317 phosphorylation post electrical stimulation, mimicking resistance exercise, in rat. However, no differences in LC3b II/I ratio or p62 were found. Smiles et al (2015) investigated the effect of a five-day energy deficit and EAA supplementation post RE on autophagy signalling and found no difference in ULK1 ser757 phosphorylation between EAA and placebo, but increased phosphorylation compared to rest. However, no study combined lowered glycogen content with resistance exercise to evaluate autophagy.

The different cell signalling responses seen in nutrient deficiency and sufficiency affects the NBAL. Since NBAL is comprised of MPS and MPB, an excessive MPB post resistance exercise might be negative for both athletes and recreational exercisers who aim to increase muscle mass for performance or function in everyday life. Both glycogen availability (Wojtaszewski et al., 2002; Jamart et al., 2013) and endurance exercise (Jamart et al., 2012; Schwalm et al., 2015) has been shown to affect autophagic markers or inductors of autophagy (i.e. AMPK). However, studies on the combination of glycogen availability and its effect on autophagy post resistance exercise, the largest stimuli for MPS (Wackerhage et al., 2019), do

as of yet not exist. Furthermore, the potential implications of EAA-supplementation to reduce autophagy has only been investigated in one study (Smiles et al., 2015) and results were only reported for LC3b-I and $ULK1^{ser757}$. Given the potential role of glycogen and exercise to affect AMPK-activity, and mTORC1´s inhibitory phosphorylation of ULK1, the purpose of this study was to evaluate the influence of glycogen availability on autophagy signalling post resistance exercise. Furthermore, since anabolic pathways are regulated by mTORC1 which also may inhibit autophagy, and mTORC1 activation increases with EAA supplementation (Apró et al, 2015a) a secondary purpose was to evaluate the effect of EAA supplementation on autophagy signalling post RE. It was hypothesized that autophagy signalling would increase due to low glycogen availability post resistance exercise and that essential amino acids would attenuate this effect.

2 Research questions

Specific research questions were:

- Does resistance exercise in the glycogen depleted state affect phosphorylation of $AMPK\alpha^{thr172}$, $ULK1^{ser555}$, and ACC^{ser79} compared to the glycogen loaded state?
- Does resistance exercise in the glycogen depleted state affect total content of LC3b-II or the LC3b-II/I ratio compared to the glycogen loaded state?
- Does EAA-supplementation influence phosphorylation of $AMPK\alpha^{thr172}$, $ULK1^{ser555}$, and ACC^{ser79} or the content of LC3b-II and the LC3b-II/I ratio compared to placebo?

3 Method

This master thesis was carried out as part in an ongoing project in Dr William Apró's research group.

3.1 Overview of study design

A randomized controlled trial with crossover design was employed. The study consisted of a screening process of volunteering subjects prior to inclusion, a familiarization process to minimize learning effects and making sure subjects could perform the study protocol, and the experimental trials where subjects performed resistance exercise with one depleted and one glycogen loaded leg (Figure 1). The familiarization process and the experimental trials were performed twice, with randomization between placebo or EAA-supplementation post

resistance exercise. Experimental trials were separated by ≈ 4 weeks. In total, each subject reported to the laboratory 13 times (Figure 1).

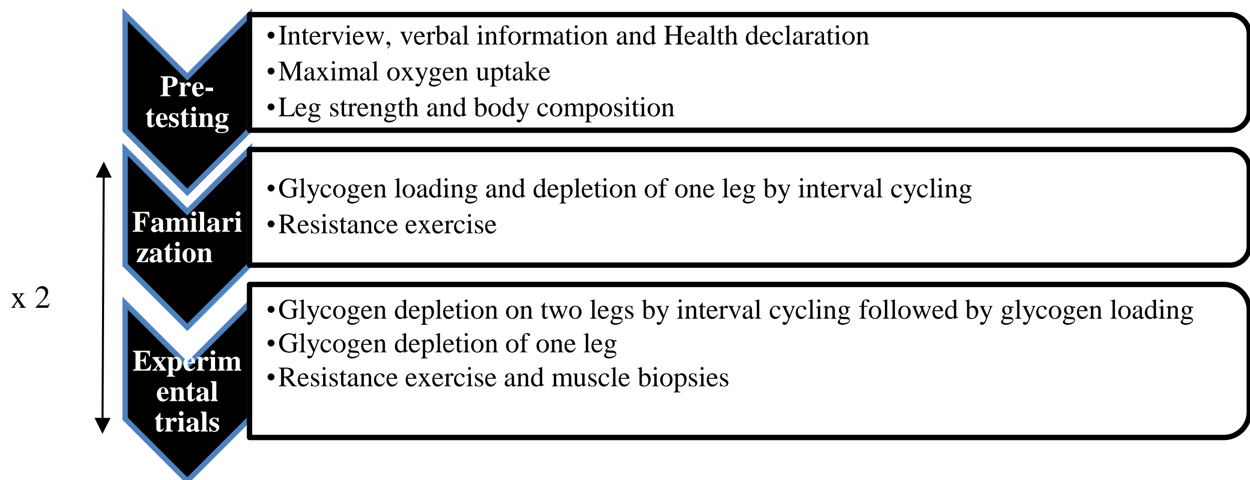

Figure 1. Overview of study design.

3.1.1 Subjects

Subjects were recruited continuously through ads posted on noticeboards at Gymnastik- och idrottshögskolan, surrounding universities and gymnasiums. Information was also spread on social media etc. In total, ten subjects (6 men/4 women) volunteered and were included in this master thesis. Subjects were included if they were healthy, non-smoking, between 18-40 years old, been training resistance exercise at least twice per week for the past year and were not consuming any dietary supplements during the study period. Subjects were excluded if they had any kind of injury or disease or were not able to complete the study protocol during the familiarization process described below. Previous research investigating similar molecular markers have shown that eight subjects have been enough to detect significant differences between conditions (Apró et al 2015b).

All subjects were given written (appendix 3) and oral information about the study and all associated risks before written informed consent (appendix 2) was obtained. Subjects filled in a health declaration before inclusion to screen for disease and injuries.

3.1.2 Preliminary testing

Preliminary testing was performed a few weeks before the trials. Subjects performed an incremental bicycle (SRM Ergometer, Germany) test prior to inclusion to determine maximal

oxygen uptake (VO_2max) and watt peak. After an initial warm up period of 8 minutes (100 watt (W) men, 70 W women), the trial started at 2-3 W/kg body weight at a cadence of 90 revolutions per minute (RPM). The load increased with five W every 15 seconds for men and three W every 15 seconds for women. Subjects continued until volitional fatigue which was defined as when subjects could maintain cadence over 80 RPM. Expired air was measured and analysed continuously (Oxycon pro, Erich Jaegar, Hoechberg, Germany). VO_2max was determined as the average of the four highest measure points in a row expressed in ml/kg/min. Watt peak was defined as the last completed work-intensity before the subjects reached failure.

On a separate day, subjects reported to the laboratory at 07.00 after an overnight fast, rested for five minutes in a supine position and body composition was measured with bio-impedance according to manufacturer's instructions (Quadscan 4000, Bodystat). Thereafter, subjects one-legged 10 repetition maximum (10 RM), defined as the maximal weight subjects could lift in full range of motion 10 times, was determined in a leg extension machine (Cybex Eagle). After three sets of warm up with successively increasing weight, subjects lifted an estimated 10 RM weight 10 times. Depending on performance, the weight was either increased or decreased the following set. Rest between sets was three minutes. When 10 RM had been determined, the contralateral leg repeated the procedure. Subjects were asked to refrain from exercise 48 hours prior to the test.

3.1.3 Familiarization

In the purpose of minimizing learning effects, subjects performed a shortened version of the study protocol approximately one week before the experimental trials. Briefly, subjects commenced glycogen loading (10 g carbohydrates/kg body weight) for a day and a half to get accustomed to this procedure. Subjects reported to the laboratory in the evening of the second day for seated interval cycling (Monark RC6, Vansbro, Sweden) with the purpose of depleting muscle glycogen in one leg. Two 2,5 kg weight plates were used as a counterweight on the contralateral side to make it easier to maintain proper cadence. The cycling protocol has been shown to effectively reduce glycogen levels significantly (van Loon et al, 2000). Furthermore, subjects performed five intervals of two minutes arm cranking with two minutes rest between sets to minimize restoration of glycogen during the night. The morning after,

subjects performed the resistance exercise protocol (described below) in a fasted state thus concluding the familiarization process.

3.1.4 Resistance exercise protocol

The morning after interval cycling, subjects arrived at 07.00 after an overnight fast for unilateral RE on a leg extension machine (Cybex Eagle). Warm up consisted of 3 sets of 10 repetitions with increasing load and two minutes rest between sets. After warm-up, subjects rested three minutes and performed 10 sets of 10 repetitions with one leg at a starting weight of the 10 RM previously determined. RE commenced with the glycogen depleted leg, followed by the glycogen loaded leg after 90 seconds of rest. Total rest time for the glycogen depleted leg was three minutes before set 2 was initiated. Help was given manually in the concentric phase when subjects were not able to lift the weight in full range of motion. The weight was decreased for the following set when subjects could not perform at least seven full repetitions on their own. Number of repetitions were matched between legs and subjects were verbally encouraged to match time under tension.

Subjects were allowed to eat habitually after the familiarization session until glycogen loading commenced during the experimental trials and asked to refrain from exercise 48 hours before the start of the experimental trials described below.

3.2 Experimental trial

The experimental trials were preceded by a three-day preparation period (day 1-3) leading up to the experimental day (day 4). Day 1, approximately 72 hours before the start of the experimental trials, subjects arrived after an overnight fast to the laboratory at ≈ 0700 for interval cycling on two legs (Monark 839E, Vansbro, Sweden) in the purpose of depleting both legs of glycogen and thereby increasing glycogen synthase activity (Wojtaszewski et al., 2003) an enzyme involved in glycogen synthesis (Burke et al., 2017). Following five minutes of warm up at 50% of watt peak (determined from VO_2max test) subjects cycled at 90% of watt peak for two minutes with a cadence of 70 RPM, followed by active rest for two minutes at an workload of 50%-watt peak. This proceeded until subjects no longer could keep the cadence at 70 RPM. Following failure at 90 %, workload was decreased to 80%-, 70%- and finally 60%-watt peak. If necessary, adjustments to the protocol were made in the purpose of

making sure the subjects could complete the cycling protocol. Following interval cycling, subjects commenced glycogen loading (10 g carbohydrates/ kg bodyweight/day) for two whole days (day 1-2). Sixty-five % of carbohydrates were provided by meals, and 35 % from carbohydrate drinks (Vitargo carboloader-powder). Meals consisted of slices of white bread for breakfast (Jättefranska, Pågen), and pasta (Strozzapreti, Zeta) with pre-prepared pasta sauces (Zeta) for lunch and dinner. Subjects were allowed to divide lunch and dinner on three meals if necessary. Vitargo powder had the flavour of orange or summer fruits (91,7% carbohydrates), was mixed with water and the daily amount was spread out on three occasions in between meals. Food and drinks were weighed and provided from the laboratory packed in portions simplifying the procedure for the subjects. Subjects were instructed not to eat or drink anything else than the food provided, excluding water, during glycogen loading. They were also asked not to consume any dietary products and refrain from exercise. Furthermore, subjects were to fill in a dietary notebook which test-leaders collected after glycogen loading. Day 3, subjects ate the same breakfast as previous days (1.6 g carbohydrates/kg bodyweight), half the amount of carbohydrates for lunch (1.2 carbohydrates/kg bodyweight) and at 15.00 two protein bars (Star Nutrition) which contained 20 g of protein, 13-15 g carbohydrates and 7.0-8.3 g fat. Subjects arrived at the laboratory at 18.00 day 3 for seated interval cycling (Monark RC6, Vansbro, Sweden) on one leg according to the same protocol described above for glycogen depletion on two legs. Workloads were determined in the same manner as before but divided by two, rounded to the closest watt-level of five because of the bicycle's settings. After the protocol, one leg would have been depleted, while the other remained glycogen loaded. To minimize restoration of glycogen in the depleted leg during the night, subjects also performed 10 intervals of two minutes arm cranking (Monark Ergomedic 891E, Vansbro Sweden) at 70 RPM with a workload of approximately 105 w (men) or approximately 70 w (women) with two minutes sitting rest in between. After the depletion protocol, the subjects were instructed not to eat or drink anything other than water until after the experiments the day after.

The next morning subjects arrived ≈ 0600 to the laboratory after an overnight fast for the experimental trials (day 4). After a baseline blood sample, venous catheters were inserted in the antecubital vein for repeated blood sampling and stable isotope infusion (13C6-phenyl-alanine) with an infusion rate of 0,005 mmol/kg/hour reaching steady state after 150 minutes. Analysis of data from isotope infusion and blood samples were not included in this thesis.

After 150 minutes infusion in a rested position, a resting muscle biopsy was obtained from the *vastus lateralis* muscle in both legs, starting with the glycogen depleted leg. Subjects then performed unilateral resistance exercise as during the familiarization process, 10 x 10 repetitions at a load of 10 RM. Exercise began with the glycogen depleted leg followed by the loaded leg, and the number of repetitions were matched between legs. Immediately after the 10th work set for the depleted leg, another biopsy was harvested, before the loaded leg completed the exercise after which another biopsy was collected from the loaded leg. Immediately thereafter, subjects were given either a drink containing 240 mg EAA/ kg bodyweight (Fight pharm) or a placebo drink in a randomized order and the test-leaders were blinded. The EAA- solution and dosage has been used previously and shown to potently stimulate mTORC1 activation (Apró et al., 2015a). The placebo drink was apple flavoured and calorie free. After one and three hours of recovery (post drink) additional biopsies were collected in each leg, always starting with the depleted leg.

3.3 Muscle Biopsies and blood sampling

In total 16 biopsies were obtained from the *vastus lateralis* muscle over the two trials, eight from each leg. Biopsies were harvested under local anaesthesia (carbocain 20 mg/ml Astra Zeneca, Sweden) with a 5 mm Bergström needle modified for suction. Each biopsy was taken from a new incision separated by 2-3 cm. Biopsies were obtained by a researcher with extensive experience of the procedure, blotted free from blood and immediately frozen in liquid nitrogen and stored at -80°C for later analysis.

Eleven ml blood was sampled at baseline, and after 30, 60, 90, 120- and 150-minutes post infusion. Blood was also sampled after the 4th - and 9th set of leg extension and immediately after termination of exercise. Post drink, blood was sampled after 10, 20, 30, 45, 60, 90, 120, 150 and 180 minutes.

3.3.1 Tissue processing and analysis

Biopsies were freeze dried and cleaned from connective tissue, fat, and blood under a light microscope. Approximately 3 mg muscle was used for glycogen analysis and 5 mg was used for Western blot.

3.4 Muscle glycogen analysis

Muscle glycogen was analysed according to Leighton et al (1989). Approximately 3 mg of muscle was dissolved in 150 µl of 1 M KOH and heated for 15-20 minutes at 70°C. 60 µl of sample was transferred to a new 1.5 ml Eppendorf tube and 7 µl glacial acetic acid was added to each sample creating an acidic environment (pH 4.8). 200 µl of 0.1 M NaAC-buffer with 0.08 mg amyloglycoisedase (Sigma-Aldrich) was added per sample. Samples were heated for 4 hours at 40°C and vortexed once per hour (Vortex Genie 2, Scientific industries). 20 µl was then transferred to a new 1.5 ml Eppendorf tube and one ml of reagent solution containing 0.65 ml TEA-buffer, 0.35 ml double distilled water (ddH_2O), 4.1 mg ATP, 0.5 mg NADP, 1 µl G-6-PDH and 1 µl Hexokinase was added to each sample. 365 µl was thereafter transferred from each tube to a 96 well cell culture plate in duplicates including blanks (1M KOH only). 30 minutes after reagent was added to the samples, the absorbance was measured spectrophotometrically (Infinite F200 Pro, Tecan) at 340 nm.

Glycogen was calculated according to following formula:

$$Absorbance\ measured - absorbance\ Blank * 150\ * \left(\frac{267}{67}\right) * 1.12\ /\ weight\ in\ mg.$$

Where 150 µl is the total volume of dissolved sample from which 60 µl was drawn and 7 µl glacial acetic acid was added. 267 µl is the total volume of the sample after sodium-acetate was added. 1.12 is the dilution factor after adding 7 µl of glacial acetic acid to 60 µl sample.

3.5 Western Blot

Western blot is a method for detection of specific proteins in a cell, tissue or sample by using specific antibodies to the protein of interest (Gosh et al., 2014) and is one of the most widely used methods for assessment of autophagy (Oh et al., 2017; Gosh et al., 2014; Klionsky et al, 2016). To determine induction of autophagy, phosphorylation status of $AMPK^{thr172}$ and $ULK1^{ser555}$ was analysed. For activity of AMPK, p-ACC was analysed at ser79. Furthermore, total LC3b-II and ratio between LC3b-II/I was used to determine autophagosome content and autophagosome synthesis respectively. Comparison between protein phosphorylation in muscle biopsies from the depleted and loaded leg was used to determine the impact of glycogen availability on autophagy signalling. Differences in protein phosphorylation between trials, in post drink biopsies, were used to evaluate the effect of EAA supplementation.

3.5.1 Muscle homogenization

Approximately five mg of cleaned muscle were homogenized in 500 µl homogenization buffer containing 2 mM HEPES (pH 7.4), 1 mM EDTA, 5 mM EGTA, 10 mM $MgCl_2$, 50 mM β-glycerol phosphate, 1% TritonX-100, 1 mM Na_3VO_4, 2 mM dithiothreitol, 20 µg/ml leupeptin, 50 µg/ml aprotinin, 1% phosphatase inhibitor cocktail and 1 % Halt Protease inhibitor cocktail. Samples were homogenized in a Bullet Blender (Next advance, New York, USA) by the addition of 0.5 mm zirconium oxide beads (Science Imaging Scandinavia) for a minimum of 6x1 minute, or until samples were visually dissolved. No sample were ran more than 8x1 minute. Samples were thereafter shaken (IKA-vibrax-VXR) for one hour at 2200 rpm at 6-7° C.

Homogenates were kept ice cold and transferred to a new 1.5 ml Eppendorf tube with safety cap and spun at 16.1 g for 10 minutes at 4° C. The resulting supernatant was transferred to a new 1.5 ml Eppendorf tube and the remaining pellet was frozen in -80° C for analysis outside the scope of this thesis. 10 µl of the supernatant was transferred to a new 1.5 ml Eppendorf tube and diluted in 40 µl of ddH_2O for analysis of protein concentration (see below). The remaining supernatant was stored at -80 °C.

3.5.2 Protein concentration

Ten µl of each diluted sample (see above) was pipetted in triplicates into a 96 well cell culture plates and compared to pre-diluted protein assay standards (Bovine serum albumin set, Thermo scientific) for determination of protein concentration. The standards generate a standard curve of protein concentration which each sample was compared to (Figure 2). Samples and standards were incubated for five minutes in 150 µl of pierce^{TM} 660 nm protein assay buffer (Thermo Scientific) and after five minutes the absorbance was measured spectrophotometrically (Infinite F200 Pro, Tecan) at 650 nm.

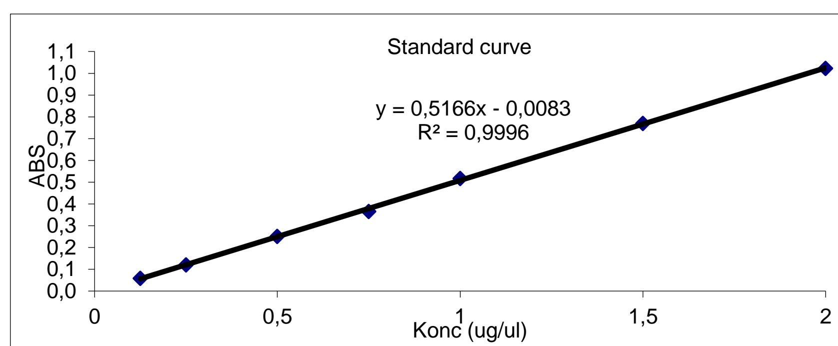

Figure 2. Example of standard curve of protein concentration.

Protein concentration was subsequently calculated according to following equation generated from the standard curve:

$$Conc = (\frac{Mean\ absorbance - Blank + constant}{slope}) * 5$$

Samples were subsequently diluted in Chaps buffer to the same protein concentration and a total volume of volume 75 µl. 25 µl of 4x Laemmli sample buffer (Bio Rad laboratories) containing 400 mM DTT was added to each sample, making the final protein concentration 1µg/µl. Samples were then centrifuged shortly and heated at 95 °C to for five minutes to denature proteins.

3.5.3 Electrophoresis and membrane transfer

Proteins were separated by size through electrophoresis on Criterion TGX precast gels (Bio Rad laboratories, California, USA) in electrophoresis buffer. 20 µl of sample (equals 20 µg protein), was loaded into each well and the gel was run for 30 minutes at 300 Volt. Proteins were then transferred to a polyvinyl difluoride membrane (PVDF) by electrophoresis in transfer buffer for 3 hours at 0,3 A. Membranes were thereafter washed five times in ddH_2O and stained with MemCode (Pierce^{TM} reversible protein stain kit, ThermoFisher Scientific), and proteins where visualized through chemiluminescence using Chemidoc^{TM} MP imaging system (Bio-Rad laboratories). Membranes were afterwards de-stained with de-stain solution and methanol and cut in accordance with suitable size for the specific proteins intended to be analysed. Thereafter, membranes were washed five times in ddH_20 and subsequently blocked for one hour in Tris buffered saline with 0.1% tween (TBST) and 5 % non-fat dry milk. Membranes were then washed for 3x3 minutes in TBST before application of primary antibodies in TBST and 2.5% non-fat dry milk or 2.5 % bovine serum albumin (BSA) and incubated overnight at 4-6°.

The morning after, primary antibodies were removed, and membranes were washed in TBST (2x1 minute, 3x5 minutes) to remove unbound antibodies. Secondary antibodies were applied for one hour in TBST and 2.5 % non-fat dry milk and subsequently washed in TBST (2x1 and 3x5 minutes). Enhanced chemiluminescence (Pierce^{TM} SuperSignal, ThermoFisher Scientific) was applied for 5 minutes before visualization of bands (Chemidoc MP, Bio-Rad laboratories). Visualized bands and MemCode were quantified using Image Lab version 6.1. (Bio-Rad laboratories). Background was subtracted locally for blots and globally for

Memcode in the software. Due to incomplete stripping of the membranes, phosphorylated proteins were related to MemCode instead of total amount of the specific proteins.

3.5.4 Antibodies

Primary antibodies from rabbit were purchased from Cell Signalling Technology. The following antibodies were diluted in TBST with 2,5 % non-fat dry milk or BSA and applied, p-AMPKα^{thr172} #2535 (1:1000), p-ULK1^{ser555} #5869 (1:500), p-ACC^{ser79} #11818 (1:1000), and total LC3b #3868 (1:1000). The Antibody against LC3b recognizes both the LC3b-I and -II isoform. Secondary antibodies against rabbit were from Cell Signalling Technology and were used at a concentration of 1:10 000.

3.6 Ethical considerations

Ethical permission was granted by the ethical review board in Stockholm (DNR:2019-00381). Subjects who volunteered to participate were given oral and written information about the study, see appendix 3, and written consent was acquired before inclusion (appendix 2). Subjects were well informed that they can terminate their participation without giving a reason at any time. Subjects were under the duration of the study insured by Patientförsäkringen and project specific insurance. Subjects were compensated after full participation.

Personal numbers were collected and held by Gymnastik- och Idrottshögskolan. To ensure that collected data could not be connected to a specific subject, subjects were coded, and the key was kept by one of the authors separated from the raw data. Blood samples and muscle biopsies were coded and stored in a biobank registered at Socialstyrelsen. Obtained samples were only used for the outlined purposes.

The study was not associated with major risks. The muscle biopsies were performed by an experienced researcher who has extensive experience from obtaining muscle biopsies and a medical doctor was always on call during each experimental trial should there be a need for medical consultation. As with all invasive methods, there is a risk for infection, and for that reason the use of sterile instruments were used to minimize the risk. Before the incision was made with sterile disposable scalpels, the skin was thoroughly cleaned. Biopsy needles were

autoclaved before usage. Furthermore, subjects were recommended not to shower for two days to keep the incision dry and thus decrease the risk for infection. The study also included infusion of a stable amino acid isotope (13C6- phenylalanine), for the purpose of tracking and evaluating protein synthesis. The amino acid used is a common amino acid which already exists in the body and is harmless. The amino acid infusate was prepared under sterile conditions. Subjects were also well informed about symptoms of infection and were asked to contact the study team if any questions arose.

In the purpose of minimizing experience of pain during the muscle biopsies, subjects were pain relieved with local anaesthesia. However, muscle biopsies may cause some discomfort. Subjects were well informed about the procedure to minimize discomfort. In total 16 biopsies were obtained, four from each leg each experimental day. The biopsies may cause tenderness in the leg for a couple of days after experimental days. To minimize risk for hematoma after muscle biopsies, pressure was applied immediately afterwards, and kept on during the day. No other major risks are associated with the study provided the subjects are healthy which was screened for during the interview and using a health questionnaire.

3.7 Statistics and analytical procedures

Tibco Statistica v. 13.5.0.17 was used for statistical analysis and parametric statistics were employed. Statistics on 10 subjects were employed for glycogen content, strength performance and p-ACC. Due to the breaking of one of the gels during the western blot procedure, only data from nine subjects were analysed for remaining proteins (AMPK, ULK1, LC3b). A dependent T-test was used to calculate differences in time (minutes) in glycogen depletion and strength performance (weight lifted). Data for phosphorylated proteins was related to the total amount of proteins loaded (MemCode) and checked for normal distribution by Q-Q plots. Skewed data was logarithmically transformed. A three-way repeated measures ANOVA was used to analyse for main and interaction effects between the different variables (supplement x glycogen content x time). In case of significance, a Tukey´s post hoc test was applied. Statistical significance was set at $p < 0,05$.

As there was no interaction effect for the whole model (supplement x glycogen content x time), glycogen content x time was analysed in case of significance. This means that the values within the depleted and loaded leg were combined over the two trials and compared in differences over time. Thus, the number of observations increased for each leg at every timepoint. These interaction effects for glycogen content x time are presented in figures termed "Combined" in the results section. This may be performed since the study involved two situations with independent observations meaning that both the mean and measures of variance (standard deviation) changes. Separate figures for the two trials (placebo and EAA) are also presented to visualize data separately, however without symbols for significance since no post hoc was applied for this interaction.

4 Results

In total 10 subjects were included in this analysis (6 men, 4 women). Included subjects had a mean maximal oxygen uptake of 47 ± 4 ml/kg/min and a mean watt peak of 295 ± 61 W. Subjects one legged 10 RM strength was on average 38 ± 13 kg for both legs. Subject characteristics are presented in table 1.

Table 1 - Subject characteristics

	Women (n=4)	**Men (n=6)**	**Total (n=10)**
	Mean ± SD	Mean ± SD	Mean ± SD
Age	28 ± 7	30 ± 5	29 ± 5
Height (cm)	162 ± 4	177 ± 6	171 ± 9
Weight (kg)	61 ± 4	79 ± 8	72 ± 12
Body fat (%)	23 ± 4	13 ± 1	17 ± 6
ml VO2/kg/min	44 ± 2	48 ± 5	47 ± 4
Watt peak	232 ± 24	337 ± 33	295 ± 61
10 RM Right (Kg)	26 ± 5	46 ± 8	38 ± 13
10 RM Left (Kg)	26 ± 5	46 ± 9	38 ± 13

4.1 Muscle glycogen

On average, the glycogen depletion protocol lasted 198 ± 27 minutes the night before the placebo trial and 199 ± 26 minutes before the EAA trial, with no significant difference between the two trials. The glycogen loading and depletion protocol resulted in significant differences in glycogen between the depleted and loaded leg at all timepoints ($p<0.001$), with an average difference between loaded and depleted of 68 % at baseline. For glycogen content at all timepoints, see figure 3. There was a main effect of glycogen content ($p<0.001$) and-time ($p<0.001$) and an interaction effect for glycogen content x time ($p=0.03$). Glycogen content in the depleted leg did not change significantly from baseline over time. Glycogen content in the loaded leg decreased significantly with 11% immediately after resistance exercise ($p=0.011$) and continued to decrease over time compared to baseline ($p<0.001$).

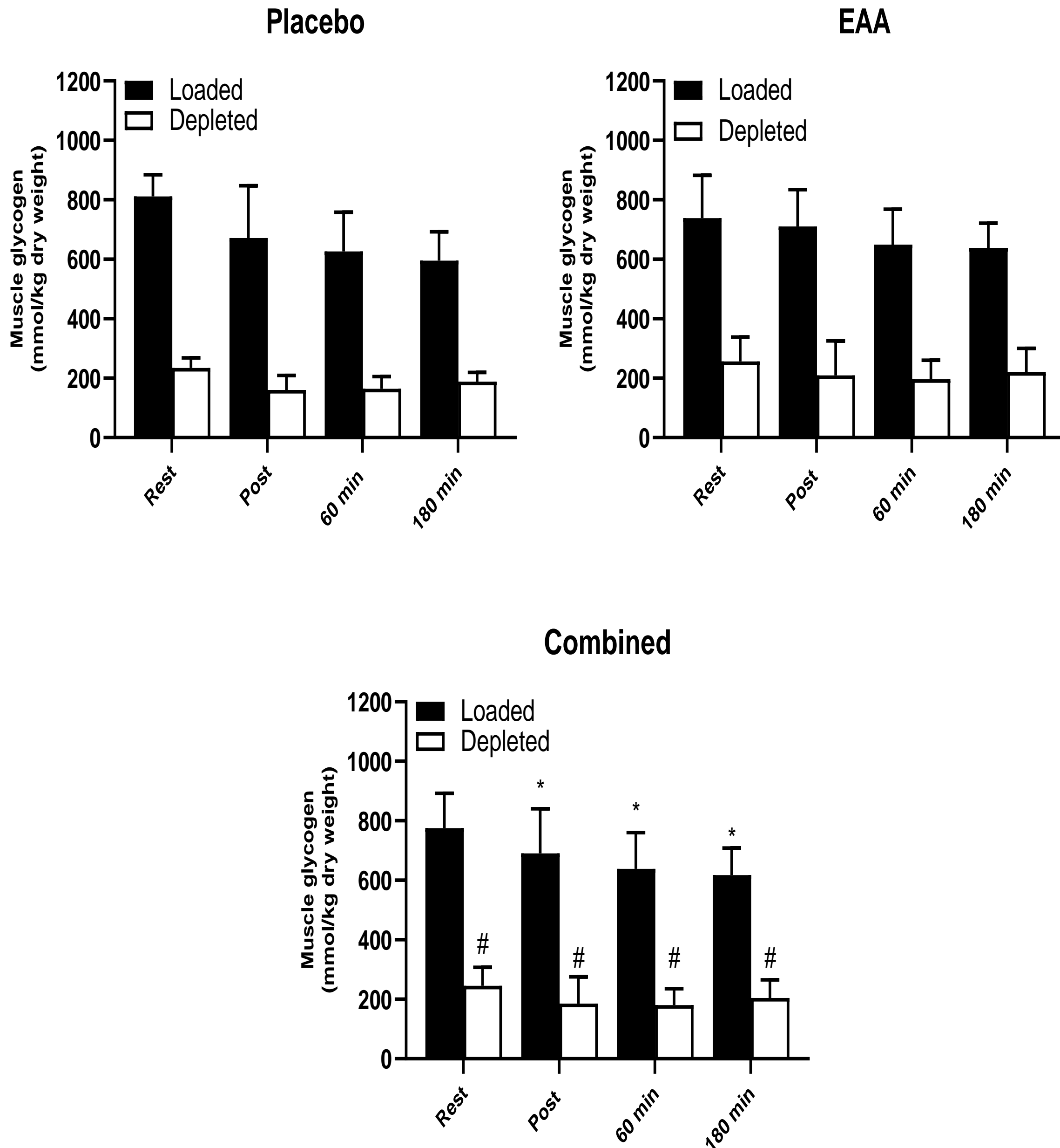

*Figure 3A (placebo), 3B (EAA) and 3C (combined graph of the loaded and depleted leg over time in both trials) – Glycogen content in loaded and depleted leg at rest (baseline), post exercise, 60- and 180 minutes post drink. Values are mean ± SD. * p<0,05 compared to rest, # p<0,05 compared to loaded leg.*

4.2 Strength performance

On average, subjects lifted 3059 ± 957 kg per leg (Men 3674 ± 671, women 2138 ± 329) during the placebo trial and 3030 ± 952 kg per leg (Men 3640 ± 666, Women 2115 ± 343) in the EAA trial (Figure 4) with no significant difference between the two. All subjects were able to match depleted and loaded leg within the trials. Two men and two women lifted less weight on the second trial (EAA-trial) compared to the first.

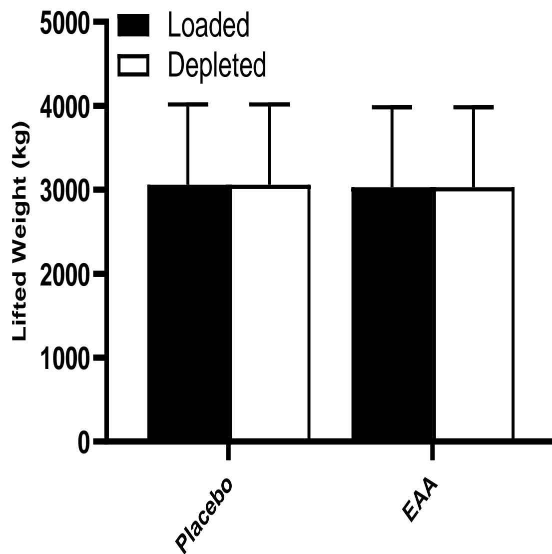

Figure 4 – Strength performance between trials and legs. Values are lifted wight (kg) over 10 sets and reported as mean ± SD

4.3 Western Blot

4.3.1 phosphorylation of AMPK^{thr172}

Statistical analysis revealed main effects of glycogen content ($p<0,001$) and interaction effects of glycogen content x time ($p<0.001$) for p-AMPK^{thr172}. There was also a trend towards an interaction for supplement x glycogen content x time, but no significant difference between situations (placebo/EAA) ($p=0.054$).

Post hoc test of glycogen content x time showed no differences between depleted and loaded leg at baseline in p-AMPK^{thr172} (Figure 5 C). p-AMPK^{thr172} increased significantly in the depleted leg post exercise with 59 % compared to baseline ($p<0.001$). The depleted leg showed a trend towards staying elevated above baseline at 60 minutes post drink, however not significantly ($p=0.09$), and returned to baseline values at 180 minutes post drink. Meanwhile, p-AMPK^{thr172} did not change significantly in the loaded leg compared to baseline during the trials.

Post exercise, p-AMPK^{thr172} was 117 % higher in the depleted leg compared to the loaded leg ($p<0.01$). p-AMPK^{thr172} in the depleted leg continued to stay elevated compared to the loaded leg at 60- ($p<0.01$), and 180 minutes post drink ($p=0.02$) with 76% and 39 % respectively.

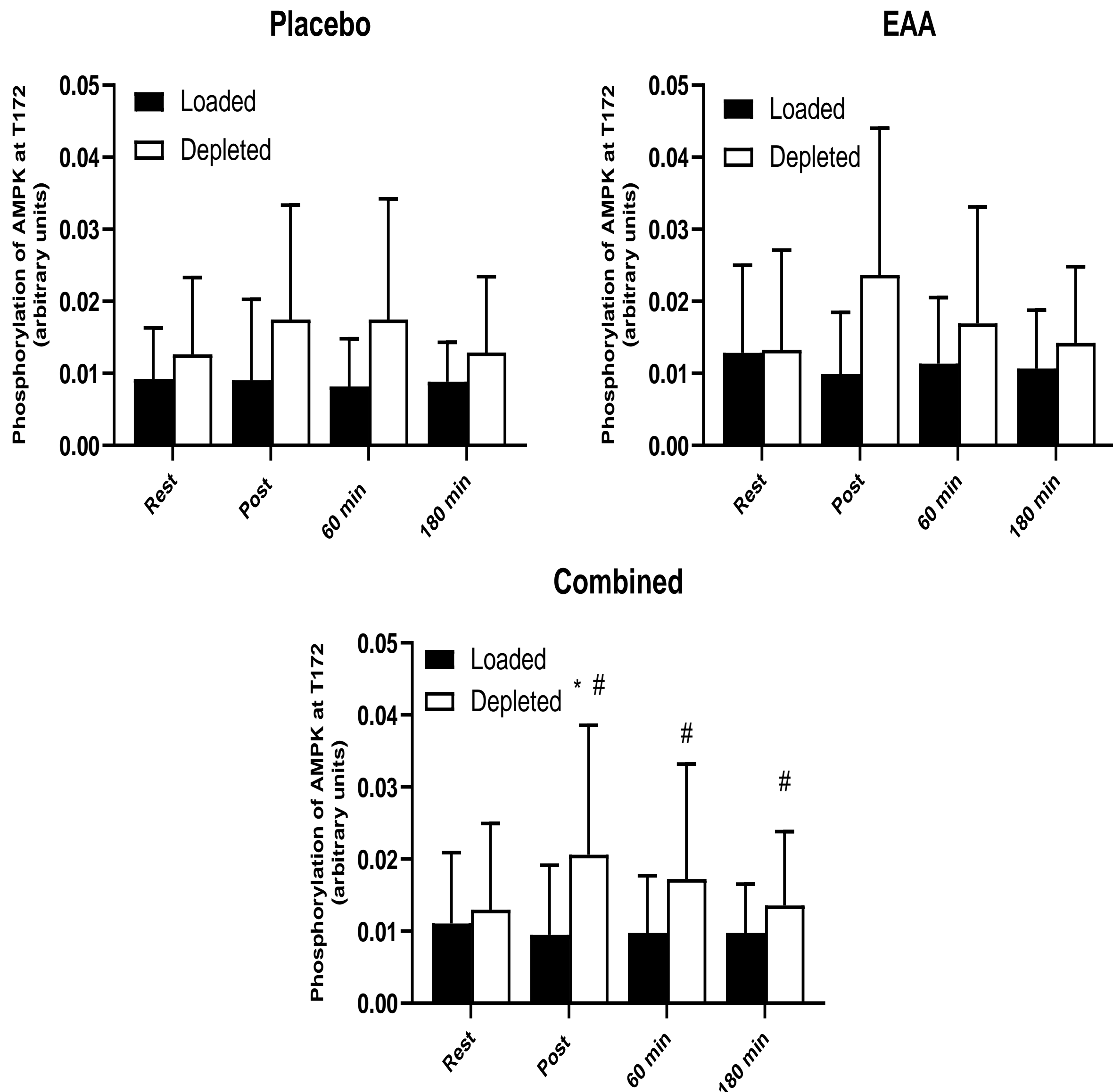

*Figure 5A (placebo), 5B (EAA) and 5C (combined graph of the loaded and depleted leg over time in both trials) – phosphorylation status of AMPK^{Thr172} related to MemCode at rest (baseline), post exercise, 60- and 180 minutes post drink. Values are mean ± SD. Arbitrary units (AU). * p<0,05 compared to rest, # p<0,05 compared to loaded leg.*

4.3.2 phosphorylation of ACC^{ser79}

Activity of AMPK was evaluated with the AMPK target Acetyl-CoA Carboxylase (ACC). There were significant main effects from leg and time on p-ACC (p<0.001). There was also an interaction of glycogen content x time (p<0.001). There was no significant difference between the placebo or EAA trial (supplement x glycogen content x time, p=0,741). Post hoc test of glycogen content x time showed no difference in p-ACC^{ser79} between the legs at baseline (Figure 6C), but a trend towards elevated phosphorylation in the depleted leg (p=0.072). Post exercise, p-ACC^{ser79} increased in the depleted leg with 173% and remained

elevated compared to baseline at 60 minutes post drink (p<0.001) before returning to baseline values at 180 minutes post drink. Meanwhile, the loaded leg did not change significantly compared to baseline over time (p=0.08 post exercise, p=0.47 at 60 minutes post drink, and p=0.78 at 180 minutes post drink).

p-ACC^{ser79} was significantly higher in the depleted leg compared to the loaded leg at all timepoints (p<0,001), except baseline (p=0.07). Post exercise, p-ACC^{ser79} was 129 % higher in the depleted leg than the loaded leg. At 60 min post drink, the differences between legs continued to increase due to decreased phosphorylation in the loaded leg. p-ACC was 445% higher in the depleted leg at 60 minutes post drink and 173 % at 180 minutes post drink.

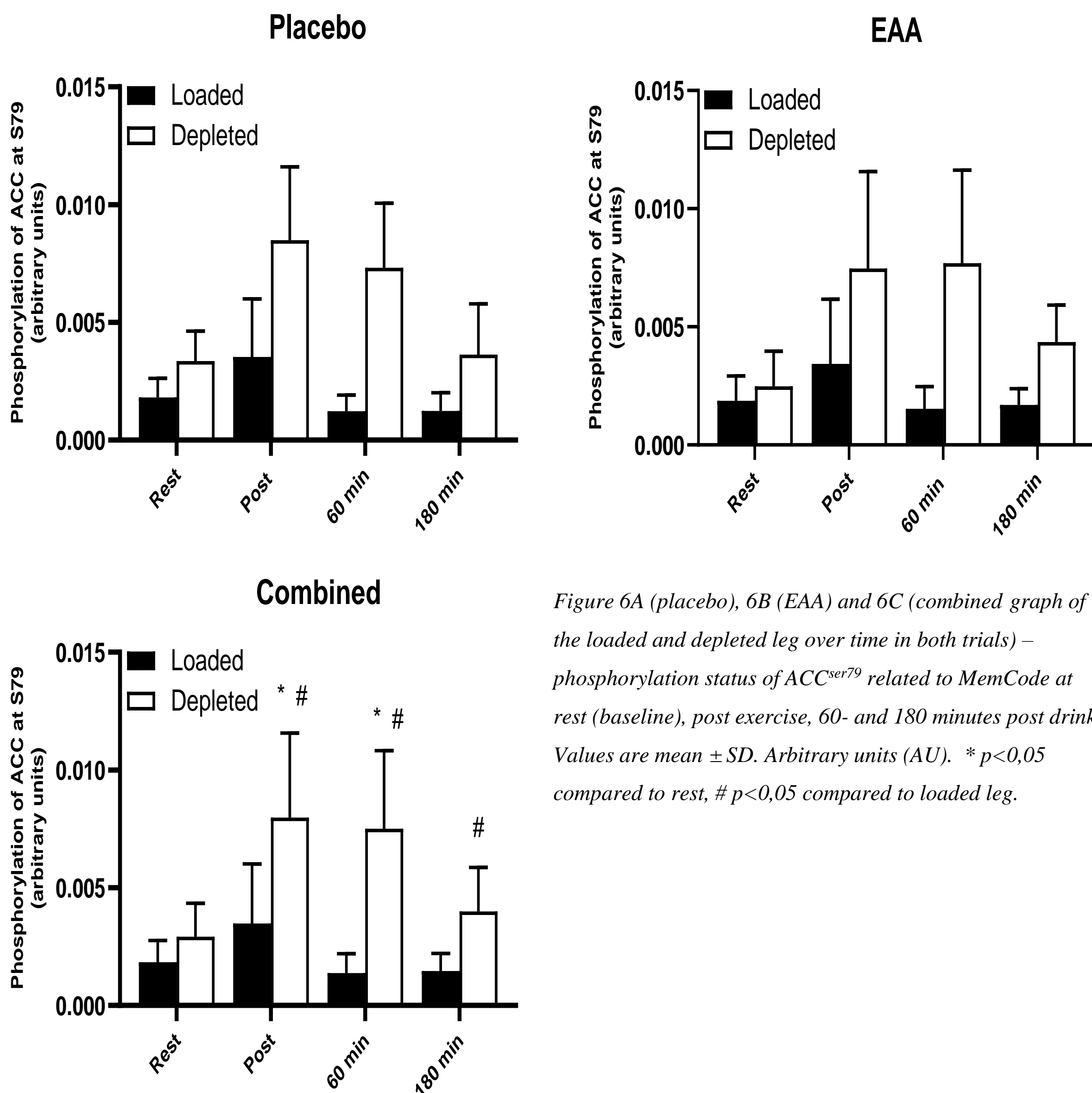

*Figure 6A (placebo), 6B (EAA) and 6C (combined graph of the loaded and depleted leg over time in both trials) – phosphorylation status of ACC^{ser79} related to MemCode at rest (baseline), post exercise, 60- and 180 minutes post drink. Values are mean ± SD. Arbitrary units (AU). * p<0,05 compared to rest, # p<0,05 compared to loaded leg.*

4.3.3 phosphorylation of ULK1ser555

There were significant main effects of glycogen content ($p<0.001$) and time ($p=0.001$) on p-ULK1^{ser555}. Furthermore, there was significant interaction effect of glycogen content x time ($p=0.042$). There was no significant difference between trials (supplement x glycogen content x time, $p=0.074$). Post hoc test of glycogen content x time revealed that there was no significant difference between legs in p-ULK1^{ser555} at baseline ($p=0.08$) (Figure 7C). The loaded leg did not change significantly over time in p-ULK1 compared to baseline. The depleted leg increased slightly in p-ULK1^{ser555} post exercise, however not significantly. At 60 minutes post exercise p-ULK1^{ser555} had increased with 123% within the depleted compared to baseline. p-^{ULK1ser555} was 135 % higher in the depleted leg than the loaded at the same timepoint ($p<0.001$). The depleted leg decreased until 180 minutes post drink and was not significantly different from baseline, however, still significantly higher than the loaded leg (59% ($p=0.006$)).

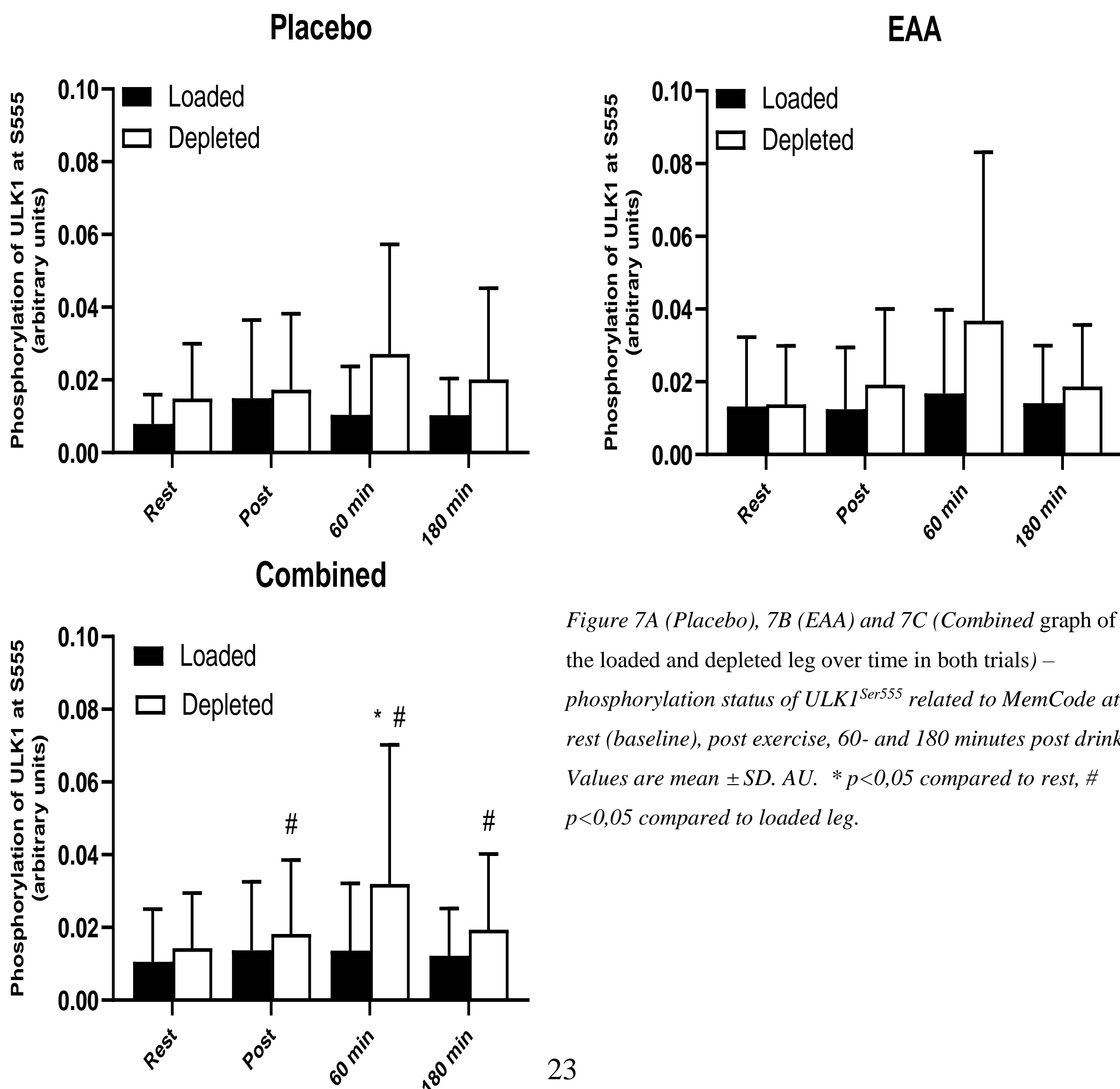

Figure 7A (Placebo), 7B (EAA) and 7C (Combined graph of the loaded and depleted leg over time in both trials*) – phosphorylation status of ULK1^{Ser555} related to MemCode at rest (baseline), post exercise, 60- and 180 minutes post drink.. Values are mean ± SD. AU. * p<0,05 compared to rest, # p<0,05 compared to loaded leg.*

4.3.4 Total LC3b-II

There were main effects of glycogen content (p=0.001) and time (p<0,001) and an interaction effect of glycogen content x time (p=0.03). There was no difference between trials for total amount of LC3b-II (supplement x glycogen content x time, p=0.253).

There was no significant difference between legs at baseline (Figure 8C). Levels of LC3b-II decreased similarly in both legs, however only significantly in the loaded leg (p=0.006; depleted leg p=0.08) with no difference between the two at post exercise or 60 minutes post drink. At 180 minutes post drink, the depleted leg had decreased with 81% from baseline (p<0.001). The loaded leg had decreased with 73% to the same timepoint (p<0.006). At 180 minutes post drink there were significantly less amount of LC3b-II in the depleted leg (35%, p=0.007).

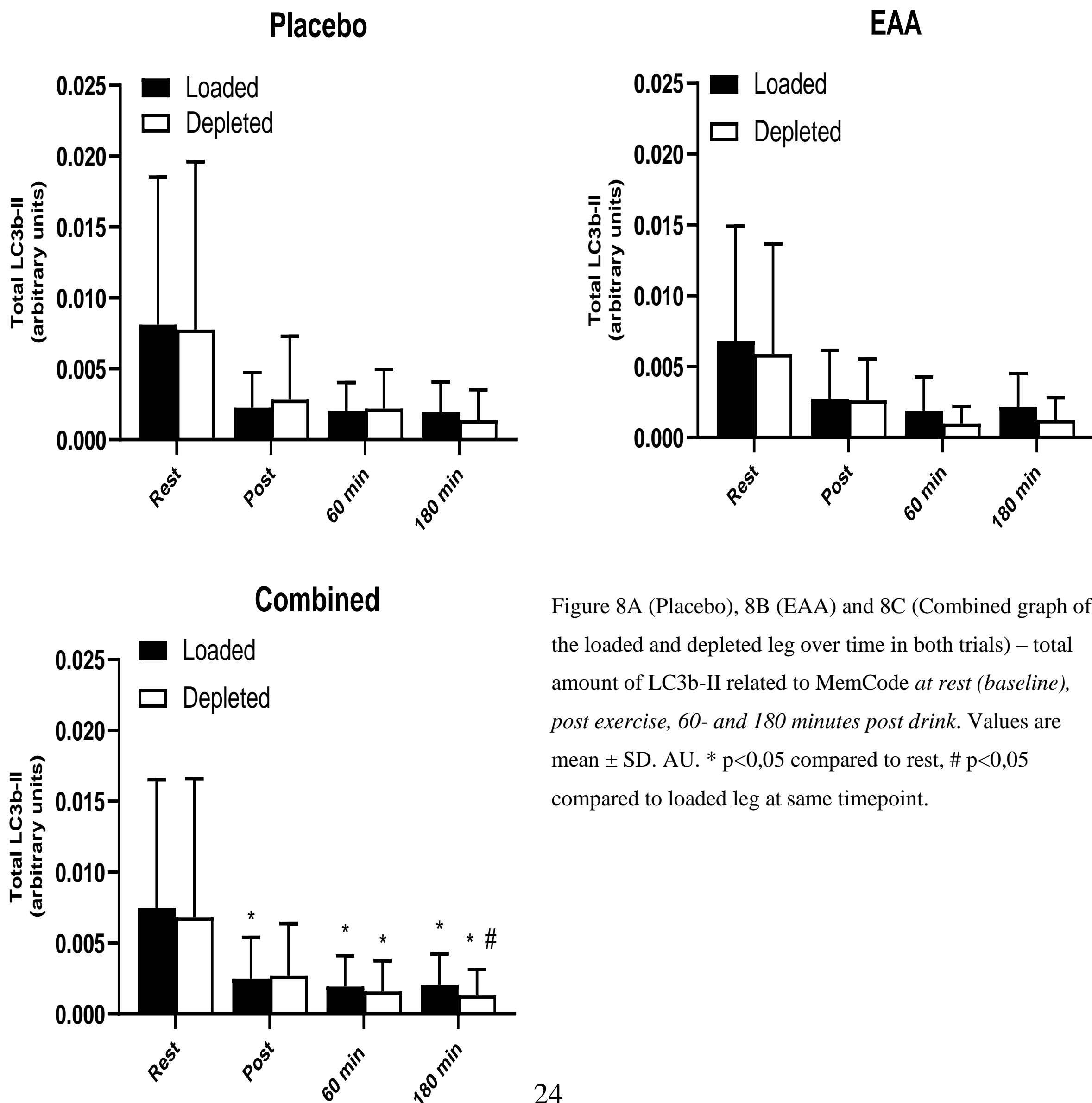

Figure 8A (Placebo), 8B (EAA) and 8C (Combined graph of the loaded and depleted leg over time in both trials) – total amount of LC3b-II related to MemCode *at rest (baseline), post exercise, 60- and 180 minutes post drink*. Values are mean ± SD. AU. * p<0,05 compared to rest, # p<0,05 compared to loaded leg at same timepoint.

4.3.5 LC3b-II/I ratio

There was main effects of glycogen content and time on LC3b-ratio (p<0.001) and an interaction effect of glycogen content x time (p=0.009) with no difference between trials (supplement x glycogen content x time, p=0,196). Post hoc test of glycogen content x time (figure 9C) showed no difference between legs at baseline. The LC3b-ratio followed a similar pattern as LC3b-II. The loaded leg decreased significantly until post exercise (p=0.013). Meanwhile, the depleted leg's decrease was not significant (p=0.16). The LC3b-ratio continued to decrease over time compared to baseline in the depleted leg (p<0.001) and the loaded leg (p<0.01). At 180 minutes post drink the depleted legs ratio had decreased with 79 % and with 69% in the loaded leg compared to baseline. The LC3b-II/I ratio did not significantly differ between legs at baseline, post exercise or 60 minutes post drink. At 180 minutes post drink the depleted leg's ratio was 54 % lower than in the loaded (p<0.001).

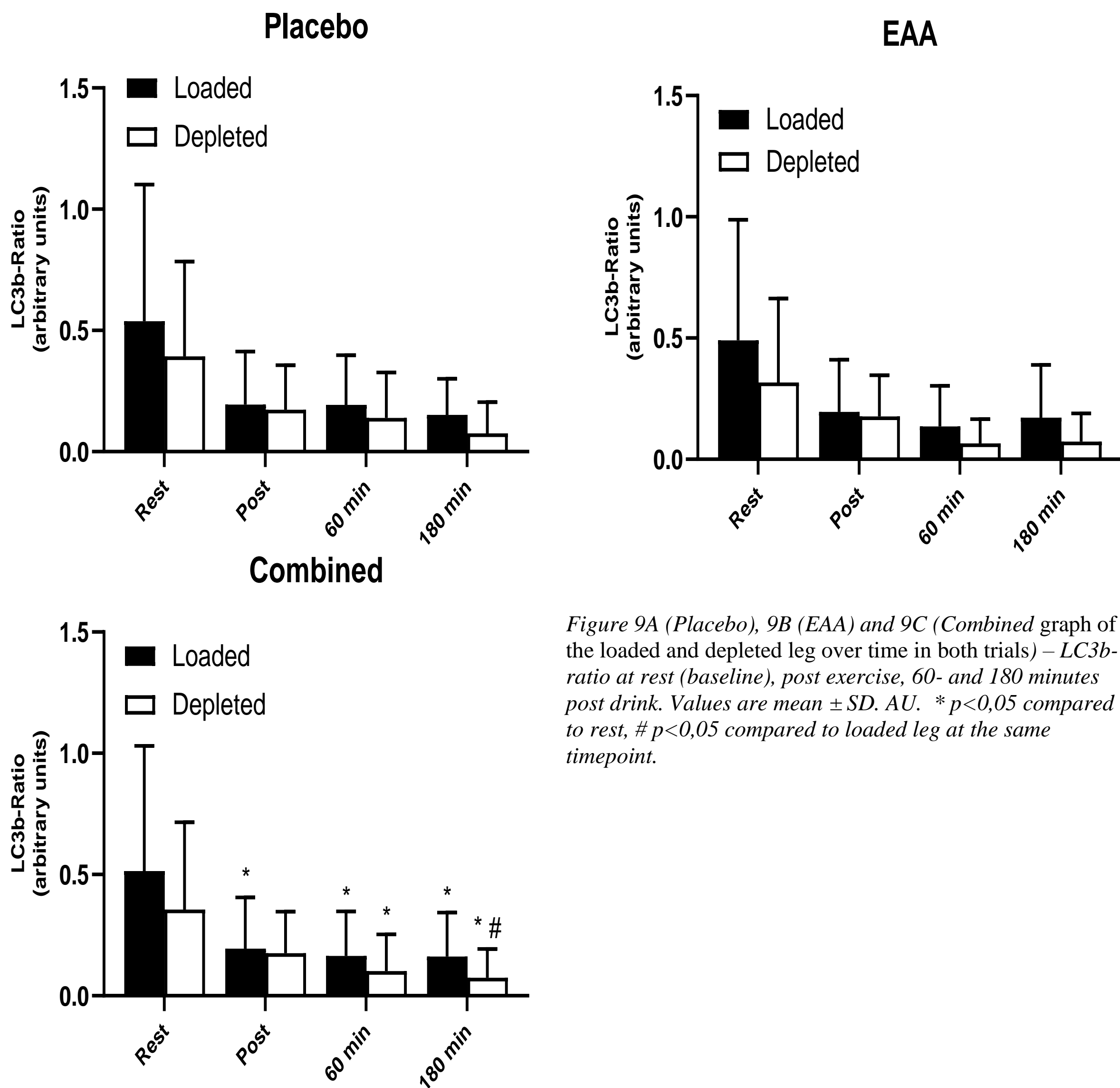

Figure 9A (Placebo), 9B (EAA) and 9C (Combined graph of the loaded and depleted leg over time in both trials*) – LC3b-ratio at rest (baseline), post exercise, 60- and 180 minutes post drink. Values are mean ± SD. AU. * p<0,05 compared to rest, # p<0,05 compared to loaded leg at the same timepoint.*

5 Discussion

The primary purpose of this study was to evaluate the influence of glycogen availability on autophagy signalling post resistance exercise. A secondary purpose was to evaluate the effect of EAA-supplementation post resistance on autophagy signalling. This is the first experimental study on the subject to my knowledge.

Similar to the results of van Loon et al (2000), the glycogen depletion protocol resulted in significantly lowered intramuscular glycogen which was required to answer the research questions. There was no significant difference in glycogen levels between trials at any timepoint making comparisons between trials possible. In summary, the results from this study suggests that a single bout of resistance exercise with low muscle glycogen increases phosphorylation of AMPKα^{thr172}, ULK^{ser555} and AMPKα activity, evaluated by p-ACC^{ser79}, compared to a glycogen loaded leg. Furthermore, results showed a significantly lower LC3b-II/I ratio in the depleted leg at 180 minutes post resistance exercise indicating a higher rate lysosomal breakdown of LC3b-II. There was no effect of EAA-supplementation on autophagy signalling.

At baseline there was no significant difference between legs in AMPK phosphorylation which is similar to the results of Camera et al (2012) who did not observe any differences in AMPKα phosphorylation the day after one-legged glycogen depletion. However, baseline p-AMPKα in these studies stands in contrast to the results of Wojtaszewski et al (2003) who found increased basal AMPK-activity after glycogen depletion. These discrepancies might have been due to the different levels of glycogen between studies. Wojtaszewski et al (2003), using another depletion protocol, lowered glycogen levels to 163 mmol/kg dry muscle after the glycogen depleting session, and reached 909 mmol/kg dry weight in the loaded session. Corresponding values in the current study was 235-256 mmol/kg dry weight in the depleted leg and 811-739 mmol/kg dry weight in the loaded leg. There is a possibility that lowering the amount of skeletal muscle glycogen further would have affected basal phosphorylation of AMPK^{thr172} in the present study. However, Camera et al (2012) decreased glycogen content to 174-185 mmol/ kg dry weight and did not observe an increased AMPKα^{thr172} phosphorylation at rest compared to 383 mmol/kg dry weight. It might be the that the relative differences between studies explains the different results of p-AMPK^{thr172} observed where Wojtaszevski et al (2003) compared a larger relative difference between situations. Furthermore, the

participants were experienced cyclist with significantly higher aerobic capacity compared to in this study which might explain these differences.

The resistance exercise protocol in the present study increased p-AMPK^{thr172} significantly in the depleted leg, with a concomitant increase in p-ACC^{ser79} which stayed elevated compared to baseline at 60 minutes of recovery, however only significantly in the depleted leg. This implies that low glycogen levels were not sufficient to increase LKB1 phosphorylation of AMPK^{thr172} at rest in the present study. However, during increasing metabolic stress, as our resistance exercise protocol, lowered glycogen stores activates catabolic kinases. Moreover, once activated, p-AMPK^{thr172} was significantly higher in the depleted leg compared to the loaded leg during three hours of recovery. As there was no significant alteration in p-AMPK^{thr172} in the loaded leg post exercise, and that the number of repetitions was matched, there is a possibility that the metabolic stress from resistance exercise was not enough to perturbate the metabolic homeostasis in the loaded leg and thus not enough to significantly increase p-AMPKα post resistance exercise. Glynn et al (2010) showed increased AMPK^{thr172} phosphorylation without dietary manipulation post resistance exercise (10 sets of 10 repetitions at 70% of 1 RM leg extension). However, glycogen content was not measured in their study which makes comparisons difficult. Regardless, this study provides information that when the same amount of work was performed, low glycogen content increases p-AMPKα in a manner that high glycogen content does not. Contrary to our results, p-AMPK^{thr172} continued to stay elevated at 2- hours post exercise compared to baseline despite supplementation of 0,5 g carbohydrates/kg lean mass and roughly 20 g of EAA at 1-hour post exercise. In this study, values within the loaded and depleted leg were not statistically different from baseline at 1- hour post exercise. The reason for this is unclear, however might be due to the fact that their participants were beginners to resistance exercise. Novice resistance exercisers usually exhibits prolonged elevations in MPS compared to advanced (Burd et al., 2009) and there might be similar effects for AMPK.

ACC^{ser79} phosphorylation increased similar to that of AMPK in the depleted leg and continued to stay elevated above baseline at 1- hour post exercise. ACC is a protein involved in lipid metabolism and once phosphorylated by AMPK, lipid oxidation increases (Kahn et al., 2005). As evident in this study, the decreased availability of glycogen as a substrate led to increased ACC-phosphorylation and probably fatty acid oxidation as glycogen did not decrease

significantly in the depleted leg after resistance exercise. There was no difference in ACC^{ser79} phosphorylation between trials post drink which is in line with the hypothesis. There is no previous experimental evidence that suggests that EAA-supplementation would affect ACC to my knowledge.

ULK1 is phosphorylated at ser555 residue by AMPK (Fritzen et al., 2016). The increase in $ULK1^{ser555}$-phosphorylation was delayed in the depleted leg compared to AMPK-phosphorylation and was only significantly different from baseline within the depleted leg at 60 minutes post drink. However, ULK1 phosphorylation was higher in the depleted leg than the loaded leg at all timepoints post exercise similar to AMPK and ACC. ULK1 is essential in the process of autophagy as it is phosphorylated by both catabolic and anabolic kinases (Martin- Rincon et al., 2018). The present study solely evaluated p-ULK1 at site 555 because of antibody availability. This site is however strongly correlated with AMPK-activity as AICAR stimulation of AMPK leads to increased p-$ULK1^{ser555}$ phosphorylation (Fritzen et al., 2016). In this study, p-$ULK1^{ser555}$ followed the same pattern as p-$AMPK^{thr172}$ with higher phosphorylation at all timepoints compared to the loaded leg. To my knowledge this is the first study to evaluate this site on ULK1 post resistance exercise making comparison with other studies difficult. The only study, to my knowledge, which evaluated ULK1 post resistance exercise is that of Smiles et al (2015), who investigated $ULK1^{ser757}$-phosphorylation which is expected to react differently to ser555 since this site is phosphorylated by mTORC1. However, endurance exercise has been shown to increase $ULK1^{ser555}$ - (Fritzen et al., 2016), and ^{ser317} phosphorylation (Schwalm, Jamart, Benoit et al, 2015). The present study shows that resistance exercise in combination with low glycogen availability increases p-$ULK1^{ser555}$ similar to endurance exercise.

AMPK phosphorylation, as well as $ULK1^{ser555}$-phosphorylation indicate an activation of the autophagic machinery in the glycogen depleted state compared to the glycogen loaded. The LC3b-ratio decreased significantly over time in both legs and was lower in the depleted leg than in the loaded at all timepoints, however, only significantly lower at 180 minutes post drink. The decrease was mainly due to a decreased amount of LC3b-II over time as there was no interaction effects for supplement or glycogen content in LC3b-I (data not shown). However, LC3b-I increased slightly, although not significantly, in the depleted leg in both trials at 180 minutes post drink and thus decreasing the ratio even more. As previously

described, a decrease in LC3b-II is related to a decreased autophagosome content which might be due to an increased lysosomal degradation of autophagosomes or decreased autophagosome synthesis (Klionsky et al, 2016). Since both LC3b-II and LC3b-I decreases post exercise, it could be interpreted as the ratio decreased due to increased lysosomal degradation, i.e. increased MPB. This is also supported by the increased phosphorylation status of upstream kinases in the present study. Data of the LC3b-ratio are consistent with previous studies on autophagy post resistance exercise with a decreased LC3b-ratio at 2 hours post exercise (Glynn et al. 2010) or at 6- and 24 hours post resistance exercise (Fry et al. 2012). In line with this, the LC3b-ratio has been shown to decrease with endurance exercise as well (Fritzen et al., 2016; Schwalm et al., 2015).

Increased autophagy could potentially lead to an increased MPB and decreased net balance post resistance exercise as the net balance is comprised of both MPS and MPB (Burd et al., 2009). However, we do not know the effect of upregulated autophagy for the other MPB systems, the ubiquitin-proteasomal system and calpain Ca^{2+}- dependent cysteine proteases. Expression of MuRF1, a protein involved in proteasomal muscle breakdown has been shown to increase post resistance exercise in combination with decreased LC3b-ratio in previous studies (Glynn et al., 2010; Fry et al., 2013). However, neither examined the effect of glycogen availability. It is also important to consider that this study examined autophagy signalling after a single bout of resistance exercise, and it cannot be concluded with certainty that the results in the current study will be the same over repetitive exposures. Furthermore, as western blot is considered to be semi-quantitative method, meaning that values are not absolute, but relative to the total amount of protein in the sample, we cannot quantify the difference and thus not know if these acute differences will generate a significant difference in muscle mass over time. A measure for quantifying the total rate of MPB and MPS could have been combined with the western blots to generate a more comprehensive view of the practical implications of performing resistance exercise with low glycogen. Future studies should also include anabolic signalling through the mTORC1 pathway, because of the potential inhibitory effect of AMPK on mTORC1 (Bolster et al., 2002). Moreover, mTORC1´s activity towards $ULK1^{ser757}$ should also be evaluated to better understand the relationship between these signalling pathways in response to nutritional interventions.

Contrary to the hypothesis, supplementation of essential amino acids had no effect on AMPK^{thr172}, ULK1^{ser555} or the LC3b-II/I ratio during the trials as there were no significant differences between trials. EAA supplementation activates mTORC1 and the amount of amino acids used has previously shown to increase mTORC1 phosphorylation with 165% post resistance exercise (Apró et al., 2015a). The mTORC1 specific site on ULK1, ser757 was not evaluated in this study, hence any conclusions about possible effects of EAA supplementation on p-ULK1 upstream of LC3b cannot be drawn. However, a potential effect of EAA-supplementation on autophagy should have been detected through changes in the LC3b-II levels or LC3b-II/I ratio between the placebo and EAA trial. Previous studies on the effect of EAA on autophagy have shown increased ULK1$^{ser\ 757}$ phosphorylation at 1- hour post resistance exercise compared to baseline and placebo at 4 hours post, however not different compared to placebo at the same timepoint (Smiles et al., 2015), indicating no effect of EAA on ULK1^{ser757} phosphorylation. Glynn et al (2010) observed a decreased amount of LC3b-II after supplementation with a mixture of carbohydrates and EAA, which means it could have been an effect either from carbohydrates, EAA, a combination, or simply a result of time since no placebo or control group was included. In this study the LC3b-ratio and the amount of LC3b-II decreased over time in both trials, regardless of supplementation of EAA. It cannot be ruled out that this thesis is underpowered for this variable, since no power calculation was made. Furthermore, there is a possibility that EAA-supplementation is a lower stimulus than glycogen availability during these circumstances, which would lead to smaller effect sizes and therefore the need for subjects would increase to be able to detect differences between conditions.

As to why EAA did not change or attenuate autophagy is unclear. Autophagy and the mTORC1-pathway are two competing and coexisting systems. Since autophagy also is a system for remodelling, re-cycling and to some degree provides energy (Martin-Rincon., 2018) it could be that the autophagic process is prioritized over anabolic processes due to the importance of intracellular substrate availability. Furthermore, as autophagy is required, at least in rodents, to maintain proper muscular function (Masiero et al., 2009) it might be detrimental to inhibit this process. Although provided with 240 mg EAA/kg/body weight, which is a powerful stimulus for anabolism via mTORC1-dependent pathways (Apró et al., 2015a) no effect was observed. It could be that both autophagy and the mTORC1-pathway are upregulated at the same time during these conditions, as lowered glycogen does not affect

anabolic pathways, as shown by Camera et al (2012). Nevertheless, mTORC1 does not seem to inhibit autophagy via $ULK1^{Ser757}$-phosphorylation when glycogen availability is low, potentially through an unknown mechanism. This is one of the reasons that future studies should include anabolic signalling in their analysis.

5.1 Method Discussion

Overall, this randomised controlled double-blind trial was well designed to describe the influence of glycogen availability on autophagic signalling and the effect of EAA-supplementation on autophagic signalling post resistance exercise. However, the study comes with a few concerns. This study employed a glycogen depletion protocol for one leg after glycogen loading with the purpose of creating large differences in glycogen content between legs. Although legs were significantly different in glycogen levels in this study, we were not able to lower glycogen levels as much as other studies using different protocols (Wojtaszevski et al., 2002; Camera et al., 2012). Furthermore, these studies provided subjects with a low carbohydrate meal post glycogen depletion, indicating that the more extreme protocol in this study leading up to the experimental trials might have been unnecessary from an ethical point of view as subjects undergoes approximately 20-21 hours without feeding. However, this procedure might have reduced the risk for confounding factors, as dietary intake of protein and fat can change the metabolic activity.

Normal glycogen content varies widely as it is affected by training status and dietary intake. However, in a recent meta-analysis, resting glycogen content was determined for groups depending on fitness level (VO_2max) and dietary carbohydrate intake. For a population similar to ours in VO_2max with moderate carbohydrate intake was the expected normal value approximately 460 ± 130 mmol/kg dry weight (Areta & Hopkins, 2018). According to the same meta-analysis, predicted values at rest were 220 ± 90 with a low (< 2 g/kg/day) carbohydrate intake on a daily basis. Thus, it is not unthinkable that resistance exercise would be performed with the same glycogen content as in this study if not adequate carbohydrate intake is maintained and/or if resistance exercise follows endurance exercise. This makes the results relevant in that aspect. On the other hand, in this study, low glycogen content was compared with a loaded leg of 811-739 mmol/kg dry weight which evidently is higher than normal. To better understand the practical implications of performing resistance exercise with

low glycogen content it would have been preferable to compare low values with normal, i.e approximately 460 mmol. However, this study was the first to describe the link between glycogen availability and autophagy which is an important first step.

The sample size of 10 subjects was rather small, however, enough to detect significant differences in the present study as well as previous studies on similar markers (Apró et al, 2015b). Since there are no previous studies investigating glycogens effect on autophagy post resistance exercise it is difficult to put the results into context. Thus, one should exercise caution when generalizing the results to other subjects and under other circumstances (for example with different glycogen content). The study might be viewed as the first piece of the puzzle in describing glycogens effect on autophagy in human skeletal muscle post resistance exercise.

Western blot is one of the most common techniques to evaluate autophagy (Klionsky et al, 2016) and generates results from the environment in the specific fibres at the moment of the muscle biopsy. Meaning that, when investigating a dynamic process, potential peaks or lows in phosphorylation could be missed due to timing issues. Samples from each subject was treated in an identical fashion, since they were analysed according to the same protocol. Ideally would the western blot be performed for all samples at the same time. However, the western blot procedure was performed at one timepoint for subject 1-5 and another for subject 6-10 potentially leading to small undetected differences in handling. The western blot procedure requires a lot of manual biochemical work which might affect the results. Results may vary because of pipetting error, inadequate transfer of proteins to the membrane or the antibody might be non-specific to the intended protein. However, the ability to pipet correctly was controlled for by using triplicates when protein concentration was decided in each sample. Also, if pipetting error still would occur this was controlled for by dividing the signal from phosphorylated proteins, with that from Memcode (signal from all proteins in the sample). Originally, phosphorylated proteins were supposed to be related to the total amount of that specific protein. However, in the present study, total proteins could not be quantified due to incomplete stripping of the membranes. Thus, MemCode was used instead to relate phosphorylation status which was a weakness. Also, the visualization of the proteins did not indicate incomplete transfer which might occur when too much protein is loaded into the gels for example (Gosh et al., 2014).

When performing a Western blot, it is important that antibodies are specific to the proteins they are intended. When analysing ULK1, especially for subject 1-4, the antibody produced some background noise, meaning that it also was bound to other parts of the membrane than intended. This made the quantification of these blots more difficult as bands were not as clearly separated as for other proteins. Statistics and values for p-ULK1^{ser555} were presented for all subjects as this seemed most appropriate. Furthermore, when statistical analysis was performed on subject 6-10 alone which had more clearly separated bands, similar results were obtained (data not shown).

In conclusion, the results of the current study showed an increase in autophagy signalling through the AMPK-ULK1 pathway post resistance exercise in the glycogen depleted state compared to a glycogen loaded. This is followed by a decreased LC3b-ratio which might have been due to an increased rate of lysosomal degradation. This suggests that performing resistance exercise in the glycogen depleted state might decrease the net balance due to increased MPB, and possibly generate a decreased rate of muscle mass accretion over time. This is relevant for athletes and individuals engaged in resistance exercise in the purpose of gaining or maintaining muscle mass. The effect of training with low glycogen does not seem to be rescued by supplementation of essential amino acids. Additional studies are needed on the autophagic response in skeletal muscle due to low glycogen availability. Future studies should be accompanied by evaluation of the mTORC1- pathways anabolic targets and its inhibitory phosphorylation of ULK^{ser757} to be able to control for anabolic pathways when describing glycogen's role for net balance post resistance exercise.

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Appendix 1

Litteratursökning

I bilagan Litteratursökning ska du återge de sökningar du har gjort för att hitta tidigare forskning inom ditt ämnesområde. Det gör inget om bilagan blir längre än en sida.

Syfte och frågeställningar

The purpose of this study was to evaluate the effect of glycogen availability on autophagy signalling post resistance exercise. A secondary purpose was to evaluate the effect of supplementation with essential amino acids on autophagy signalling post resistance exercise.

Vilka sökord har du använt?

Ämnesord och synonymer svenska	Ämnesord och synonymer engelska
Autofagi, träning, styrketräning skelettmuskulatur, människa, glykogentillgänglighet.	Autophagy, exercise, resistance training, skeletal muscle, human, glycogen availability

Var och hur har du sökt?

Databaser och andra källor	Sökkombination
PubMed	181220 Autophagy skeletal muscle Limits: human, review 190102 Autophagy, resistance training Limits: Clinical trial 190102 Autophagy skeletal muscle Limits: clinical trial 190209 Autophagy and nutrition Limits: Clinical trial, full text 190209 Autophagy and “endurance exercise” Limits: Full text Autophagy and “resistance training” Limits: Full text
Discovery	190123 Glycogen loading muscle.

	Limits: Full text, Peer reviewed 190220 Glycogen availability and resistance training. Limits: Peer reviewed

Kommentarer

Previous research with the same purpose does not exist, which is why it was hard to find articles on the subject. Furthermore, the search terms needed to be wide to be able to find articles. I received a few articles from my supervisor, and articles has been added from reference lists.

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.

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.