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Master thesis

Glucose ingestion during resistance training does not augment ribosome biogenesis in young moderately trained adults

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Kristian Lian
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Disadvantages due to Covid-19

Due to restrictions regarding social distancing and lock downs caused by the Covid-19 pandemic, we were not able to recruit and include as many participants as we planned to the study. The pandemic was also a contributing factor to the endocrine analysis not being completed, as they were supposed to be performed at Sykehuset Innlandet Hospital Trust.

Abstract

Introduction: Responses to resistance training (RT) are not uniform across the population, and individuals responding poorly to RT show a blunted ability to produce novel ribosomes. High glucose treatment has been observed to augment rDNA transcription, however its effect on RT-induced adaptations remains quite unexplored.

Methods: Sixteen healthy moderately trained participants were included and randomized to having one leg perform unilateral resistance training with glucose supplement (16) and the other with placebo (16), alternating training every other day. Participants remained in an overnight fasted state, only receiving glucose/placebo and protein until after completion of the daily intervention. Resistance training consisted of 3 sets of 10 repetitions maximum unilateral leg press and knee extension. Micro biopsies were sampled pre (T1/T2) and post (T3/T4) the intervention, and maximal unilateral isometric and isokinetic knee extension force and torque were measured pre (T0), during (days 4, 5, 8, 9) and post (T3/T4, day 13) the intervention.

Results: There were no difference in accumulation of total RNA ($p = 0.499$) between glucose and placebo (26% and 22% increase, respectively). This was also evident in expression of mature rRNA (18S: $p = 0.584$, 28S: $p = 0.740$, 5.8S: $p = 0.935$, 5S: $p = 0.790$, 47S: $p = 0.502$), despite a robust increase in both glucose and placebo (34-43% and 33-41%, respectively). Furthermore, there were no differences between glucose and placebo in mean maximal unilateral isometric knee extension force and isokinetic peak torque during the intervention (Isometric: $p = 0.336$, 240 d/s: $p = 0.527$) and following last RT session (Isometric: $p = 0.442$, 60 d/s: $p = 0.377$, 240 d/s: $p = 0.154$), with the exception of the 60 d/s test prior to last RT session ($p = 0.037$).

Conclusion: Based on our findings, glucose supplement during RT does not augment RT-induced ribosome biogenesis compared to placebo supplement, nor does it affect peak torque during a five-session RT intervention, or enhance muscular recovery following a single session of RT.

Table of contents

Abbreviations

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-dependent protein kinase
Akt	Protein kinase B
ATP	Adenosine triphosphate
bb	Bullet blender
CaMKII	Calcium/calmodulin-dependent protein kinase
CHO	Carbohydrate
CI	Confidence interval
c-MYC	Cellular myelocytomatosis oncogene
Ct	Cycle threshold
DXA	Dual-energy X-ray absorptiometry
E	Efficiencies
ERK1/2	Extracellular signal-regulated kinase 1/2
ETS	External transcribed spacer
Fig	Figure
GEC	Glomerular epithelial cells
GLU	Glucose
HEK293	Human embryonic kidney 293 cells
IGF-1	Insulin-like growth factor 1
ITS	Internal transcribed spacer
Kcal	Kilo calories
KDM2A	Lysine-specific demethylase 2A
LKB1	Liver kinase B1
Log	Logarithmic scale
MAPK	Mitogen-activated protein kinase
mmol/L	Millimoles per liter
MPS	Muscle protein synthesis
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin

mTORC1	Mammalian target of rapamycin complex 1
MuRF	Muscle RING Finger
NAD+	Nicotinamide-Adenine-Dinucleotide
PIC	Pre-initiation complex
PI3K	Phosphoinositide 3-kinase
PLAC	Placebo
Pol I	Ribonucleic acid polymerase I
Pol II	Ribonucleic acid polymerase II
Pol III	Ribonucleic acid polymerase III
Pre-rRNA	Precursor Ribonucleic acid
PRO	Protein
P70S6K	Ribosomal protein S6 kinase beta-1
qPCR	Quantitative polymerase chain reaction
rDNA	Ribosomal Deoxyribo nucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RPE	Rate of perceived exertion
R-proteins	Ribosomal proteins
RT	Resistance training
SD	Standard deviation
SIRT1	Sirtuin 1
SL-1	Selectivity factor 1
SUV39H1	Histone-lysine N-methyltransferase
Tab	Table
TIF-IA	Transcription initiation factor IA
TSC2	Tuberous sclerosis complex 2
T0	Pre intervention days (-7 to -1), 1RM and humac testing, DXA analysis
T1	Pre intervention muscle biopsy and blood sampling leg 1
T2	Pre intervention muscle biopsy and blood sampling leg 2
T3	Post intervention muscle biopsy and blood sampling leg 1, humac testing
T4	Post intervention muscle biopsy and blood sampling leg 5, humac testing

UBF	Upstream binding factor
UCE	Upstream control element

1. Theory

Regular resistance training results in increased muscle mass (hypertrophy) and muscle strength, representing adaptations to the stimulus of mechanical strain. Unfortunately, responses to resistance training are not uniform across the population. In fact, studies have shown that RT-induced muscle growth varies widely between individuals, with 10-15% showing considerable impaired growth (Álvarez et al., 2018; Mann, Lamberts, and Lambert, 2014; Thalacker-Mercer et al., 2013). This may be due to genetics, epigenetics, or unfavorable internal physiological conditions. Recently, tweaking training modalities and -protocols have shown to elicit different responses in different populations (Hammarström et al., 2020), but we know little about what kind of resistance training to prescribe to individual phenotypes. As several studies have found variations in muscle growth response to standardized RT protocols (Hammarström et al., 2020; Schoenfeld, Ogborn, and Krieger, 2017; Stec et al., 2016), other means than RT *per se* seem necessary to circumvent this discrepancy. Indeed, ingestion of nutrients such as protein and creatine supplementation are effective in optimizing RT (Cermak et al., 2012; Lanhers et al. 2015, 2017). However, it remains largely unknown if other nutritional adjuvants such as glucose can increase the efficacy of RT, which is surprising as glucose is the preferred energy substrate of the contracting skeletal muscle during strenuous exercise, and the major energy source of cells via ATP synthesis (Hargreaves et al., 2018; Mul et al., 2015). Glucose availability seems important to regulate central processes to muscle growth, for instance ribosome biogenesis which drives MPS (Tanaka and Tsuneoka, 2018; Figueiredo and McCarthy, 2019). This regulation may in theory occur in two ways: I) directly via effects on ATP-synthesis or glucose as a signaling molecule, or II) indirectly via insulin (Tanaka and Tsuneoka, 2018). Interestingly, variations in RT-induced responses have been linked to ribosome biogenesis, where individuals responding poorly to RT also experience blunted ribosome biogenesis after 5 RT sessions (Hammarström et al., 2020; Stec et al., 2016). Hence, there may be a relationship between an individual´s capacity for ribosome biogenesis and trainability, thus investigating the possible effects of glucose on ribosome biogenesis is warranted.

1.1 Individual variations in trainability

The discrepancy observed in RT-induced muscle growth is proposed to stem from unequal levels of trainability/readiness of the muscle cells to adapt to a growth inducing stimulus (Hammarström et al., 2020; Stec et al., 2016; Thalacker-Mercer et al., 2013). Trainability is affected by baseline- and training characteristics, blood variables, indices of mTOR signalling, and the total RNA content of the muscle cells (Hammarström et al., 2020; Iadevaia, Liu, and Proud, 2014). Untrained individuals generally benefit from a low-volume RT protocol (Cannon and Marino, 2010; Mitchell et al., 2012), and as training status progress, meta-analyses favor a moderate-volume RT protocol furthering gains in muscle mass and -strength (Krieger, 2009; Rhea et al., 2003; Schoenfeld, Ogborn, and Krieger, 2017). In the study by Hammarstrøm et al. (2020), a unilateral model providing a within-subjects design, proved to elicit prominent gains in muscle mass and -strength, especially with a moderate RT-volume (Hammarström et al., 2020). Furthermore, total RNA, rRNA, hence by proxy ribosome biogenesis, has been suggested to be volume sensitive (Hammarström et al., 2020). Precursor rRNA expression has been observed to increase shortly following a single session of RT (Figueiredo et al., 2016), and both total RNA accumulation and rRNA expression has been reported to significantly increase following 2 weeks of moderate volume RT (Hammarström et al., 2020). Despite this, only ~50% of the participants in Hammarström et al. (2020) experienced true beneficial effects of increasing RT-volume, therefore higher training volumes alone do not convert low responders to high responders. As both Hammarstrøm et al. (2020) and Stec et al. (2016) suggested a relationship between ribosomal content and extent of response to RT, ribosome biogenesis in regards to muscle plasticity has become an interesting topic.

1.2 Mechanisms of muscle plasticity

Muscle cells are the most plastic cells of the human body, adapting to mechanical strain by increasing intracellular protein content and thereby growing in size. Ribosomes are the motors of MPS, as such detrimental to muscle plasticity (Figueiredo and McCarthy, 2019; Tanaka and Tsuneoka, 2018). Ribosomes are composed of two subunits, the large 60S and the small 40S (Figueiredo and McCarthy, 2019; Tanaka and Tsuneoka, 2018). 60S is formed by three rRNAs (28S, 5.8S, 5S) and 47 r-proteins, 40S is formed by one rRNA (18S) and 33 r-proteins (Figueiredo and McCarthy, 2019; Tanaka and Tsuneoka, 2018). Ribosomal RNA represents 80-

90% of the cell's total RNA content, meaning that changes in total RNA content typically is used as a marker of ribosome biogenesis. When more ribosomal RNA is transcribed and translated into ribosomes, the capacity for translating mRNA into protein is increased (Figueiredo and McCarthy, 2019; Tanaka and Tsuneoka, 2018). This could be compared to a restaurant hiring more chefs, whereas the ribosomes serve as chefs, translating the mRNA being the recipe of the protein to be synthesized. As the ribosomal content of the cell, is believed to represent a roof for protein synthesis (Iadevaia, Liu, and Proud, 2014), the possible mechanisms of ribosome biogenesis has been a topic of great interest. It is now suggested that the acute increase in MPS minutes to hours following an RT session is caused by increased efficiency of existing ribosomes (translational efficiency), while increases following hours and days is a product of increased translational capacity (Figueiredo, 2019; Kimball, Farrell, and Jefferson, 2002). Substantiating the likely role of translational capacity in MPS, is the fact that no positive relationship has yet to be observed between acute changes (translational efficiency) in MPS and chronic muscle mass gains, along with methodological and technical progression in the field (Figueiredo 2019). These progressions allow high resolution measurement of rRNA expression, providing yet another robust marker of ribosome biogenesis, and means to investigate the synthesis of ribosomes in relation to exercise training further.

Synthesis of new ribosomes occur when rDNA is transcribed into 47S pre-rRNA, further processed into mature rRNAs, ultimately forming the ribosomes (Figueiredo and McCarthy, 2019), and requires approximately 80% of the cell's energy and materials (Schmidt, 2004). The processing mainly occurs within the nucleus of muscle cells, in a structure formed by chromosomal loops of rDNA called the nucleolus (Drygin, Rice and Grummt, 2010). R-proteins are translated in the cytoplasm, then imported to the nucleolus to associate with their respective ribosomal subunit, as a vital part of ribosomal maturation (Henras et al., 2015; Moss et al., 2007). The maturation of ribosomes take place in both the nucleolus and nucleoplasm, ultimately leading to the final step ocurring in the cytoplasm, where they assemble to ribosome units (Henras et al., 2015; Moss et al., 2007). This process occurs upon a stimulus, for instance RT, involving multiple cellular pathways, suggested to be c-Myc protein, mTORC1, and MAPK, converging upon and promoting the expression and transcription of rDNA (Kusnadi et al., 2015; Mayer and Grummt, 2006; Zhao, Fröding and Grummt, 2003). Ribosomal DNA transcription

into 47S pre-rRNA is a key step in ribosome biogenesis, initiated by assembly of the pre-initiation complex (PIC) at the rDNA promoter (Grummt, 2003; Russell and Zomerdijk, 2015). PIC is composed of the upstream binding factor (UBF), selectivity factor 1 (SL-1) complex (containing TATA-binding proteins), transcription initiation factor TIF-IA, and the RNA polymerase I (Pol I) (Grummt, 2003). UBF bind to the upstream control element (UCE), SL-1 bind to the core promoter region of rDNA, recruiting Pol I through TIF-IA, stabilized by UBF (Russell and Zomerdijk, 2005). The assembly of the PIC initiates Pol I transcription of rDNA, transcribing rDNA to 47S pre-rRNA containing 18S rRNA, 28S rRNA and 5.8S rRNA (Grummt, 2003; Russell and Zomerdijk, 2015). 47S pre-rRNA is processed by cleavage enzymes, removing internal and external transcribed spacers (ITS and ETS), leaving three mature rRNAs (18S, 28S, 5.8S) (Henras et al., 2015). The 5S rRNA is expressed, transcribed and matured outside of the nucleolus, by Pol III (Mayer and Grummt, 2006). Lastly, r-proteins are transcribed by Pol II, imported into the nucleus to be assembled along with their respective mature rRNAs (Mayer and Grummt, 2006).

In vitro, rodent, and human studies have linked ribosome biogenesis to skeletal muscle hypertrophy (Figueiredo and McCarthy, 2019). Synergist ablation in rodents showed that: I) increases in 47S pre-rRNA precedes hypertrophy and is associated with higher transcription factor activation and chromatin remodeling, suggesting a connection between higher availability of precursor rRNA and ribosome biogenesis due to exercise (Gordon et al. 2016; Kirby et al., 2016, 2015; Walden et al., 2012), and II) strong correlations between hypertrophic responses and translational capacity rather than translational efficiency (Nakada et al., 2016). Measured by reduced total RNA abundance, in vitro studies on non-muscle cell cultures showed that inhibition of ribosome biogenesis reduced translational capacity and protein synthesis (Nader, McLoughlin, and Esser, 2005). Furthermore, muscle wasting and low MPS has been observed in cancer patients, and impaired hypertrophy in healthy untrained individuals, both associated with a blunted ribosome biogenesis and thus, a reduced ability to increase total content of ribosomes (Hammarström et al., 2020; H.-G. Kim et al., 2021; Stec et al., 2016). In a time course perspective, elevated 47S pre-rRNA has been observed following a single RT session, passing baseline after 4h, continuing to rise through 24h and staying elevated up to 48h, while augmented rRNA expression required multiple sessions (Figueiredo et al., 2016). Considering this, short-

term RT may give valuable information on ribosomal content of the muscle cell. Notably, some discrepancy exists in the field, as IGF-1-induced myotube hypertrophy has been observed independent of ribosome biogenesis (Figueiredo and McCarthy, 2019). Altogether, there seem to be a growing body of evidence supporting ribosome biogenesis and translational capacity as driving and determining factors in long-term skeletal muscle growth induced by RT, possibly regulating the extent of muscular hypertrophy. As such, ribosome biogenesis may also represent a source to individual variation in RT-adaptations (Hammarstöm et al., 2020; Stec et al., 2016).

1.3 Mechanisms of individual variations in RT adaptations

Indeed, recent studies have shown a possible connection between individuals' skeletal musculature´s ability to adapt to RT and the ribosome content of the muscle cells, as measured by total RNA and rRNA (Hammarström et al., 2020; Stec et al., 2016). Interestingly, Stec et al. (2016) found that ribosome biogenesis could possibly be a key process regulating the extent of RT-induced myofibre hypertrophy, while Hammarstrøm et al. (2020) demonstrated that poor response to increased RT volume coincided with a blunted ribosome biogenesis. More specifically, Stec et al. (2016) observed substantial differences between response groups (non, moderate and extreme), whereas only extreme responders to RT increased total RNA and rRNA compared to their baseline measures. Another marker of ribosome biogenesis, c-Myc, increased significantly compared to baseline in moderate- and extreme responders, although not in the non-group (Stec et al., 2016). The findings by Stec et al. (2016) and Hammarstrøm et al. (2020) suggest that difference in augmentation of RT-induced ribosome biogenesis affects MPS, thus affecting the ability to maximize hypertrophic adaptation to RT over time. In addition, a study by Thalacker-Mercer et al. (2013) suggested that skeletal musculature in those who respond poorly to RT is inhibited from growth, while in extreme responders it is primed for growth. The explanation for this is far from elucidated and has been suggested to stem from differential regulation of protein accretion and stem cell activity (Thalacker-Mercer et al., 2013). It seems apparent that low responders share a common blunted ability for ribosome biogenesis, though the reason for this and how to circumvent it, seem more elusive. On that note, rDNA content of the muscle cell may represent a possible explanation to some of the variation in ribosome biogenesis, as an individual with only 60 copies of rDNA would have less potential for ribosome biogenesis than an individual with 1590 copies (Figueiredo and McCarthy, 2019; Tanaka and Tsuneoka, 2018). Based on current knowledge, there is little to be done about the copies of rDNA an

individual carries, and higher training volumes does not convert low responders to high responders. Hence, investigating the regulation of rDNA transcription in response to different stimuli has received increasing attention, for instance via high and low glucose treatments (Mariappan et al., 2011; Zhai et al., 2012). Using glucose as an adjuvant might have beneficial effects on the internal physiological milieu during RT (Kusnadi et al., 2015; Tanaka and Tsuneoka, 2018), and may therefore prove a promising strategy in participants experiencing low RT adaptations.

1.4 Glucose and resistance training induced ribosome biogenesis

Exogenous glucose is not strictly vital to our survival, but it is the preferred energy substrate of contracting muscles, especially during exercise requiring rapid ATP-synthesis (Hargreaves and Spriet, 2018; Mul et al., 2015) such as RT, as well as in proliferating and transformed cells (Altman and Dang, 2012). After degradation through the digestive system, glucose is transferred to the blood stream, elevating the levels of plasma glucose, commonly known as blood sugar. The plasma glucose level at rest is regulated by the endocrine system, whereas insulin secretion facilitates glucose uptake from the blood for glycogen storage, while glucagon secretion facilitates mobilization of glycogen storage and secretion of glucose into the blood stream (Mul et al., 2015). At rest, elevated plasma glucose levels stimulate insulin secretion from the ß-cells of pancreas, which in turn stimulate glucose uptake and glycogen storage in muscle and liver (Mul et al., 2015). To avoid energy depletion, glucagon stimulates glucose turnover in the liver, secreted into the bloodstream and supplied to working skeletal muscle tissue. During exercise training, both insulin and muscle-contraction stimulate glucose uptake from the bloodstream into the muscle cells (Hargreaves and Spriet, 2018; Mul et al., 2015). At onset of exercise activity and increased energy demand, intramuscular glycogen is broken down to glucose and synthesized to ATP (Hargreaves and Spriet, 2018; Mul et al., 2015). During ATP synthesis, glucose is first broken down through glycolysis, resulting in two pyruvate molecules and 2 ATP per glucose molecule (Kim, Buel, and Blenis, 2013). The pyruvate is either secreted as lactic acid or sent to Kreb´s cycle, depending on oxygen availability, for further energy metabolism via oxidative phosphorylation (Kim, Buel, and Blenis, 2013). Glucose as an energy substrate has been well investigated. In addition, recent studies indicate it as a possible mediator of anabolic signaling (Tanaka and Tsuneoka, 2018).

We know little about how glucose *per se* impacts the regulation of ribosome biogenesis in human skeletal muscle, but there have been studies conducted on glomerular epithelial cells (GEC) of mice in cell cultures (Mariappan et al., 2011) and some human cell cultures (human HEK293T) (Zhai et al., 2012) investigating high glucose vs. low glucose treatment. Mariappan et al. (2011) observed augmented rDNA transcription stimulation upon high glucose treatment, as a result of ERK1/2 and mTORC1-p70S6K regulated UBF activation. This resulted in increased ribosome biogenesis and ultimately hypertrophy of the GEC´s (Mariappan et al., 2011). Zhai et al. (2012) observed high glucose treatment to lead to recruitment of SL-1 and TIF-IA to the rRNA promoter by PIH1 and SNF5-Brg1 complex association. The SNF5-Brg1 complex is proposed to increase acetylation of several histones increasing rDNA availability, while SL-1 and TIF-IA increases Pol-1 activity, together enhancing rDNA transcription during high glucose treatment (Zhai et al., 2012). Nutrient depletion on the other hand, has been observed to repress ribosomal gene transcription in yeast (Kos-Braun, Jung, and Koš, 2017). In fact, Zhai et al. (2012) observed that glucose starvation led to dissociation of PIH1 from SNF5-Brg1, repressing rDNA transcription. Furthermore, low energy level induced increase in AMP/ATP ratio is known to activate the AMP-dependent protein kinase (AMPK). AMPK is suggested to affect PIC-assembly in two ways; I) Negatively regulating mTORC1 directly or indirectly through TSC2 activation, reducing TIF-IA and Pol I interaction, and II) phosphorylating TIF-IA, impairing its interaction with SL-1 and thus PIC-assembly (Hoppe et al., 2009). Moreover, SIRT1 and SUV39H1 have been observed in repression of rDNA transcription, by triggering heterochromatin formation which silences transcription (Murayama et al., 2008). Based on these observations, high glucose treatment is suggested to promote rDNA transcription (Mariappan et al., 2011; Zhai et al., 2012), while low glucose treatment and glucose starvation is proposed to repress rDNA transcription (Zhai et al., 2012; Hoppe et al., 2009). In this perspective, mTORC1 seem to be the primary upstream pathway regulating ribosome biogenesis in response to glucose. Indeed, mTORC1 is proposed as a nutrient regulator linking the availability of growth factors and amino acids to rDNA transcription (Kusnadi et al., 2015), as well as being energy-sensitive (Kim, Buel and Blenis, 2013). AMPK on the other hand, is an established crucial cellular energy sensor, switching off/on energy-consuming anabolic processes and energy-generating catabolic processes depending on energy availability to maintain cellular equilibrium (Hoppe et al., 2009). Through directly affecting intracellular energy status, glucose therefore indirectly regulate AMPK activity,

ultimately impacting mTORC1 and downstream targets regulating ribosome biogenesis (Hoppe et al., 2009; Murayama et al., 2008; S. G. Kim, Buel, and Blenis, 2013; Kusnadi et al., 2015). As such, ribosome biogenesis and protein synthesis are greatly impacted by AMPK-activity and dependent on a positive cellular energy status (Hoppe et al., 2009). Based on these findings, it seems apparent that glucose may indeed be crucial to supply intracellular energy levels and thereafter inhibit AMPK-dependent repression of rDNA transcription, possibly also to inhibit epigenetic down-regulation of ribosome biogenesis (SIRT1 and SUV39H1-dependent) (Hoppe et al., 2009; Kusnadi et al., 2015; Murayama et al., 2008; Tanaka and Tsuneoka, 2018).

There are equivocal findings on insulin´s impact on MPS, whereas insulin is proposed to either increase synthesis or decrease breakdown (Abdulla et al., 2016; Tanaka and Tsuneoka, 2018). Tanaka et al. (2018) propose insulin to mediate an anabolic signal through the PI3K-Akt-mTORC1 pathway, while Abdulla et al. (2016) reviewed 25 meta-analyses finding no significant effect of insulin alone on MPS. Insulin did have a significant effect on MPS when amino acid delivery was increased, thereby suggesting the insulin-induced increase in MPS to be dependent on a simultaneous increase in amino acid delivery (Abdulla et al., 2016). The aforementioned review also found that insulin impacts muscle protein breakdown, especially when amino acids are scarce, possibly having an anti-catabolic effect (Abdulla et al. 2016). Cell culture studies on mouse adipocytes have provided some evidence that polymerase I transcription and release factor (PTRF)/Cavin-1 promotes rDNA transcription, induced by insulin and repressed by fasting (Liu and Pilch, 2016). It seems plausible that insulin may act in an additive manner in concert with amino acid ingestion to increase MPS (Abdulla et al., 2016), although it has yet to be determined whether it has any effect on ribosome biogenesis *per se* in muscle cells. Perhaps the simultaneous ingestion of glucose (thereby elevated insulin) and amino acids lead to higher mTORC1 activity, which may ultimately up-regulate rDNA transcription factors and/or translation of c-Myc, a protein regulating the PIC (Abdulla et al., 2016; Kusnadi et al., 2015).

Taken together, it seems likely that glucose plays an important role in ribosome biogenesis as an energy substrate contributing to cellular energy status, thus regulating AMPK activity. However, quite uncertain whether it conveys an anabolic signal as a signaling molecule *per se* to stimulate mTORC1, or through insulin. However, the importance of amino acid delivery along with insulin

to stimulate increased MPS is well documented (Abdulla et al., 2016). As such, we investigated the outcome of ingesting glucose during RT on ribosome biogenesis, combined with protein ingestion in the morning and following RT. Our main purpose was to investigate the effects of glucose ingestion during 5 RT sessions on ribosome biogenesis in moderately trained participants. Changes in ribosome biogenesis were measured by accumulation of total RNA and expression of the ribosomes four mature rRNA´s (18S, 28S, 5.8S, 5S) as well as the 47S precursor RNA. In addition, as a secondary outcome, the possible effects of glucose during resistance training on muscular recovery post resistance training were measured as temporal changes in unilateral isometric knee extension force and isokinetic knee extension torque. On that note, our hypotheses were:

1) Resistance training will lead to an accumulation of total RNA and rRNA, whereas glucose may lead to higher accumulation and expression than placebo.
2) Glucose supplementation during resistance training will enhance muscular recovery, leading to a more rapid recovery post session than placebo and thus higher isometric knee extension force and isokinetic knee extension torque.

2. Introduction

Regular resistance exercise training (RT) results in increased muscle mass (hypertrophy) and muscle strength, representing adaptations to the stimulus of mechanical strain. Unfortunately, responses to resistance training are not uniform across the population. In fact, studies have shown that RT-induced muscle growth varies widely between individuals, with 10-15% showing considerable impaired growth (Álvarez et al., 2018; Mann, Lamberts, and Lambert, 2014; Thalacker-Mercer et al., 2013). This may be due to genetics, epigenetics, or unfavorable internal physiological conditions. Recently, tweaking training modalities and -protocols have shown to induce different responses in different populations (Hammarström et al., 2020), but we know little about what kind of resistance training to prescribe to individual phenotypes. Meta-analyses provide a sound basis favoring a moderate volume of RT over low volume RT in regards to increasing muscle growth and muscle strength (Krieger, 2009; Rhea et al., 2003; Schoenfeld, Ogborn, and Krieger, 2017), substantiated by a recent study by Hammarstrøm et al. (Hammarström et al., 2020). However, Hammarstrøm et al. (2020) found in their study that although a moderate volume led to more prominent gains in muscle mass and -strength than a low volume, approximately 50% of the individuals did not experience true benefits of the increased training volume. Together with other studies finding variations in muscle growth response to standardized RT protocols (Schoenfeld, Ogborn, and Krieger, 2017; Stec et al., 2016), it would seem apparent that increasing the resistance training volume does not convert low-responders to high-responders, and that other means than RT *per se* seem necessary to circumvent this discrepancy.

Indeed, ingestion of nutrients such as protein and creatine supplements are effective in optimizing RT (Cermak et al., 2012; Lanhers et al., 2015; Lanhers et al., 2017). However, it remains largely unknown if other nutritional adjuvants such as glucose can increase the efficacy of RT, which is surprising as glucose is the preferred energy substrate of the contracting skeletal muscle during strenuous exercise, and the major energy source of cells via ATP synthesis (Tanaka and Tsuneoka, 2018). Glucose availability seems important to regulate central processes to muscle growth, i.e. ribosome biogenesis which drives muscle protein synthesis (MPS) (Tanaka and Tsuneoka, 2018; Figueiredo and McCarthy, 2019). Interestingly, variations in RT-induced responses have been linked to ribosome biogenesis, where individuals responding poorly to RT

also experience blunted ribosome biogenesis (Hammarström et al., 2020; Stec et al., 2016). Hence, there may be a relationship between an individual´s capacity for ribosome biogenesis and trainability, thus investigating the possible effects of glucose on ribosome biogenesis is warranted. First and foremost, glucose greatly impact the energy status of the cell, which ribosome biogenesis is dependent upon, requiring up to 80% of the cell's energy and materials (Schmidt, 2004). Changes in the energy status of the cell affect ribosome biogenesis regulators such as the PI3K-Akt-mTOR pathway, as mTORC1 is suggested to be energy-sensitive (Kim, Buel and Blenis, 2013). Furthermore, mTORC1 is thought to control nutrient availability and link the availability of growth factors and amino acids to rDNA transcription, giving it a central role in ribosome biogenesis (Kusnadi et al., 2015). Another important player in the energy balance of the cell, the activity of the AMP-dependent protein kinase (AMPK), is regulated by intracellular ATP/AMP ratio (Hoppe et al., 2009). Based on ATP/AMP ratio, AMPK switches off/on energy-consuming anabolic processes and energy-generating catabolic processes depending on energy availability, such as mTORC1 (Hoppe et al., 2009). Viewing recent literature, it is suggested that high energy status promotes ribosome biogenesis, and low energy status inhibits ribosome biogenesis (Kusnadi et al., 2015; Mariappan et al., 2011; Tanaka and Tsuneoka, 2018; Zhai et al., 2012). It does however remain uncertain whether glucose affects ribosome biogenesis through cellular energy levels alone, as a signalling molecule *per se*, or indirectly via insulin. These theories remain quite unexplored in human skeletal muscle cells. Factors involved in the pre-initiation complex (PIC) is thought to receive cues from pathways (mTORC1, MAPK) regulated by hormonal and nutritional signals (Figueiredo, 2019), perhaps glucose directly or indirectly affect these pathways. Lastly, there are equivocal findings on insulin´s impact on ribosome biogenesis and MPS. Recent meta-analysis suggest insulin to act in concert with amino acid intake to enhance MPS, and that insulin may reduce muscle protein breakdown when amino acids are scarce (Abdulla et al., 2016).

As it is uncertain what effects glucose has on ribosome biogenesis, we investigated the outcome of ingesting glucose during RT on ribosome biogenesis, in addition to protein ingestion in the morning and following RT. Our main purpose was to investigate the effects of glucose ingestion during 5 RT sessions on ribosome biogenesis in moderately trained participants. Changes in ribosome biogenesis were measured by accumulation of total RNA and expression of the

ribosomes four mature rRNA´s (18S, 28S, 5.8S, 5S), as well as the 47S precursor RNA. In addition, as a secondary outcome, the effects of glucose during resistance training on muscular recovery post resistance training were measured as temporal changes in unilateral isometric knee extension force and isokinetic knee extension torque. On that note, our hypotheses were:

1) Resistance training will lead to an accumulation of total RNA and rRNA, whereas glucose may lead to higher accumulation and expression than placebo.
2) Glucose supplementation during resistance training will enhance muscular recovery, leading to a more rapid recovery post session than placebo and thus higher isometric knee extension force and isokinetic knee extension torque.

3. Materials and methods

This data set is a part of a larger project involving several investigators and other outcomes not covered here. All participants were informed about the potential discomforts and risks associated with the study and gave their informed consent prior to study enrollment. The project was approved by the regional ethical committee (REK, ID nr. 153628), pre registered at clinicaltrials.gov (Identifier: NCT04545190) and conducted according to the Helsinki declaration.

3.1 Participants

Sixteen male and female participants (20-33yrs, Tab 1) were recruited to the study through facebook advertisement and word of mouth and taken through the selection process (Fig 1). The eligibility criteria were non-smokers and moderately trained (i.e. 2-8 resistance training sessions per 14 days for the last six months). Exclusion criteria were previous injury leading to impaired strength, inability to perform resistance training and symptoms, and a medical record of metabolic disorders including hyperglycemia, i.e. fasting venous plasma glucose ≥6.1 mmol/L and/or 2-hour glucose tolerance ≥7.8 mmol/L, and/or HbA1c >42 mmol/mol. Our goal was to recruit 20 participants to the study, however due to the advents of Covid-19, we were not able to do so. Sixteen participants commenced the intervention, during which three dropped out. One participant had a sick child, and was unable to resume the intervention, two participants experienced muscular discomfort connected to heavy resistance training (Fig 1). Baseline characteristics (Tab 1) were measured by means of DXA (Prodigy Advance PA+302047, Lunar, San Francisco, CA, USA) at Day -1, the last day preceding the RT intervention.

3.2 Experimental design

The study was designed as a 12-day double-blinded randomized controlled trial, with a unilateral RT protocol (Fig 2). Participants were randomized to either commence training with glucose (GLU, 30g per bolus) or placebo (PLAC, 0g per bolus), alternating RT and supplement from one day to another (Fig 3). After randomization to either commencement with glucose or placebo, participants were further randomized to commence the intervention with their dominant or non-dominant leg. Half the participants commencing with glucose were randomized to start RT with their dominant leg (n=8), the other half randomized to start RT with their non-dominant leg (n=8), the same was true regarding the participants commencing with placebo. Each subject was

linked to an ID number, kept on a list with personnel not involved in any other aspect of the study than the randomization.

Table 1: Baseline characteristics of participants. BMI = body mass index. A = characteristics per sex, B = characteristics per leg. Values are mean ± standard deviation.

A

Variable	Female (n=7)	Male (n=9)
Age (yrs)	24.6 ± 4.8	23.7 ± 1.8
BMI	23.2 ± 1.4	25.2 ± 2.5
Fat mass (kg)	17 ± 5.7	14.9 ± 6.1
Fat free mass (kg)	52.2 ± 6.8	64.4 ± 4.6
Height (cm)	172.1 ± 5.8	176.7 ± 5
Lean body mass (kg)	49.5 ± 6.5	61.1 ± 4.5
Body weight (kg)	68.5 ± 3.5	78.4 ± 6.1

B

Variable	Left leg	Right leg
Fat mass	3.3 ± 1.4	3.4 ± 1.4
Lean mass	9.7 ± 1.4	9.9 ± 1.4
Total mass/kg	13.7 ± 1.5	14 ± 1.4

The randomization list was not revealed until after completion of data sampling and analysis of main outcome measures. In addition, participants ingested protein prior to (25g) and after completion (25g) of RT every day, as protein supplementation has been shown to enhance muscle growth in response to RT (Cermak et al. 2012; J. Kim and Guan 2019). Performance tests (Test, Fig 2) were performed prior to (Days -7 and -5, and T0, both legs, Fig 2), during (Days 4, 5, 8 and 9, Fig 2), prior to and after session six (T3/T4, RT#1 leg; T4/Day 13, RT#2 leg, Fig 2), a total of 10 days (Fig 2). Performance tests consisted of unilateral isometric knee extension force, unilateral isokinetic knee extension torque, and 1RM leg press and knee extension. Unilateral isometric and isokinetic knee extension tests were used to investigate muscle force development prior to the intervention to post fifth RT session, in addition to investigating the muscular recovery following the sixth RT session. 1RM tests served as a basis for calculation of an approximate 10RM as well as accustoming participants to the exercise protocol.

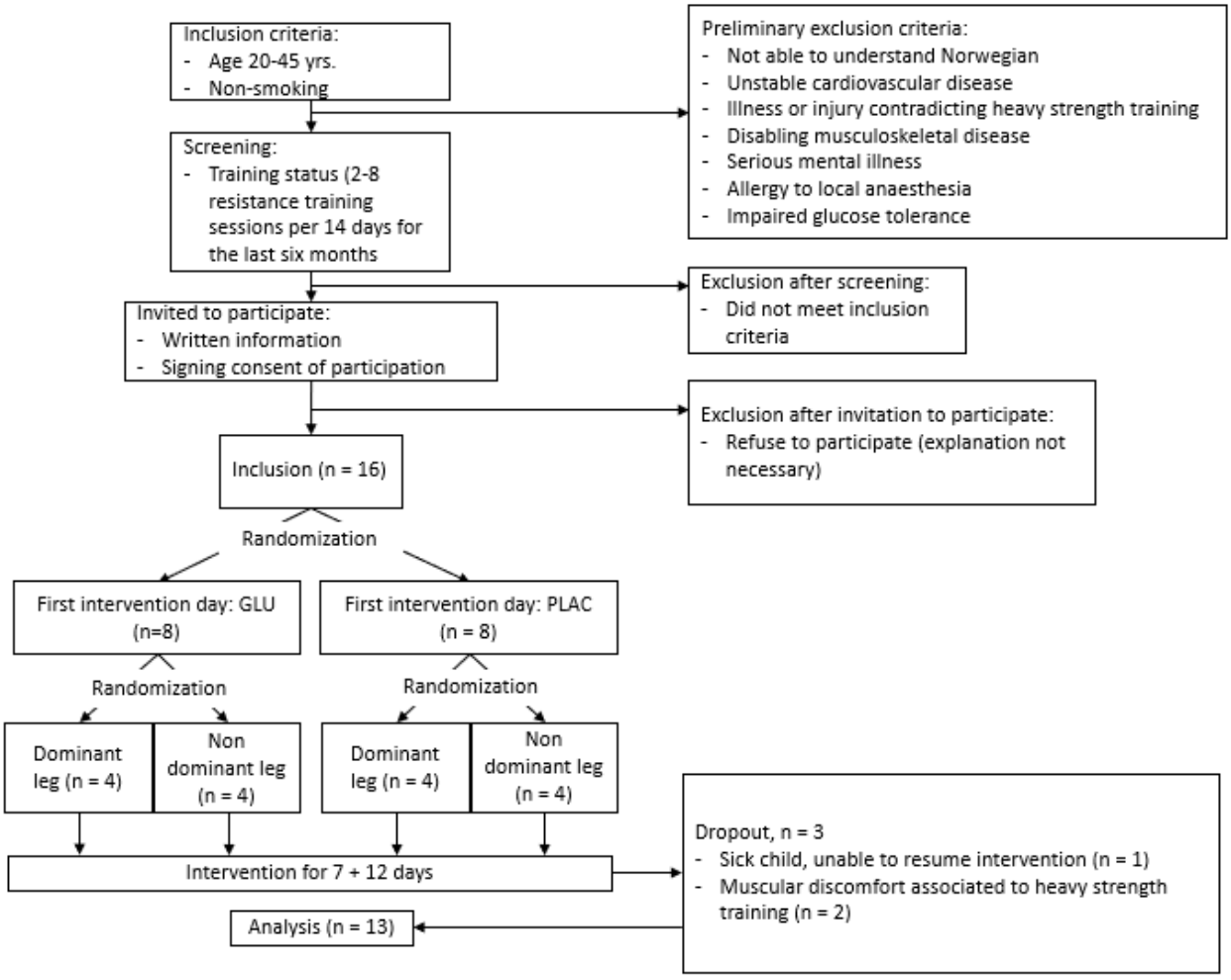

Figure 1. Flowchart of the selection process. The order in which participants performed the two intervention blocks was determined in a planned randomized fashion. GLU = glucose, PLAC = Placebo.

Six RT sessions were conducted with glucose and six with placebo, allowing a within-subjects analysis of the effects of glucose ingestion, arguably removing biological diversity between individuals as a confounding factor. The first five RT session were used to investigate main outcome measures (total RNA, rRNA), whereas the sixth RT session were used to explore secondary outcomes (muscular recovery, plasma glucose levels). Participants were asked to abstain from resistance- or high intensity training of the legs from Day -7 and forwards until the intervention was concluded, to ensure reliability of pre-intervention strength data and minimal interference from other training sources. If participants did conduct exercise training outside of the protocol, they were asked to ensure equal loading on pairwise consecutive days.

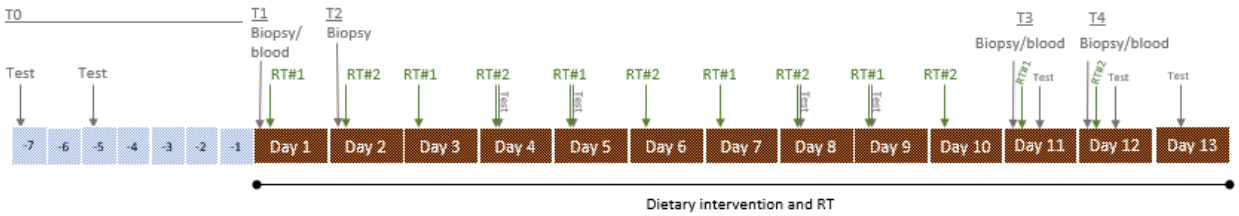

Figure 2. Overview of the intervention, with 12 days of concomitant dietary intervention and resistance training (RT), preceded by 7 days of testing. T0 = timepoint 0 (testing days prior to intervention), T1/T2 = biopsy prior to resistance training for leg #1 and #2, respectively, T3/T4 post intervention testing of leg #1 and #2, respectively, including post sixth RT session. Test = physical performance test (unilateral 1RM leg press, knee extension, isometric knee extension force, isokinetic knee extension torque) testing on days 4, 5, 8, 9, T3, T4 will not include 1RM leg press and knee extension, Biopsy = microbiopsy, RT#1 = resistance training leg #1, RT#2 = resistance training leg#2.

3.3 Dietary intervention

The dietary intervention spanned the whole day, divided into three periods: I) From awakening until two and a half hours after RT, II) from two and a half hours after RT until 2200hrs, and III) from 2200hrs until awakening. During period I, participants ingested protein and glucose/placebo only. Glucose/placebo was ingested at three time-points: 30 minutes prior to RT (0830hrs, 30g vs. 0g glucose), immediately prior to RT (0900hrs, 30g vs. 0g glucose), and immediately after completion of RT (~0930hrs, 30g vs. 0g glucose). Protein was ingested 2hrs prior to RT (0700hrs, 25g) and immediately after completion of RT (~0930hrs, 25g). In the afternoon (1800hrs-1900hrs) during period II (~1200hrs-2200hrs), participants ingested glucose or placebo (3x30g vs. 3x0g glucose) opposite to the supplement they received during RT, to ensure a balanced intake of glucose during the entirety of intervention days. Apart from this, participants ingested a self-chosen diet in period II, registered in MyFitnessPal or similar applications. The self-chosen diet was repeated on pairwise consecutive days (i.e. on days 1-2, 3-4, etc.), to ensure similar premises for resistance training responses between the two legs. During period III (2200hrs-0700), participants remained in an overnight fasted state. The daily onset of the dietary intervention (i.e. first ingestion of PRO supplement) varied for individual participants, between 0600hrs and 0900hrs to allow multiple participants to complete the protocol in the same time period. Individual participants commenced the intervention at the same time of day on every test day (pre and post) and intervention day.

To ensure blinding of participants, boluses of glucose (30g glucose) (Glucosum monohydricum, Merck KGaA, Darmstadt, Germany) and placebo (~0.3g Stevia rebaudiana extract) (Steviosa, Soma Nordic AS, Oslo, Norway) were diluted in 300ml Fun Light juice (Orkla, Oslo, Norway). Thus, the only difference between the boluses was glucose/steviosa content. Stevia contains the natural sweetener erythritol, and they both serve as suitable placebo sweeteners, neither increasing blood glucose levels nor inducing an insulin response (Tey et al. 2017; Wölnerhanssen et al. 2020). A blinded taste test was conducted to investigate whether participants were able to disclose the contents of the glucose and placebo beverages or not. Participants were offered four glasses, two contained 75ml of the glucose supplement and two contained 75ml of the placebo supplement, ingested in a randomized order. Participants were not allowed to go back and forth between glasses before guessing its content, hence they noted their guess on each separate glass and moved on to the next glass. Participants were awarded with 1 point per correct guess, with 4 points being the highest possible score. The average score of the taste test was 2,08 points (i.e. ~50%), meaning the participants likely were not able to detect the difference between the two beverages. Protein supplement was ingested as 25g Whey Protein Isolate boluses (Proteinfabrikken, Stokke, Norway), diluted in 150ml water. During training, participants were free to ingest water ad libitum.

3.4 Assessment of muscle strength

Performance tests (Test) were performed prior to (Days -7 and -5, and T0, both legs), during (Days 4, 5, 8 and 9), after session 5 and finalization of the intervention (T3/T4, RT#1 leg; T4/Day 13, RT#2 leg), a total of 10 days (Fig 2). Maximal isometric knee extension force and maximal isokinetic knee extension torque were tested with a HUMAC Norm dynamometer (CSMi, Stoughton, Massachusetts, USA). Participants were strapped to the chair using a 4-point harness, and the knee joint was aligned to the rotational axis of the dynamometer. The thigh of the leg being tested was strapped tight in the chair, while the leg was strapped tight to the dynamometer arm two fingers above the ankle joint. All chair positions were controlled and recorded at pre-intervention tests (days -7 and -5), then repeated for every subsequent test. Maximal isokinetic torque was measured at speeds 60 d/s, 240 d/s, 2 x 3 repetitions each, whereas the first set of each was sub-maximal as a warm-up. Lastly, maximal isometric force was measured at knee-angle 60, for a maximum of 10 seconds and one repetition per test. The highest peak torque values were carried forward for further analysis. During days 4, 5, 8, and 9 humac tests were conducted 1

hour prior to RT on the leg that performed RT the previous day. During days 11 and 12 (T3 and T4), humac tests were performed at four timepoints: I) 45 min prior to resistance training session, II) 30 min after finalization of the session, III) 2hrs after finalization of the session, and IV) 23hrs after finalization of the session. Test I at T4 included testing of both legs, to determine 23hrs post exercise test of leg 1 and pre-exercise test of leg 2.

Assessment of unilateral 1 repetition maximum (1RM) leg press and knee extension were conducted at pre-intervention testing (days -7 and -5, Fig 2). Participants warmed up with 10 minutes of cycling on an indoor exercise bicycle, and a specific warm-up before each of the exercises, following adjustment and standardization of the equipment. Back position, foot position (leg press and knee extension) and knee angle (leg press) were noted for the following resistance training. To standardize the 1RM leg press test, the equipment was marked at the point where each individual participant reached 45 degrees in the eccentric phase of the exercise. The leg not performing the exercise was kept still, in a natural position touching the ground. The specific warm-up consisted of 3 sets (10, 6, and 3 repetitions respectively), with 2 minutes pause in-between. Participants were given a 3 minute pause between specific warm-up and 1RM testing, and 3 minutes between each 1RM attempt. 1RM leg press was defined as the maximal load lifted in a controlled fashion, with a knee angle of 45 degrees, and within a 2.5 kg range. Attempts were participants did not reach 45 degrees in the eccentric phase were not approved. 1RM knee extension followed the same specific warm-up and pause protocol. Following equipment adjustment, the leg not performing the exercise was strapped to not interfere, and a point of full knee extension was visually established. 1RM knee extension was defined as maximal load lifted in a controlled fashion, reaching full extension of the knee joint, within a 1.25kg range. Attempts with too much hip movement or beneath full extension were not approved.

3.5 Resistance training protocol

Resistance training consisted of three sets of each unilateral leg press and knee extensions exercises, conducted as 10 repetition maximum (10RM). For each participant, equipment adjustments were controlled and noted at pre-intervention testing (days -7 and -5, Fig 2), and used throughout the intervention. Warm-up consisted of 5-10 minutes moderate cycling on an indoor exercise bicycle. In addition, before the respective exercises, participants conducted 2 sets

of 10 repetitions as warm-up sets at ~50% and ~70% of 1RM. During working sets, participants completed every set with an intensity equivalent to 10RM. Rest time between sets was 2 minutes. Loading/resistance during RT sessions were progressively modified to ensure adequate exercise stimulation throughout the intervention. All sessions were monitored by trained personnel. The minimal requirement was that the same trainer accompanied the same participant on pairwise consecutive days, if by some reason the trainer could not accompany the participant through the whole intervention. RPE (0-10 point scale) was logged before every RT session, session score (0-10) was logged 15min after RT sessions. Training volume (load and reps) was also logged for every RT session.

3.6 Sampling of muscle tissue and blood

Muscle biopsies were sampled from m. vastus lateralis using well-established procedures. Muscle biopsy sampling was performed under local anesthesia (Xylocaine, 10 mg ml−1 with adrenaline 5 µg ml−1, AstraZeneca AS, Oslo, Norway) using a 12-14-gauge needle (Universal Plus, Mermaid medical AS, Stenløse, Denmark), operated with a spring-loaded biopsy gun. After biopsy sampling, muscle tissue was divided into two aliquots for determination of total RNA/expression of rRNA, and were snap frozen in isopentane (-80 °C) and stored at -80°C until further analysis. For each participant, muscle biopsies were sampled at four time points (figure 2): I/II) before the intervention (2hrs before training, time point T1, pre RT#1 leg, T2, pre RT#2 leg), and III/IV) before the sixth training session from the RT#1 leg (2hrs before training, T3) and before the sixth training session from the RT#2 leg (2hrs before training, T4). At each time-point, two samples were taken from the same incision, for total RNA and rRNA analysis. To standardize this procedure, all individual participants had biopsies taken at the same time of day, in an overnight-fasted state.

To measure glucose levels in blood with and without glucose intake/training, blood was collected by means of finger stings on days with biopsy sampling. At T1 (pre-test, Fig 2), one finger sting was taken, to provide a baseline for each participant. At T3 and T4 (post-test, Fig 2), finger stings were collected at 7 time points: I) before protein ingestion (0700hrs), II) 45 minutes after protein ingestion (0745hrs) III) 1.5hrs after protein ingestion (0800hrs, i.e., immediately before GLU/PLAC intake), IIII) 2hrs after protein ingestion (0900hrs, i.e., immediately before training), IV) in the middle of RT (~0915hrs), V) immediately after training (~0930hrs), and VI) 2hrs after

completion of training (~1130hrs). Finger stings were analyzed with in-house equipment (BIOSEN C-Line, EKF diagnostic GmbH, Barleben). Blood samples were also collected coinciding with finger stings, except from in the middle of the RT session, to analyze endocrine variables. The blood analyses were not completed in due time to include in this data set.

3.7 Total RNA extraction

Two muscle biopsy samples were taken for total RNA extraction per leg per time point, and total RNA was extracted in two duplicates per muscle biopsy. Thus we had two duplicates per leg per time point, giving a total of eight RNA samples per participant. RNA was extracted using TRIzol according to the manufacturer's protocol. Briefly, muscle tissue was homogenized in 300µl TRIzol using 0.5mm RNase-free Zirconium beads (~50 ul) (Next Advanced, Averill Park, NY, USA) and a bullet blender (bb). Samples were ran 1min at speed 10, then put on ice for 1min and bb one more round at speed 12 for 1 min. If not fully dissolved, samples were ran additional rounds until fully homogenized. Thereafter, an additional 700 µl TRIzol was added, and samples were ran 1 min at speed 3 in bb for mixing, before 5 min incubation at room temperature. 200 µl chloroform (Sigma-Aldrich, Missouri, USA) was added, samples were shaken for 15sec, followed by 2-3min incubation at room temperature. After incubation, samples were inverted by hand and centrifuged in Heraeus™ Fresco™ 21 Microcentrifuge (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 12000g, 15min, 4°C. The upper aqueous phase (450 µl) was transferred to a new tube, containing 500 µl isopropanol (VWR International, Pennsylvania, USA), mixed, and incubated for 10min at room temperature. Samples were centrifuged at 12000g for 10min at 4°C, precipitating an RNA pellet. The pellet was washed twice with 1000 µl 75% cold ethanol; centrifuged for 5 min at 7500g, 4°C. Thereafter, ethanol was removed and the pellet was air dried for 10min (or until dried). The pellet was suspended in 30 µl DEPC-treated water, and incubated for 10 min at 55°C. For assessment of RNA-content and -purity 5µl RNA, from the RNA stock, was aliquoted with 5µl TE-buffer (1:2), for assessing via spectrophotometry. All samples had a 260nm to 280nm ratio > 1.9. The RNA stock was stored at -80°C until further analyses.

3.8 cDNA synthesis and quantitative polymerase chain reaction

RNA was reverse transcribed using Super Script IV Reverse Transcriptase (Invitrogen), according to the manufacturer's instructions. Briefly, 1µl 10 mM dNTP mix, 0.5µl anchored

oligo-dT, 0.5µl random hexamer primers (Thermo Scientific), a maximum of 9µl template RNA and 2 µl nuclease free water were mixed, vortexed en briefly spun down. Samples were then heated at 65°C for 5 minutes, followed by at least 1 minute incubation on ice. For the next step, 4µl 5x SSIV Buffer, 1µl 100 mM DTT, 1 µl RNase OUT, and Super Script IV Reverse Transcriptase (Invitrogen) were mixed, vortexed and briefly spun down and added to the samples. Samples were incubated for 10 minutes av 23°C, 10 minutes at 50-55°C and 10 minutes at 80°C. All samples were reverse transcribed and diluted to 1:50 prior to quantitative real-time polymerase chain reaction (qPCR). Lambda was used as external reference gene, added in the RNA extraction (2µl per extraction). qPCR reactions were run on a fast-cycling real-time detection system (Applied Biosystems 7500 fast Real-Time PCR Systems, Life Technologies AS), with a total volume of 10 µl, containing 2 µl of cDNA, gene-specific primers (0.5 µM final concentration) and a commercial master mix (2X SYBR Select Master Mix, Applied Biosystems, Life Technologies AS)(Hammarström et al. 2020). qPCR reactions consisted of 40 cycles (3 s 95°C denaturing and 30 s 60°C annealing) (Hammarström et al. 2020). Raw fluorescence data were exported from the platform-specific software and amplification curves were modelled with a best-fit sigmoidal model using the qPCR-package (Ritz & Spiess, 2008) written for R (Hammarström et al. 2020; Team 2018). For overview of primers, see Table 2.

3.9 Data handling and statistical analysis

A priori power calculations showed that 20 participants would be enough to grant a statistical power of 80% (= 0.05), with an expected drop out of 20%. As no studies existed to guide our power calculations, we had to assume that the effects of glucose ingestion on ribosome biogenesis may equate to the effects of increasing resistance training volume from low to moderate (Hammarström et al. 2020). All raw data were imported and analyzed in R (Team 2018). Individual participants leg results were categorized as either glucose or placebo according to the randomization. Total RNA and rRNA were analyzed by post to pre log-fold change score difference comparisons, using a linear mixed effects model with mean-centered baseline values as a co-variate and subsequent calculation of estimated marginal means. Total RNA was normalized by muscle biopsy weight, qPCR values were normalized by muscle biopsy weight and external reference gene (Lambda). Total RNA and rRNA changes were calculated as log-fold change score per mg wet muscle weight.

Table 2: Primer sequence and performance. Average cycle thresholds (Ct) and priming efficiencies were calculated from all qPCR reactions.

Gene	Primer sequence (forward and reverse)	Ct mean (SD)	*E*
18S ribosomal RNA	5'-TGCATGGCCGTTCTTAGTTG-3'	9.73 (0.768)	1.82
	5'-AACGCCACTTGTCCCTCTAAG-3'		
28S ribosomal RNA	5'-TGACGCGATGTGATTTCTGC-3'	11.0 (0.968)	1.88
	5'-TAGATGACGAGGCATTTGGC-3'		
5.8S ribosomal RNA	5'-ACTCTTAGCGGTGGATCACTC-3'	15.8 (0.747)	1.81
	5'-GTGTCGATGATCAATGTGTCCTG-3'		
5S ribosomal RNA	5'-TACGGCCATACCACCCTGAAC-3'	18.4 (0.639)	1.83
	5'-GGTCTCCCATCCAAGTACTAACC-3'		
47S ribosomal RNA	5´-CTGTCGCTGGAGAGGTTGG-3´	26.1 (1.90)	1.81
	5´- GGACGCGCGAGAGAACAG-3´		
Lambda external reference gene (F2R2)	5´- AAGACGACGCGAAATTCAGC-3´	23.2 (1.50)	2.02
	5´- TGGCATTCGCATCAAAGGAG-3´		
Lambda external reference gene (F3R3)	5´- TCGCGGCGTTTGATGTATTG-3´	23.8 (0.890)	1.81
	5´- TGACGCAGACCTTTTCCATG-3´		

Blood glucose levels, dietary data, training volume, training intensity, RPE, and muscular strength/recovery were analyzed by multiple time-point log-fold change scores comparisons, using the same linear mixed effects model, adding time as an explaining factor in addition to supplement. The linear mixed effect model was design with the lmer function of the lme4 package (Bates et al. 2014) in R, using the lmerTest function in addition to procure p-values. A paired sample t-test was used to analyze baseline characteristics of the two legs. All data were log-transformed for analysis to control for heteroscedasticity. For figure illustration, values were either reverse transformed or calculated as absolute changes. Mean-centered baseline values were used to correct for regression to the mean, which potentially occurs when the same participants are repeatedly tested. Regression to the mean entails that scores close to the upper or lower limit of a participants potential score would be replaced with a score closer to the mean, e.g. high baseline values are shifted downwards while low baseline values are shifted upwards, creating a negative association between initial values and change. Estimated marginal means were calculated from the linear model, enabling acquisition of least-square means, showing the means for all involved groups adjusted to means of other potential factors in the model. Values are

presented as mean ± SEM unless otherwise stated. Alpha-level was set to $\alpha = 0.05$. Complete data sets and scripts are downloadable here; *https://github.com/Kristianlian/master_degree*

4. Results

At baseline there were no significant differences between glucose and placebo in muscle mass, isometric peak torque, isokinetic peak torque (60 and 240 d/s), total session volume, training intensity, total RNA content, or rRNA expression (Tab 3). Furthermore, on pairwise consecutive days, were no significant differences between glucose and placebo in calorie, carbohydrate, fat or protein ingestion (Tab 4). During the sixth training session, RT with glucose ingestion led to significant increases in plasma glucose levels between glucose and placebo prior to resistance training, during the exercise and immediately after the exercise (Fig 3B, 120min: 43 ± 4% [p = 0.00000], 135min: 32 ± 4% [p = 0.00000], 150min: 32 ± 4% [p = 0.00000]). At 2 hours post exercise glucose measured significantly lower plasma glucose levels than placebo (8 ± 4%, p = 0.03).

Table 3: Baseline characteristics of the intervention legs, organized per supplement. Variables: Iso 60 = isokinetic 60 d/s, Iso 240 = isokinetic 240 d/s, training intensity = load/rm*100. Nm = newton meters, kg = kilograms, %1RM = percentage of 1 repetition maximum, ng/mg = nanogram per milligram wet muscle tissue, nf.expr = expression normalized by weight and external reference gene. rRNA data are presented as log transformed. Glucose n = 13, placebo n = 13.

Variable	Glucose (n=13)	Placebo (n=13)	P-value	95% CI
Isometric peak torque (Nm)	269.08	259.85	0.420	-14.855 - 33.316
Iso 60 peak torque (Nm)	193.15	198.31	0.363	-17.036 - 6.729
Iso 240 peak torque (Nm)	123.85	124.62	0.773	-6.438 - 4.900
Training volume (kg)	5262.12	5351.34	0.675	-540.824 - 362.362
Training intensity (%1RM)	63.26	63.56	0.867	-4.241 - 3.601
Total RNA (ng/mg muscle tissue)	10912.01	12427.06	0.106	-3403.587 - 373.482
18S rRNA (nf.expr)	-3.64	-3.59	0.725	-0.379 - 0.272
28S rRNA (nf.expr)	-4.74	-4.72	0.877	-0.366 - 0.316
5.8S rRNA (nf.expr)	-7.31	-7.20	0.384	-0.397 - 0.164
5S rRNA (nf.expr)	-9.05	-8.90	0.265	-0.412 - 0.125
47s pre-rRNA (nf.expr)	-13.22	-13.39	0.329	-0.195 - 0.537

Table 4: Daily mean dietary intake of macro nutrients during each training day per supplement leg. Glu = glucose, Plac = placebo. Training day 1-training day 12 are combined pairwise to day 1-6: Day 1 = training day 1/2, Day 2 = training day 3/4, Day 3 = training day 5/6, Day 4 = training day 7/8, Day 5 = training day 9/10, Day 6 = training day 11/12. Values are mean ± standard deviation. *P* = difference between Glu and Plac. Glucose n = 12, placebo n = 12. Day 6: Glu = 11, Plac = 11.

Time	Suppl.	Kcal	CHO (g)	Fat (g)	PRO (g)	PRO (g/kg)
Day 1	Glu	2493.4 ± 539.2	315 ± 86.9	78.1 ± 23.9	171 ± 40.8	2.4 ± 0.5
Day 1	Plac	2349.2 ± 576.4, $p = 0.369$	315.6 ± 78.1, $p = 0.979$	73.5 ± 20.9, $p = 0.654$	168.6 ± 44.6, $p = 0.737$	2.4 ± 0.5, $p = 0.700$
Day 2	Glu	2582.2 ± 569.8	369.8 ± 74.7	80.9 ± 41	173.2 ± 33.9	2.5 ± 0.4
Day 2	Plac	2587.4 ± 653.3, $p = 0.974$	367.8 ± 82.4, $p = 0.930$	66.6 ± 24.6, $p = 0.169$	170.6 ± 43.4, $p = 0.715$	2.4 ± 0.5, $p = 0.654$
Day 3	Glu	2574.2 ± 532.6	352.2 ± 50	78.7 ± 31.6	171.4 ± 41.1	2.4 ± 0.5
Day 3	Plac	2522 ± 560, $p = 0.744$	354.8 ± 53.2, $p = 0.909$	80.2 ± 28.7, $p = 0.883$	172.4 ± 37.2, $p = 0.888$	2.5 ± 0.5, $p = 0.870$
Day 4	Glu	2480.6 ± 573.4	323.8 ± 91	77.3 ± 34	167 ± 32.4	2.4 ± 0.4
Day 4	Plac	2444.8 ± 591.5, $p = 0.823$	316.2 ± 93.7, $p = 0.738$	79.3 ± 32.7, $p = 0.845$	163.6 ± 36.6, $p = 0.634$	2.3 ± 0.5, $p = 0.599$
Day 5	Glu	2420.6 ± 724.6	327.2 ± 92.1	82.8 ± 44.3	157.6 ± 34.2	2.2 ± 0.4
Day 5	Plac	2488.2 ± 680, $p = 0.672$	344.4 ± 95.5, $p = 0.451$	87.9 ± 42.3, $p = 0.618$	164.2 ± 33 $p = 0.357$	2.3 ± 0.4, $p = 0.329$
Day 6	Glu	2168 ± 535.5	304 ± 70.4	89.8 ± 32.4	156 ± 24.6	2.2 ± 0.2
Day 6	Plac	2257.5 ± 510.9, $p = 0.617$	302.5 ± 69.1, $p = 0.953$	82.3 ± 34.2 $p = 0.658$	153.8 ± 24.6, $p = 0.778$	2.2 ± 0.2, $p = 0.771$

4.1 Total RNA and ribosomal RNA

RT with glucose led to no difference in mean log-fold change from baseline to post compared with placebo in total RNA (Fig 4A, $p = 0.499$), or rRNA (Fig 4B, 18S: $p = 0.584$, 28S: $p = 0.740$, 5.8S: $p = 0.935$, 5S: $p = 0.790$, 47S: $p = 0.502$). Between baseline and post-sampling there was an accumulation of total RNA, whereas glucose increased by 26% and placebo increased by 22%. This accumulation was also observed in rRNA expression in both glucose and placebo, where expression increased between 34-43% in glucose and 33-41% in the four rRNAs and 37 and 59% in 47S pre-rRNA, respectively.

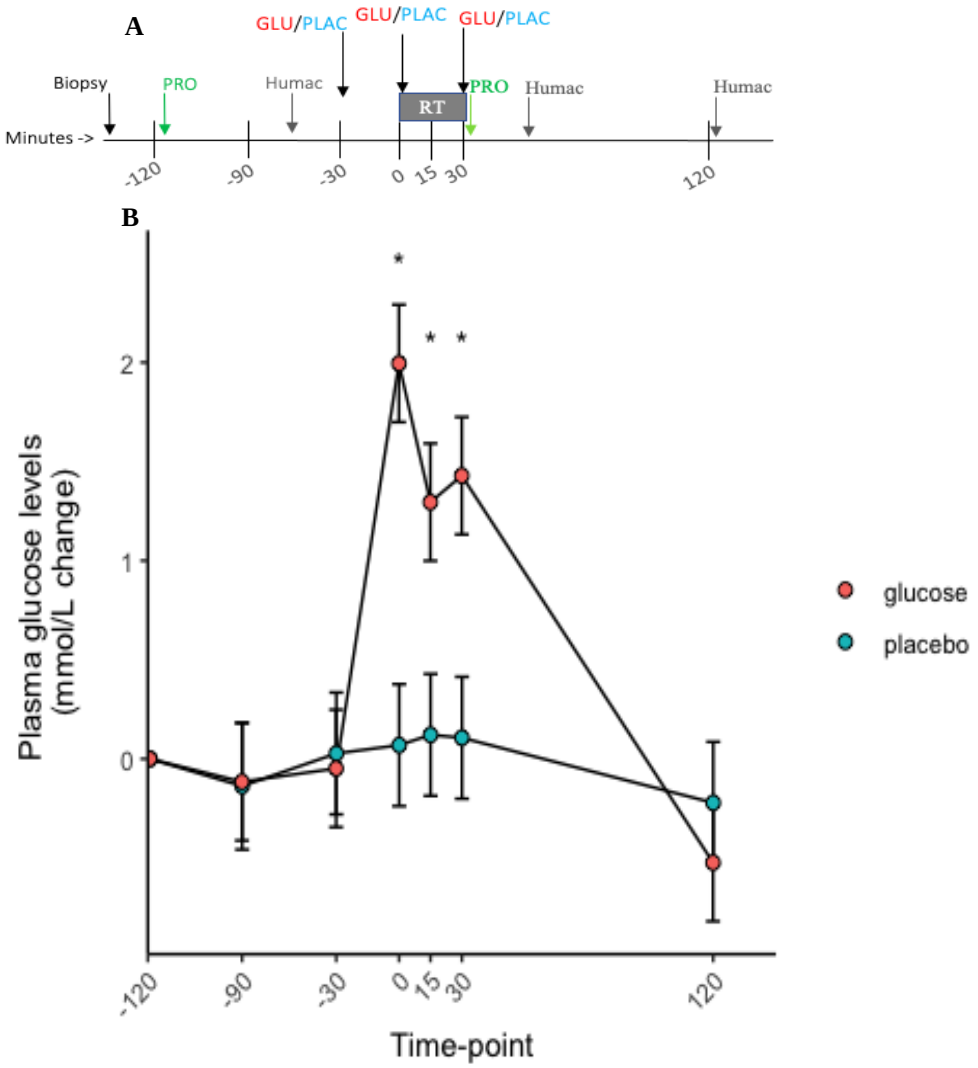

Figure 3: A) Timeline for training day 6 (combination of pairwise consecutive training day 11 and 12). Biopsy = muscle biopsy taken pre supplementation, PRO = protein supplementation, Humac = strength test (isometric knee extension, isokinetic knee extension 60 and 240 d/s), GLU/PLAC = glucose/placebo supplementation, RT = resistance training. Minutes: -120 = 2hrs prior to RT, -90 = 90min prior to RT, -30 = 30min prior to RT, 0 = onset of RT, 15 = 15min following onset of RT, between leg press and knee extension, 30 = 30min post onset of RT, 120 = 2hrs following onset of RT. B) Mean mmol/L change in plasma glucose levels per supplement group during training day 6. Time-points: 0, 45, 90, 120, 135, 150 and 270min post protein ingestion, respectively. Finger stings were taken at all time-points, blood samples were taken at all time-points except from 135. Values are presented as estimated marginal means of mmol/L change ± 95% CI. $p < 0.05$ between groups. Glucose n = 13, placebo n = 13.

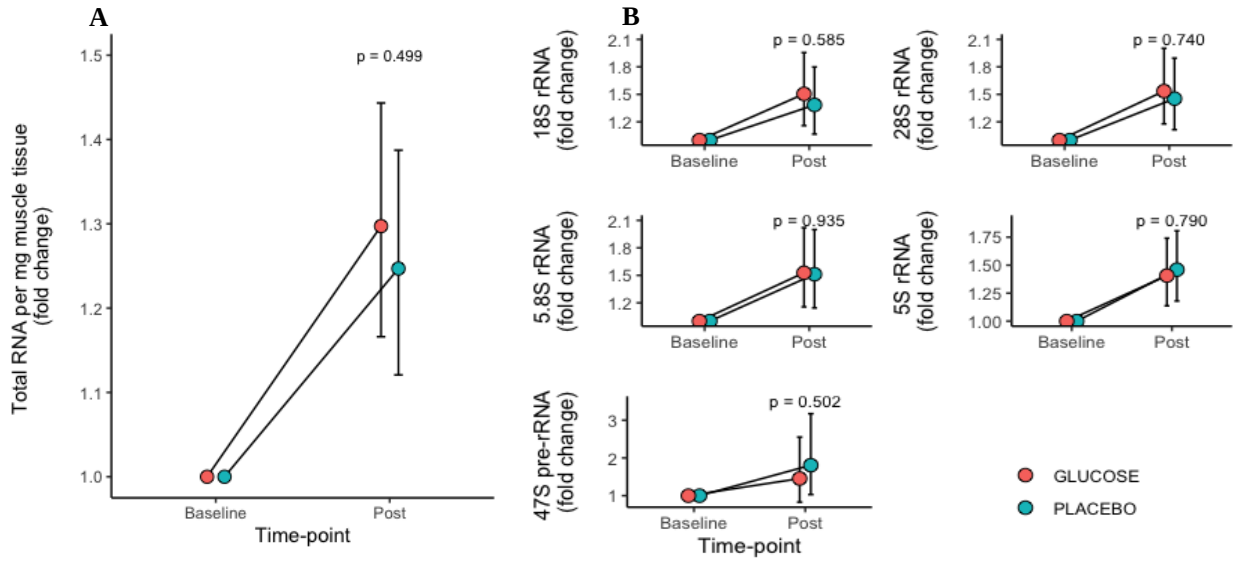

Figure 4: A) Changes in Total RNA, B) Changes in ribosomal RNA. Baseline = Training day 1/2, Post = Training day 11/12. Both total RNA and rRNA were analyzed with duplicates, with two duplicates per biopsy (two biopsies per time point). Total RNA and rRNA changes were calculated as log-fold change score per mg wet muscle weight, rRNA was normalized by reference gene (Lambda). Mean change scores of the duplicates were calculated before modelling and transformed to the log-scale. Values are estimated marginal means fold change per leg ± 95% CI. p = between groups statistic, glucose compared to placebo. Glucose n = 13, placebo n = 13.

4.2 Resistance training and strength testing

There were no significant differences in mean changes of total session volume, training intensity or RPE between glucose and placebo at any of the time-points (Fig 5, $p = 0.740$, $p = 0.917$, $p = 0.645$, post change compared to baseline, respectively). We did observe interactions between time and changes, whereas total session training volume increased from 13-18% compared to baseline. These increases in training volume were accompanied by changes ranging from 5-18% in training intensity and 2-13% in RPE (Tab 5).

The maximal isometric knee extension force and isokinetic knee extension peak torque at 240 d/s showed no differences between glucose and placebo after five RT sessions (Fig 6A, Isometric: $p = 0.336$, 240 d/s: $p = 0.527$, post change compared to baseline, respectively). There was a significant difference observed post fifth RT session, where glucose decreased 9% less than placebo (Fig 6A, $p = 0.037$). After six RT sessions, there were no differences in the same tests

between glucose and placebo (Fig 6B, Isometric: $p = 0.442$, 60 d/s: $p = 0.377$, 240 d/s: $p = 0.154$, compared to baseline, respectively).

Table 5: RPE changes through training days. Training days are presented as combined pairwise consecutive, linked to their respective sessions. Session 1 = Training day 1/2, Session 2 = Training day 3/4, Session 3 = Training day 5/6, Session 4 = Training day 7/8, Session 5 = Training day 9/10, Session 6 = Training day 11/12. Values are presented as mean ± standard deviation. *p* = no difference in change between glucose and placebo. Glucose n = 13, placebo n = 13.

Supplement	Baseline	Session 2	Session 3	Session 4	Session 5	Session 6
Plac	9.1 ± 1	9.2 ± 1.1	9.2 ± 0.6	9.4 ± 1	9.6 ± 0.7	10 ± 0
Glu	8.2 ± 1.7	8.8 ± 1.5	9.1 ± 1.7.	9.1 ± 1.6	9.3 ± 1.7	9.5 ± 1
		$p = 0.627$	$p = 0.863$	$p = 0.754$	$p = 0.636$	$p = 0.644$

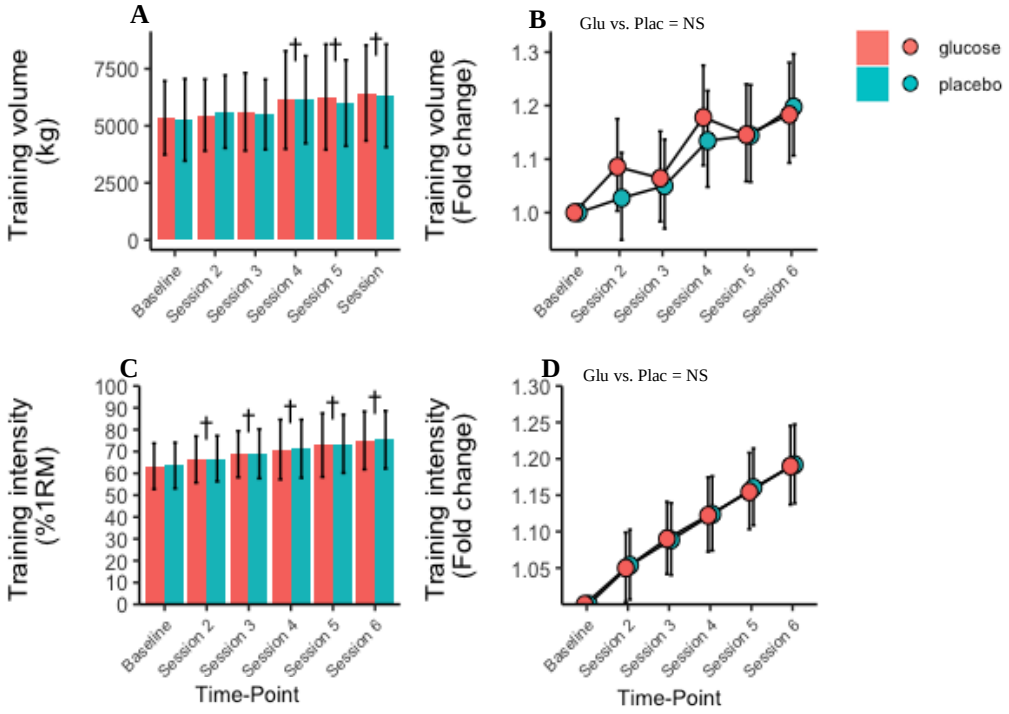

Figure 5: Training volume and training intensity from baseline until resistance exercise session 6 (training day 11 and 12). Baseline = Training day 1/2, Session 2 = Training day 3/4, Session 3 = Training day 5/6, Session 4 = Training day 7/8, Session 5 = Training day 9/10, Session 6 = Training day 11/12. A) Changes in total session volume per leg, values are mean kg ± standard deviation. B) Fold change in total session volume per leg, values are estimated marginal means fold change ± 95% CI. C) Changes in training intensity per leg calculated as %1RM, values are mean % ± standard deviation. D) Fold change in training intensity per leg, values are estimated marginal means fold change ± 95% CI. † = $p < 0.05$ in both glucose and placebo, compared to baseline. Glu vs. Plac = NS: no significant difference between legs at any of the time points. Glucose n = 13, placebo n = 13.

The mean development in peak torque at all three velocities changed little from baseline until Session 4 in both glucose and placebo (+4-7% change), decreasing at the test prior to Session 6 (9-18% change) (fig 6A). The test after RT session 6 (Fig 6B) showed a slight reduction in isometric peak torque 30 minutes following RT (2-4% change), slowly regaining at 2 hours (+3% change) and 23 hours (+3-6% change), in both glucose and placebo compared to baseline. Isokinetic peak torque at 60 d/s and 240 d/s showed no reduction in mean peak torque from baseline until 23hrs following RT, in both glucose and placebo (+0,1-11%). There was observed an effect of time in peak torque at 23 hours post RT in isokinetic 60 d/s (Fig 6B), whereas the placebo group increased 5% more from baseline compared to glucose, however, there were no significant difference between them.

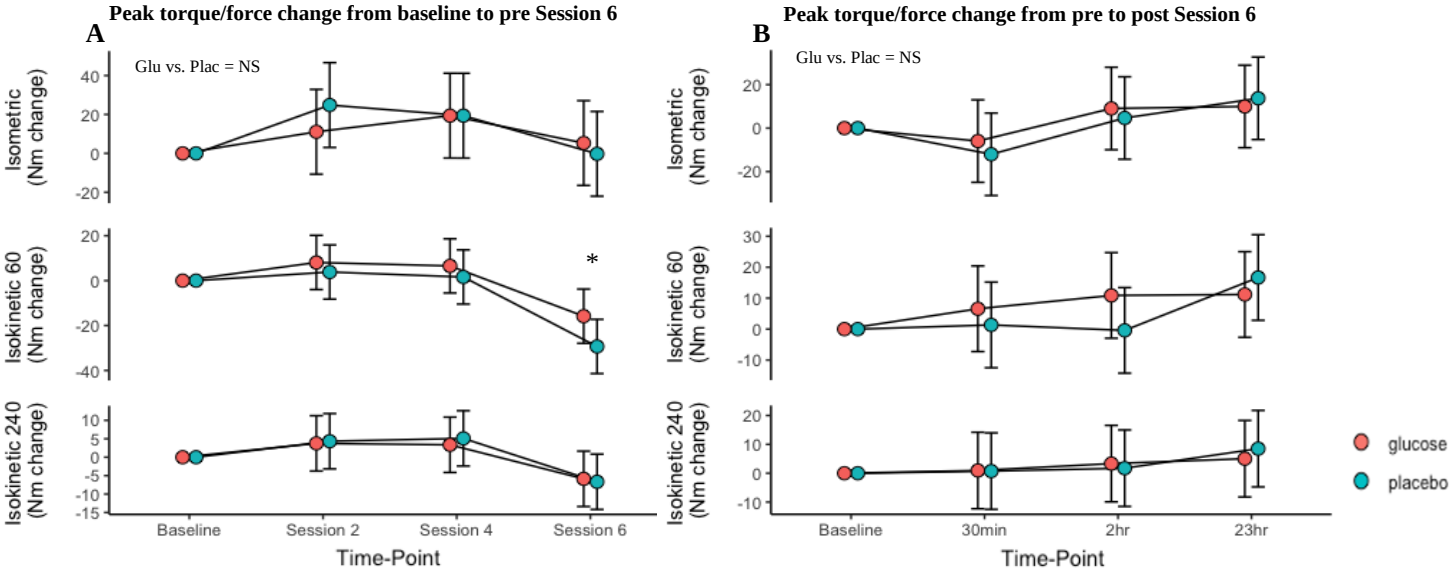

Figure 6: Peak torque changes in maximal isometric knee extension and maximal isokinetic knee extension at 60 and 240 d/s, respectively. A) Humac tests during the intervention, Baseline = Day -1 (prior to intervention), Session 2 = prior to training, training Day 4/5, Session 4 = prior training, training Day 8/9, Session 6 = prior to training 11/12. B) Baseline = prior to training, training day 11/12, 30min = 30 min after RT, 2hr = 2hrs after RT, 23hr = 23hrs after RT. Values are change in estimated marginal means of newton meters (Nm) ± 95% CI. * = $p < 0.05$ between groups. Glu vs. Plac = NS: no significant difference between legs at any of the time points, except from Session 6, 60 d/s Fig A. Glucose n = 13, placebo n = 13.

5. Discussion

The main findings of the present study were that glucose ingestion during five RT sessions did not affect muscle biology markers measured as total RNA and rRNA, or markers of muscle functionality such as muscle recovery, total session volume, and training intensity in response to RT compared to placebo in moderately trained young adults. There are several studies suggesting high glucose treatment to be important to markers of ribosome biogenesis (Mariappan et al., 2011; Tanaka and Tsuneoka, 2018; Zhai et al., 2012), however the perspective of exogenous glucose ingestion during RT on subsequent adaptations in humans has remained unexplored. Due to this unexplored nature in the field, our hypotheses and purposes were quite exploratory and in light of these previous suggestions, it is interesting that we did not observe any difference between glucose and placebo ingestion in total RNA and rRNA, as markers of ribosome biogenesis.

To our knowledge, no previous study has investigated glucose ingestion during RT on ribosome biogenesis in human skeletal muscle cells. The current model on ribosome biogenesis states that changes in accumulation of total RNA and rRNA early after onset of an RT-period predicts long-term responses in RT-adaptation (Figueiredo, 2019). Therefore, the lack of effects of glucose on total RNA and rRNA after two weeks of moderate volume RT do indicate that glucose *per se* does not contribute to positive long-term adaptations in muscle growth. Firstly, the time frame of the study has previously shown to serve as a predictor of long-term responses, as recent studies have shown augmentation of ribosome biogenesis by total RNA and rRNA following multiple session of RT (Figueiredo et al., 2016; Hammarström et al., 2020). Figueiredo et al. (2016) observed increases in 47S pre-rRNA measurable after a single RT session, passing baseline after 4hrs, peaking at 24hrs and remaining elevated until 48hrs post exercise. Other mature rRNA where first measurable after multiple sessions (Figueiredo et al., 2016). In addition, Hammarstrøm et al (2020) demonstrated that the most prominent augmentation in ribosome biogenesis markers occurred 2 weeks following moderate-volume unilateral RT. Secondly, translational capacity was recently deemed an important factor in long-term RT-induced adaptations, as increased ribosome content provides a higher possible basal MPS, giving regulation of ribosome biogenesis a central role in muscle growth (Figueiredo, 2019). This is further substantiated by association between blunted ribosome biogenesis and poor responses to

RT (Hammarström et al., 2019; Stec et al. 2016), in addition to the lack of relationship between translational efficiency and chronic gains in muscle mass (Figueiredo, 2019). Hence, two weeks of moderate-volume unilateral RT should have been sufficient to observe differences between glucose and placebo. This is substantiated by our observations on augmented accumulation of total RNA and expression of rRNA, supporting our study design as efficient and eliciting the expected gains in markers of ribosome biogenesis. As there were no differences in total RNA and rRNA between glucose and placebo in the present study, it is therefore reasonable to assume that there were no differences in ribosome biogenesis by proxy. Therefore, since glucose appeared not to augment ribosome content after two weeks of RT compared to placebo, as in the present study, it is unlikely that glucose *per se* is beneficial to long-term adaptations in RT. Although ribosome biogenesis still may represent a source to the individual variations observed between participants previously (Hammarström et al., 2019; Stec et al., 2016), the cause of these variations remains elusive, as glucose ingestion during RT does not seem to ameliorate this.

Indeed, our findings suggest that glucose ingestion *per se* during RT does not induce changes in the internal physiological milieu of human skeletal muscle cells with anabolic effects. The current model of increased ribosomal content suggests that increased PIC assembly, hence Pol I recruitment, augments rDNA transcription and subsequently rRNA maturation, leading to increased ribosome content (Figueiredo, 2019; Tanaka and Tsuneoka, 2018). Herein, glucose has been suggested as a signal mediator affecting upstream pathways of ribosome biogenesis by conveying an anabolic signal through insulin, or contribution to the cellular energy levels (Kusnadi, 2015; Tanaka and Tsuneoka, 2018). As the blood analyses were not completed in due time for this thesis, plasma insulin and its relative contribution to our results remains uncertain. Together with PIH1, the mTORC1 and ERK1/2 pathways are proposed to up-regulate PIC assembly in high energy conditions, being energy-sensitive (Mariappan et al., 2011; Kim et al., 2013; Tanaka & Tsuneoka, 2018; Zhai et al., 2012), while AMPK, possibly SIRT1, is suggested to repress PIC assembly in low energy conditions (Hoppe et al., 2009; Murayama et al., 2008). These suggestions are based on in vitro studies of rodent, yeast, and human non-muscle cell cultures, observing high glucose treatment to augment rDNA transcription by increased UBF and SL-1 activity, while low glucose treatment, glucose starvation, or nutrient depletion stimulated AMPK-dependent TIF-IA and chromatin regulation suppression (Kos-Braun, Jung, and Koš,

2017; Mariappan et al., 2011; Zhai et al., 2012). In addition to UBF and SL-1, assembly of the PIC requires TIF-IA and thus Pol I recruitment, proposed to be induced by amino acid intake in an mTOR-S6K-dependent manner and possibly by direct c-Myc activation (Kusnadi et al., 2015). As such, ingesting both glucose and protein should theoretically potently stimulate PIC assembly, through increasing energy supply and nutrient availability and as a result, rDNA transcription. However, this did not seem apparent in the present study, as glucose *per se* did not induce augmented ribosome biogenesis, despite significant elevation of plasma glucose levels when exercising with glucose, compared to no changes in plasma glucose levels with placebo. Notably, the upstream pathways and their downstream targets where not directly measured, hence this is an interpretation from observations in the available biological data and plasma glucose measurements. Based on our results, the effect of glucose on ribosome biogenesis observed in non-muscle cell culture studies on high glucose vs. low glucose treatment/glucose starvation (Mariappan et al., 2011; Tanaka & Tsuneoka, 2018; Zhai et al., 2012), does not seem to extend to human skeletal muscle cells supplied with either glucose or placebo.

A possible explanation for this, is that both glucose and placebo may have had sufficient levels of nutrients and energy during and following RT, thus mTORC1 had sufficient nutrient availability and was not inhibited by AMPK due to energy deficiency (Hoppe et al., 2009, Kusnadi et al., 2015). Indeed, mTORC1 is proposed to be energy sensitive in itself (Dennis et al., 2001), thus it might have been directly affected by the higher glucose supply in glucose compared to placebo, during RT (Mariappan et al., 2011). However, recent studies suggest the energy sensing capabilities of mTORC1 to occur through AMPK, as mTORC1´s energy sensitivity may be too low for ATP (Kim, Buel and Blenis., 2013; Tao, Barker, Shi, Gehring and Sun, 2010). This would arguably make AMPK the primary regulator of glucose´s potential impact on ribosome biogenesis. Hence, ribosome biogenesis may have been equally stimulated in glucose and placebo, which explains the accumulation of total RNA and rRNA observed in both. Importantly, as intramuscular glycogen content was not measured in the present study, whether cellular energy levels were equal or not remains unknown. However, macro-nutrient intake was similar between glucose and placebo on pairwise consecutive days, plasma glucose levels did not indicate energy deficiency during RT and there were no differences observed in ribosome biogenesis. As such, potent mTORC1 stimulation without AMPK-dependent inhibition seems a plausible explanation.

Together with previous studies on this topic (Mariappan et al., 2011; Zhai et al., 2012), it therefore seems that glucose do not in itself carry an anabolic signal, though it may still be important in the aspect of supplying cells with energy, inhibiting AMPK (Hoppe et al., 2009). Thus, physiological levels of plasma glucose may be sufficient to supply the energy needed for ribosome biogenesis following RT. These lacking differences between glucose and placebo ingestion in the biological data are further supported by the functional data.

Overall, glucose did not affect isometric force or isokinetic peak torque at 240 d/s, or muscular recovery following the sixth and last RT sessions, measured as temporal changes in isometric force and isokinetic peak torque. Generally, the mean isometric force and isokinetic peak torque seemed to slightly decrease during the intervention, especially between session 4 and 6, and then regain after the intervention. Furthermore, there were no differences observed between glucose and placebo in total session volume and training intensity. Both glucose and placebo lifted higher total session volumes at higher percentages of their 1RM at the last session compared to baseline, as expected from earlier reports on the moderate-volume RT protocol (Krieger, 2009; Rhea et al., 2003; Schoenfeld, Ogborn, and Krieger, 2017), in addition to a more recent study implementing the unilateral protocol (Hammarström et al., 2020). Interestingly, we did observe a significant difference between glucose and placebo in mean isokinetic peak torque at 60 d/s prior to the sixth RT session, where glucose decreased peak torque 9% less than placebo. However, there were no other humac tests showing tendencies of differences, and therefore it seems unlikely that there was a true effect of glucose on isokinetic peak torque at 60 d/s. These results may add to previous observation of no differences between co-ingestion of carbohydrate and protein compared to protein alone, on MPS and recovery acutely following RT (Koopman et al., 2007; Staples et al., 2011). As a whole, the functional data indeed point towards an exhausting, yet efficient RT protocol, especially in light of the biological data. As a side note, an interesting observation made by the other master´s thesis on this project, where that the concentration of MuRF protein was significantly lower at post in glucose compared to placebo. This may indicate a possible effect of glucose on protein degradation (Wilkes et al., 2009), however whether this contributes to growth or not has been a topic of debate (Figueiredo, 2019).

A possible limitation to the study was the dropout of three participants. Power calculations showed that 16 participants would be sufficient to measure differences between supplement ingestion, thus our statistical power may have been impacted by dropouts. This brings up the question if the effect of glucose was there, and we were not able to detect it, introducing some uncertainty to our analyses. However, the data set as a whole revealed no tendencies indicating a true effect of glucose, reducing the likeliness of a type 2 error due to lacking statistical power. Moreover, as the study was specifically designed to investigate differences in changes within-participants due to glucose ingestion, it was limited to this perspective. Hence, the effects of time observed only serve as supplementary to the efficiency of the design, without a negative control group to either confirm or refute them. Lastly, the blood analyses were not finished in due time for this thesis, which would have provided more background information to our biological data. Taken together, baseline measure, changes in functional data and plasma glucose data indeed point toward an efficient study design while supporting the biological data.

6. Conclusion

In conclusion, exogenous glucose ingestion during RT does not augment ribosome biogenesis following 2 weeks of moderate-volume resistance training in moderately trained young male and female adults. Neither does it affect maximal isometric knee extension force or isokinetic knee extension peak torque, nor enhance post resistance training recovery within 24hrs.

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Appendix

Høgskolen i Innlandet

[28.08.2020]

VIL DU DELTA I FORSKNINGSPROSJEKTET

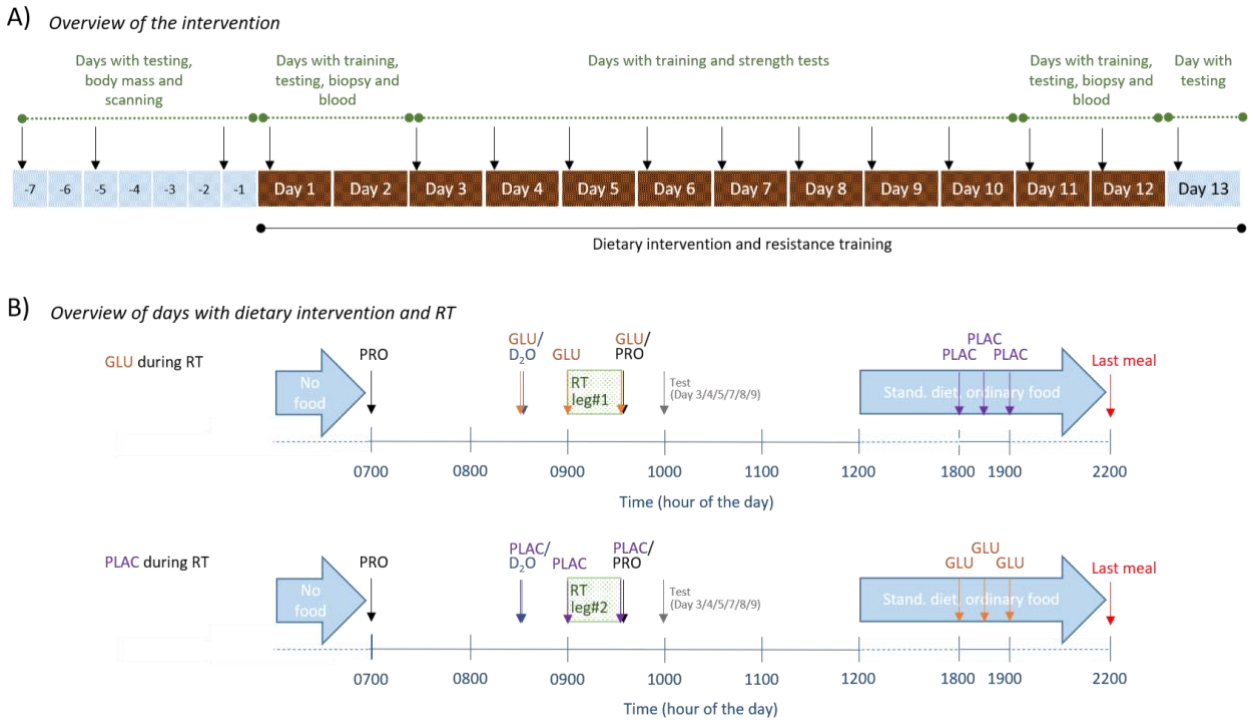
RIBOSE – EFFEKTER AV GLUKOSEINNTAK UNDER STYRKETRENING PÅ RIBOSOMAL BIOGENESE I SKJELETTMUSKEL?

formålet med prosjektet og hvorfor du blir spurt

Dette er et spørsmål til deg om å delta i et forskningsprosjekt hvor formålet er å skaffe ny kunnskap om hvorvidt karbohydratinntak (før, under og etter styrketrening) er positivt for treningsutbyttet. I dette skrivet finner du informasjon om målene med prosjektet og hva deltakelse vil innebære for deg.
Bakgrunn. Styrketrening har en rekke positive effekter på menneskekroppens funksjoner og er et av de viktigste virkemidlene for å bedre folkehelsen. Ikke nok med at det forebygger livsstilssykdommer som for eksempel hjerte-karsykdom, det gir også styrke og utholdenhet til å beherske dagliglivets utfordringer. Mange av de positive effektene er direkte knyttet til muskelvekst, som i sin tur kontrolleres av hendelser i muskelcellene. Dessverre opplever mange av oss begrenset muskelvekst etter en periode med styrketrening (og dermed ei heller den foreskrevne helseeffekten). Det er derfor av stor interesse å finne nye måter å trene på som kan tilrettelegge for muskelvekst for alle. Dette må nødvendigvis involvere tiltak som kan endre på hendelsesforløpet i muskelcellene. En mulig strategi kan være nye kombinasjoner av trening og næringsinntak. Vi vet allerede at proteininntak i forbindelse med trening er et effektivt (og ofte nødvendig) tiltak for å oppnå muskelvekst. I denne studien ønsker vi å undersøke om dette også gjelder inntak av karbohydrater.

Formål. I denne studien skal vi undersøke effekten av fem styrketreningsøkter med og uten inntak av karbohydrat (heretter kalt hhv GLUKOSE og PLACEBO) på hendelsesforløpet i muskelcellene. Studien skal gjennomføres som et randomisert dobbeltblindet kryssforsøk (se Figur 1 for oversikt over studien), der samtlige deltaker skal gjennomføre styrketrening med inntak av GLUKOSE og PLACEBO: trening med inntak av GLUKOSE skal gjennomføres annenhver dag gjennom treningsperioden (på den ene foten), mens trening med inntak av PLACEBO skal gjennomføres på alternerende dager (på den andre foten). På alle treningsdager skal du også innta proteinsupplement før og etter trening (for å sikre optimale betingelser i kroppen, i henhold til gjeldende anbefalinger). Alle treningsøkter vil bli veiledet av kyndig treningspersonell. Hverken du eller treningsveilederen vil vite hvilke dager du inntar GLUKOSE og hvilke dager du inntar PLACEBO.

I forkant av og underveis i treningsperioden skal vi ta en rekke prøver og gjennomføre en rekke tester, inklusive muskel-, spytt- og blodprøver, kroppsmassemåling og styrketester. Disse målingene gjør det mulig å undersøke effekter av trening med og uten karbohydratinntak på muskelcellenes karakteristikker (som for eksempel cellers form og utseende, genuttrykk, og proteinforekomst og -funksjon), blodets sammensetning (som for eksempel hormoner, glukose og betennelsesprosesser), muskelstyrke og muskelmasse/-mengde. Underveis i treningsperioden skal du også drikke tungtvann (litt hver dag). Dette skal gjøre det mulig å måle nyproduksjon av muskelcellenes byggeklosser (proteiner) gjennom treningsperioden.



Figur 1. A) Oversikt over treningsperioden. Dager med oppmøte på høgskolen er markert med svart pil (↓). B) oversikt over treningsperioder med og uten karbohydratinntak (GLUKOSE/GLU vs. PLACEBO/PLAC).

For den enkelte deltaker vil prosjektet vare i 20 dager, fordelt på en innledende 7 dagers testperiode og en 13 dagers trening- og testperiode. Det vil dermed være relativt tidkrevende. I prosjektperioden skal du ha til sammen 16 oppmøter på høgskolen (se Figur 1A). Samtlige aktiviteter som krever fysisk oppmøte vil bli gjennomført under tilsyn og veiledning av bachelor- og/eller masterstudenter. Disse skal skrive bachelor- eller masteroppgaver basert på resultatene. Du må også tilpasse kostholdet til prosjektprotokollen. Næringstilskuddene som inntas i prosjektperioden vil bli delt ut av testpersonell. *Detaljert informasjon om studiens innhold og tidsforløp vil bli gitt i informasjonsmøte eller i dialog med den enkelte deltaker. Der vil det også være mulig å stille spørsmål*. Du kan når som helst ta kontakt med prosjektansvarlig professor Stian Ellefsen hvis du har spørsmål knyttet til prosjektet.

Hva innebærer PROSJEKTET for deg?

For forenklet oversikt over aktivitetene i studien, se Figur 1.

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

Gjennomføring. Studien vil innebære 16 fysiske oppmøtedager på testlaboratoriet ved Høgskolen i Innlandet, campus Lillehammer (Figur 1). Forut for oppstart av treningsperioden skal du møte 3 ganger for gjennomføring av fysiske tester (maksimal muskelstyrke), kroppsmassemåling (Dual-energy X-ray absorptiometry, DXA) og inntak av tungtvann (moderat dose). Gjennom den 13 dager lange trenings- og testperioden skal du møte daglig for gjennomføring av styrketrening (med inntak av GLUKOSE eller PLAC), inntak av tungtvann (små doser), og for gjennomføring av eventuelle fysiske tester (dag 3-5, dag 7-9 og 11-13) og muskel- og blodprøvetaking (dag 1-2 og dag 11-12). Det vil kun være aktuelt med ett oppmøte per dag, så sant dette lar seg gjøre for deg. Tidsomfanget vil variere fra dag til dag, oppad til ~5 timer på Dag 11 og 12 og nedad til 30 minutter på dag 13 og 1 time på Dag 3, 6, 7 og 10. Treningsprogrammet skal gjennomføres som 3 sett med styrketrening i øvelsene kneekstensjon og

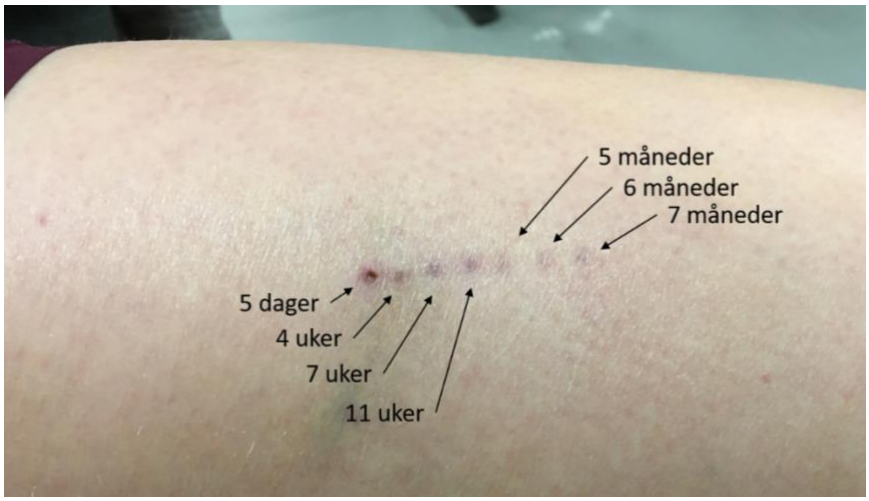
beinpress, med 10 repetisjoner maksimum i hvert sett, og vil ta ca 30 minutter. Under den 13 dager lange trenings- og testperioden skal du også følge et bestemt kostholdsprogram. Dette innebærer at du de siste ni timene før treningsøktene ikke skal innta annen mat og drikke enn proteinshake / GLUKOSE / PLACEBO / tungtvann (f.o.m. Dag -1 t.o.m. Dag 12). F.o.m. tre timer etter påbegynt styrkeøkt skal du holde et selvvalgt kosthold. I dette tidsrommet skal du registrere mat- og drikkeinntak på appen MyFitnessPal. På parvise dager skal du ha identisk kosthold (eksempelvis Dag 1+2, Dag 3+4, etc). Dette for å hindre at forskjeller i kosthold påvirker effektene av GLUKOSE og PLAC. Du vil få bistand til å utarbeide en balansert kostholdsplan dersom du trenger dette.

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

- Måling av kroppsmassesammensetning ved DXA. Denne testen gjennomføres forut for treningsperioden (Dag -1; fastende)
- Daglig inntak av proteinshake (2 doser per dag): 2 timer forut for treningsøkt og umiddelbart etter avsluttet treningsøkt
- Daglig inntak av Fun Light med tilsatt glukose eller placebo (GLUKOSE eller PLAC; 6 doser per dag): 3 doser i forbindelse med trening og 3 doser på kveldstid
- Daglig inntak av tungtvann (Dag -1, 5.25 ml per kg fettfri kroppsmasse; Dag 1-12, 0.53 ml per kg fettfri kroppsmasse).
- Daglig gjennomføring av ettbeins tung styrketrening i kneekstensjon og beinpress (3 x 10RM i hver øvelse). De to beina trenes på alternerende dager
- Maksimale styrketester i beinpress og kneekstensjon før og etter treningsperioden (Dag -7, -4, 11 og 12)
- Statisk styrke i kneekstensjon (MVC) og isokinetiske tester før, underveis og etter treningsperioden (Dag -7, -4, -1, 3, 4, 5, 7, 8, 9, 11, 12 og 13)
- Spyttprøver gjennom hele treningsperioden (Dag -1 t.o.m. 12; for å måle innhold av tungtvann i kroppen)
- Blodprøver på Dag 1, 2, 11 og 12 (fastende alle dager + forut for og i etterkant av treningsøkt på dag 11 og 12)
- Muskelvevsprøver på dag 1, 2, 11 og 12 (fastende alle dager; fire prøver fra hvert bein).

Mulige fordeler og ulemper

Du vil lære mye om egen kropp og vil få ny kunnskap om styrketrening og effekter av styrketrening. Positive effekter av styrketrening inkluderer økt muskelstyrke og -mengde, bedre helse og sannsynligvis også økt velvære. Du vil få tilgang til tester du normalt ikke har tilgang til. Styrketrening vil kunne føre til skader. Risikoen er imidlertid liten i dette prosjektet, især fordi du vil få tett oppfølging av treningskyndig personell under alle treningsøkter/tester. Det er ingen kjente helsekonsekvenser knyttet til inntak av tungtvann i de doser som anvendes i studien. Lett svimmelhet kan forkomme. For å unngå svimmelhet, vil inntak av den første (og største) dosen fordeles over to timer. Du vil få oppfølging av testpersonalet i perioden hvor svimmelhet kan inntreffe. Noen synes muskelbiopsier og blodprøver er ubehagelig. Muskelbiopsiene tas med den skånsomme mikrobiopsimetoden. Noen synes likevel biopsiene er ubehagelig. Man vil typisk bli litt støl i muskelen 1-2 dager i etterkant, først og fremst på grunn av små blødninger i muskulaturen. Stølhetsfølelsen varer i ytterst få tilfeller i inntil 3-4 uker. I svært få tilfeller kan også biopsitaking føre til tydelig arrdannelse eller føre til at følelsen i huden forsvinner for en lengre periode. Vi har ikke observert tilfeller av slike negative sideeffekter ved vårt laboratorium med våre prosedyrer (2500 biopsitakinger fordelt over en tiårsperiode). I figur 2 ser du et typiske eksempler på biopsiarr, slik de ser ut mellom 5 dager og 7 måneder etter biopsitaking. Biopsitaking er forbundet med noe infeksjonsfare. Risikoen er svært liten ved bruk av prosedyrene som benyttes i dette prosjektet. Du vil få klare instrukser om hvordan du skal behandle såret i etterkant av prøvetagningen.



Figur 2. Typisk arrdannelse etter mikrobiopsitaking av muskelvev fra låret. De angitte tidspunktene indikerer tid siden biopsitaking.

Frivillig deltakelse og mulighet for å trekke ditt samtykke

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Det vil ikke ha noen negative konsekvenser for deg hvis du ikke vil delta eller senere velger å trekke deg. Dersom du trekker tilbake samtykket, vil det ikke forskes videre på dine helseopplysninger og ditt biologiske materiale. Du kan også kreve at dine opplysninger i prosjektet slettes eller utleveres innen 30 dager, og at det biologiske materialet destrueres. Adgangen til å kreve destruksjon, sletting eller utlevering gjelder ikke dersom materialet eller opplysningene er anonymisert. Denne adgangen kan også begrenses dersom opplysningene er inngått i utførte analyser, eller dersom materialet er bearbeidet og inngår i et annet biologisk produkt
Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte prosjektleder (se kontaktinformasjon på siste side).

Hva skjer med OPPLYSNINGENE om deg?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet under formålet med prosjektet, og planlegges brukt til 31.12.2023. I etterkant av prosjektet vil det biologiske materialet (og tilstøtende opplysninger) bli overført til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» (REK-ID: 2013/2041), dersom du samtykker til dette. Eventuelle utvidelser i bruk og oppbevaringstid kan kun skje etter godkjenning fra REK og andre relevante myndigheter. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert. Du har også rett til å få innsyn i sikkerhetstiltakene ved behandling av opplysningene. Du kan klage på behandlingen av dine opplysninger til Datatilsynet og institusjonen sitt personvernombud.
Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger (=kodede opplysninger). En kode knytter deg til dine opplysninger gjennom en navneliste. Det er kun prosjektleder Stina Ellefsen og Postdoktor Håvard Hamarsland som har tilgang til denne listen. Opplysningene om deg vil bli oppbevart i fem år etter avslutning av den generelle biobanken (31.12.2038) prosjektslutt av kontrollhensyn.

Deling av OPPLYSNINGER og overføring til UTLANDET

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

Hva skjer med prøver som blir tatt av deg?

Prøvene som tas av deg skal oppbevares i en forskningsbiobank tilknyttet prosjektet.
I etterkant av prosjektslutt vil de bli overført til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» (REK-ID: 2013/2041), situert ved Høgskolen i Innlandet/Sykehuset Innlandet. Dette er frivillig og du kan velge å avstå fra at dine prøver oppbevares videre i den generelle biobanken (eget samtykkeskjema). TrainOME-prosjektet er igangsatt for å avdekke sammenhenger mellom individers tilpasningsevne til trening, også kalt trenbarhet, og kroppslige/cellulære særtrekk. Gjennom den generelle biobanken skal prøvene analyseres sammen med prøver fra en rekke andre prosjekter, hvor den overordnete målsettingen er å studere faktorer som er bestemmende for generell trenbarhet. Dette innebærer generell analyse av cellebiologiske og genetiske trekk som for eksempel cellers form og utseende, arvematerialets sammensetning (inklusiv DNA-sekvens og epigenetisk modifisering), proteinsyntese, proteinforekomst og -funksjon, RNA-uttrykk og -regulering, hormonforekomst, kroppens indre miljø (metabolomet), og mange flere mål. Deler av materialet vil kunne bli sendt til utlandet for analyse. Merking vil i slike tilfeller være begrenset til identifikasjonsnummer; dvs. de vil bli sendt i kodet tilstand. Ubenyttet materiale vil bli returnert til Lillehammer i etterkant av analysene. Det biologiske materialet vil bli anonymisert innen 31.12.2038, hvorpå det vil bli destruert innen fem år. Forskningsdata som har blitt utledet av materialet vil deretter bli oppbevart i anonymisert tilstand på sikker server på ubestemt tid, sammen med øvrige data innhentet i prosjektet. Professor Stian Ellefsen er hovedansvarshavende for forskningsbiobanken.

Genetiske undersøkelser

Det vil bli innhentet informasjon om din genetiske sammensetning. Denne informasjonen skal primært gi innsikt i sammenhengen mellom individuelle responser på styrketrening, målt som muskelvekst, og individuell genetisk variasjon. Målsetningen er å forstå hvorfor noen responderer bedre på styrketrening enn andre. Dette perspektivet er forankret i målsettingen til den generelle biobanken "Trainome - humane cellers tilpasning til trening og miljø" (REK-id: 2013/2041), hvortil prøvene skal overføres etter prosjektlutt dersom du samtykker til dette. Forståelse for hvilken rolle ulike gener spiller for muskelvekst er på et tidlig stadium. Det er derfor ikke mulig å gi genetisk veiledning basert på analysene i studien. Det skal ikke gjøres analyser som kobler enkeltmutasjoner til bestemte helseutfordringer. Genetiske data er unike og er derfor i prinsippet ikke anonyme, selv om koblingsnøkkelen som kobler deg til dine data blir slettet. Alle genetiske data (inkludert transkriptomdata) skal oppbevares på sikker server hos Tjenester for sensitive data (TSD).

Forsikring

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

Godkjenninger

Regional komité for medisinsk og helsefaglig forskningsetikk har gjort en forskningsetisk vurdering og godkjent prosjektet (søknadsid: 153628).
Høgskolen i Innlandet og prosjektleder Stian Ellefsen er ansvarlig for personvernet i prosjektet.
Vi behandler opplysningene basert på ditt samtykke.

KONTAKTOPPLYSNINGER

Dersom du har spørsmål til prosjektet eller ønsker å trekke deg fra deltakelse, kan du kontakte:
Prosjektleder: professor Stian Ellefsen (tlf: 61288103, epost: *stian.ellefsen@inn.no*), *eller*
Prosjektmedarbeider: postdoktor Håvard Hamarsland (tlf: 93445916, epost: *havard.hamarsland@inn.no*)
Dersom du har spørsmål om personvernet i prosjektet, kan du kontakte personvernombudet ved institusjonen: *anne.lofthus@inn.no*
Datatilsynets e-postadresse er *personvernombudet@nsd.no*

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

Sted og dato	Deltakers signatur
	Deltakers navn med trykte bokstaver