Glucose ingestion before and after resistance training does not augment ribosome biogenesis in healthy moderately trained young adults

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## Introduction

Responses to systematic resistance training (RT) vary widely between individuals, with as many as 10-15% showing impaired muscle growth in response to standardized training interventions (ALVAREZ18;MANN14;THALACKER13). Such inability to benefit from RT may be explained by genetic and/or epigenetic disposition, affecting the internal physiological milieu in terms of ability to mount an anabolic response to RT (**figueiredo21?**, **thalacker13?**). Although RT program modifications such as altered exercise volume or intensity may induce more favorable adaptations in some individuals, such benefits are not uniform between individuals (1, **krieger09?**, **schoenfeld17?**). For example, although changes in training volume generally induces greater benefits of RT, not all individuals benefit from such modifications (1). Therefore, other means than modification of RT variables seems necessary to optimize individual responses.

Nutritional supplements such as protein and creatine effectively optimize RT adaptations (**cermak12?**, **lanhers15?**, **lanhers17?**, **morton18?**). However, it remains unknown if other nutritional adjuvants such as glucose can increase the efficacy of RT. This is surprising, as glucose is the preferred energy substrate of the contracting skeletal muscle during strenuous exercise and a major supplier of energy to cells via ATP synthesis (**mul15?**, **tanaka18?**). Furthermore, energy availability is a decisive factor in *de novo* synthesis of ribosomes (**kusnadi15?**, **moss07?**, **tanaka18?**) which in turn determines muscle growth by increasing the muscles translational capacity (**figueiredo19?**, **hammarstrom20?**, **tanaka18?**, **stec16?**, **vonwalden19?**). Therefore, investigating the effects of combined RT and glucose ingestion may provide valuable insight into a potential additive effect of glucose and RT on ribosome biogenesis.

Transcription of ribosomal RNA (rRNA) by Pol I is considered the limiting step in synthesizing new ribosomes (2). Multiple signaling pathways converge to regulate rRNA transcription, including the mammalian target of rapamycin (mTORC1) signal-transduction pathway and c-Myc (**kusnadi15?**, **vonwalden19?**, **tanaka18?**). mTOR receives input from growth factors, hormones, mechanical loading, and nutrients to balance protein synthesis through multiple mechanisms based on cellular energy levels (**hoppe09?**). Importantly, mTOR and its downstream target S6K1 serve as a mediator from insulin signaling in skeletal muscle(3). The mTORC1 pathway contributes to ribosome biogenesis through the regulation of translation of ribosomal proteins and to forming of the preinitiation complex (PIC) that marks the initiation of rRNA transcription (**figueiredo19?**, **vonwalden19?**). Parallel to mTORC1 (4, 5), c-Myc increases ribosomal biogenesis directly and through transcriptional control of the upstream binding factor (UBF) (6, **sanijUBFLevelsDetermine2008?**). Additionally, UBF phosphorylation, required for interaction with the rDNA promoter, is increased by high glucose in a mTORC1 dependent manner (rapamycin sensitive) in kidney glomerular epithelial cells(7). Independently from UBF and mTORC1, high glucose was shown to lead to chromatin remodeling, which promotes rRNA transcription in cell cultures(8). Together these observations indicate a potential role of glucose in positively affecting muscle rRNA synthesis.

The purpose of this investigation was to test the hypothesis that glucose supplementation before and after RT would increase markers of ribosomal abundance following five RT sessions. Secondly we aimed to describe the association between UBF and total RNA abundance in human skeletal muscle.

## Methods

### Materials and methods

All participants gave their informed consent prior to study enrollment. The study 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.

### Participants

Sixteen healthy male and female participants (20-33yrs, Table 1) were recruited to the study through social media advertisement and word of mouth. The eligibility criteria were non-smokers and moderately trained (i.e. 2-8 RT sessions per 14 days for the last six months). Exclusion criteria were previous injury leading to impaired strength, inability to perform resistance exercise training, 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. Of the sixteen participants that commenced the intervention, three participants dropped out. One due to sickness and inability to resume, two participants experienced muscular discomfort connected to heavy resistance training. Lean mass (Tab 1) was measured using DXA (Prodigy Advance PA+302047, Lunar, San Francisco, CA, USA) on Day -1, the last day preceding the RT intervention.

### Experimental design

The study was designed as a 12-day double-blinded randomized controlled trial, with a alternating unilateral RT protocol (Fig 1A). Participants were randomly allocated to exercise either their dominant or non-dominant leg with glucose or placebo before and after RT, and whether they started RT with glucose or placebo. Thus, participants exercised every day, alternating between e.g., one leg + glucose and the other leg + placebo (1A). One person was exclusively responsible for the randomization code and supplement distribution, blinding both investigators and participants in regard to which leg exercised with glucose and placebo. Glucose (Glucosum monohydricum, Merck KGaA, Darmstadt, Germany) and placebo (Steviosa, Soma Nordic AS, Oslo, Norway) was masked by mixing with 300ml Fun Light juice/saft (Orkla, Oslo, Norway). A blinded taste test revealed that the participants were not able to disclose the contents of the provided boluses (30g glucosum monohydricum vs. ~0.3g Stevia rebaudiana extract). To ensure equal conditions during training sessions, participants exercised and tested at the same time of day, +/- 1hr (check this) with the same supervisor, in addition to recording and repeating their daily macro nutrient intake (protein, fat, carbohydrate) and total calories on pairwise consecutive days.

Six RT sessions were conducted with glucose and six with placebo, allowing a within-subjects analysis of the effects of glucose ingestion before and after RT. Data from the first five RT sessions was used to investigate main outcome measures (total RNA, rRNA and protein) and leg muscle strength, whereas data from the sixth RT session was used to explore secondary outcomes (muscular recovery, plasma glucose levels). Participants were asked to avoid resistance- or high-intensity training of the legs from Day -7 (Fig 1A) and onwards, until completion of the intervention and post-testing, to ensure the reliability of pre-intervention strength data and minimal interference from other training sources. If participants did conduct exercise training outside of the prescribed protocol, they were asked to ensure equal loading on pairwise consecutive days.

### Dietary intervention

The dietary intervention spanned the whole day, divided into three periods: I) From awakening until 2.5 hours after RT, II) from 2.5 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 in period I: 30 minutes before RT (0830hrs, 30g vs 0g glucose), immediately before RT (0900hrs, 30g vs. 0g glucose), and immediately after RT (~0930hrs, 30g vs. 0g glucose). Protein was ingested 2hrs before RT (0700hrs, 25g) and immediately after RT (~0930hrs, 25g). In the afternoon (1800hrs-1900hrs, period II) participants ingested glucose or placebo (3x30g vs. 3x0g glucose) opposite to the supplement they received during RT, to ensure a balanced daily intake of glucose. Apart from this, participants ingested a self-chosen diet during 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 treatments. During period III (2200-0700hrs), participants remained in an overnight fasted state. The daily onset of the dietary intervention (i.e., first ingestion of PRO supplement) varied between participants, from 0600hrs to 0900hrs to allow multiple participants to complete the protocol simultaneously. The protein supplement was ingested as 25g Whey Protein Isolate boluses (Proteinfabrikken, Stokke, Norway), diluted in 150ml of water. During sessions, participants were free to ingest water ad libitum.

### Assessment of muscular strength

Strength tests were performed prior to (Days -7 and -5, and -1, both legs) and during the intervention (Days 4 and 8 leg 1, 5 and 9 leg two), after session 5 and after finalization of the intervention (Day 11/12 leg 1 and 12/13 leg 2) (Fig 1A). Maximal isometric and isokinetic knee extension torque were measured with a Humac Norm Dynamometer (CSMi, Stoughton, Massechusetts, USA). Individual positions were recorded and standardized from pre-intervention tests (days -7 and -5). Isokinetic peak torque was measured at speeds of 60- and 240 d/s, 2x3 repetitions each, with the first set of each exercise as a sub-maximal warm-up. Isometric peak torque was measured at knee-angle 60, for a maximum of 10 seconds and two repetition per test. The highest peak torque values were used in the final analyses. During days 4, 5, 8 and 9 (Fig 1D, day 4 and 5 = Post 2RT, day 8 and 9 = Post 4RT), humac tests were conducted one hour before RT with the leg performing RT the previous day. During days 11 and 12, humac tests were performed at four time points: I) 45min before RT (1D, Post 5RT), II) 30min after the last RT session (1D, 30 min post 6RT), III) 2hrs after the last RT session (1D, 2h post 6RT), and IV) 23hrs after the last RT session (1D, 23h post 6RT). Test I at day 12/13 included testing of both legs, representing 23hrs post RT session test of one leg and post session 5 test of the other leg.

Assessment of unilateral one repetition maximum (1RM) leg press and knee extension was conducted at pre-intervention testing during familiariztion (days -7 and -5, Fig 1D). The participants performed a general warm-up with 10min cycling on an indoor exercise bicycle. In addition, a protocol consisting of 1x10, 1x6 and 1x3 repetitions, was used as spesific warm-up before each of the tests. All positions were controlled and recorded at the first 1RM test and repeated for the RT sessions. Maximal leg press strength was defined as the maximal load lifted in a controlled fashion, with a knee angle of 45 degrees (start), within a 2.5kg range. Attempts, where participants did not reach 45 degrees during the eccentric phase, were not approved. Maximal knee extension testing followed the same specific warm-up and pause protocol and was defined as maximal load lifted in a controlled fashion, reaching full extension of the knee joint, within a 1.25kg range. Attempts with exaggerated hip movement or beneath full extension were not approved. Two minute breaks were given during specific warm-up, 3min breaks were given between 1RM attempts.

### Resistance training protocol

Resistance training consisted of three sets of unilateral leg presses and three sets of knee extensions, with an intensity of 10 repetition maximum (10RM). As a general warm-up, the participants cycled on an indoor exercise bicycle for 5-10min. In addition, before the respective exercises, two 10-repetition warm-up sets were completed at ~50% and ~70% of 10RM. Breaks between working sets was 2min, and progressive loading was used to ensure adequate exercise stimulation throughout the intervention. For safety and standardization purposes, all sessions were monitored by trained personnel. To further ensure equal exercise premises for the two legs, each participant was accompanied by the same trainer in each session. If this was not possible throughout the intervention, each participant had the same trainer on pairwise consecutive days as a minimum. The rate of perceived exertion (RPE, 0–10-point scale) was logged before every session, and the session score was logged 15min after each session. Lastly, training volume (i.e., load and repetitions) was logged for every session.

### Sampling of muscle tissue and blood

Muscle biopsies were sampled from m. vastus lateralis using well-established procedures (1). Briefly, muscle biopsy sampling was performed under local anaesthesia (Xylocaine, 10mg ml-1 with adrenaline 5μg 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 the biopsy sampling, muscle tissue was divided in two aliquots for determination of total RNA/expression of rRNA and two aliquots for protein content measurement, snap frozen in isopentane (-80°C) and stored at -80°C until further analyses. Muscle biopsies were sampled at four time points: I/II) Pre-intervention (Fig 1A, 2hrs before training, Day 1 = leg 1, Day 2 = leg 2), and III/IV) after fifth RT session (1A, Day 11 = leg 1, Day 12 = leg 2), two hours before sixth RT session. At each time point, two samples were taken from the same incision. To standardize this procedure, all individual participants had biopsies taken at the same time of day, in an overnight fasted state.

To measure blood glucose levels with and without glucose ingestion/training, blood was collected by means of finger draws on days with biopsy sampling. One finger draw was taken at day 1 (Fig 1A) to serve as a baseline. At day 11 and 12 (Fig 1A), finger draws were collected at 7 time points: I) Immediately prior to 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 draws were analysed with in-house equipment (BIOSEN C-Line, EKF diagnostic GmbH, Barleben). Venous blood samples were collected from the antecubital vein, coinciding with the finger draws except 45min after protein ingestion and in the middle of the RT session, to analyse endocrine variables.

### Total RNA extraction and quantitative real-time reverse transcription polymerase chain reaction

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, a total of eight RNA samples per participant. Total RNA was extracted using TRIzol according to the manufacturer’s protocol. An exogenous RNA control (λ polyA External Standard Kit, Takara Bio Inc., Shiga, Japan) was added at a fixed amount (0.04 ng ml-1 of Trizol reagent) to enable analysis of target gene expression per unit tissue weight (ELLEFSEN2008,2014). Briefly, muscle tissue was homogenized in TRIzol using 0.5mm RNase-free Zirconium beads (~50 ul) (Next Advanced, Averill Park, NY, USA) and a bullet blender (bb). Chloroform (Sigma-Aldrich, Missouri, USA) was used for phase-separation, and an RNA pellet was precipitated with isopropanol (VWR International, Pennsylvania, USA). For assessment of RNA content and -purity 5µl RNA, from the RNA stock, was eluted in TE-buffer (1:2), for assessment via spectrophotometry. All samples had a 260nm to 280nm ratio > 1.9. The RNA stock was stored at -80°C until further analyses. 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 and 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 at 23°C, 10 minutes at 50-55°C and 10 minutes at 80°C. All samples were reverse transcribed and diluted to 1:50 before quantitative real-time polymerase chain reaction (qPCR). Lambda was used as an 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).

### Protein extraction and immunoblotting

Total protein was extracted using the Minute Total Protein Extraction Kit for Muscles (Invent Biotechnology), according to the manufacturer’s protocol, optimised for our lab. Wet muscle was freeze-dried for 24hrs and dissected before extraction. The tissue was homogenized with a plastic rod in 80mg protein extraction powder (Invent Biotechnology) and 100ul iced cell lysis buffer (Denaturing Buffer, Invent Biotechnology), and centrifuged at top speed for 1 min. The supernatant was aliquoted into 2x25ul tubes to run samples in duplicates, and total protein concentrations were determined in a 1:10 dilution (Pierce Detergent Compatible Bradford Assay Reagent, Thermo Fisher Scientific, Oslo, Norway). The protein samples were diluted to 20.25 µg µl−1 total protein with lysis buffer and 4X Laemmli sample buffer (Bio-Rad Laboratories AB, Oslo, Norway) containing 2-mercaptoethanol. Twenty microlitres from each protein sample were pooled as a normalisation factor for gel separation. All protein samples were incubated at 95°C and stored at -20°C until further analysis. The protein samples (20.25µg total protein) were separated at 250V for 50min using 4-20% Tris-Glycine gels (Thermo Fisher Scientific), then transferred to PVDF membranes with wet transfer at 300mA for 3hrs. Both gel electrophoresis and protein transfer were performed at 4°C. Following the wet transfer, membranes were stained with a MemCode reversible total protein stain (MANUFACTURER) and then blocked for 1hr at room temp with a blocking buffer of Tris-buffered saline (TBS; 20 mM Tris, 150 mM NaCl) with 5% non-fat dry milk and 0.1% Tween-20. Primary and secondary antibodies were purchased from Santa Cruz Biotechnology (Texas, USA) UBF:(UBF-9, sc-13125), rpS6:(Ribosomal protein S6 C-8, sc-74459), and Thermo Fisher Scientific () c-Myc:(c-Myc 9E10), goat anti-mouse for c-Myc (goat anti-mouse IgG1 (y1) horseradish peroxidase conjugate), and anti-mouse (anti-mouse IgG1 horseradish peroxidase conjugate). Antibodies were diluted in a 5% non-fat dry milk blocking buffer (TBS, 5% non-fat dry milk, 0.1% Tween-20). Membranes were incubated overnight with primary antibodies, and for 1hr with secondary antibodies. Between blocking and primary antibody staining, membranes were washed 1x5min, between primary and secondary staining, and after secondary staining, membranes were washed 3x5 with TBS-Tween (TBS; 20mM Tris, 150mM NaCl, 0.1% Tween). Following the last TBS-T wash, membranes were incubated 1x5min with enhanced chemiluminescent substrate (ECL, SuperSignal West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific). Membrane blocking, secondary antibody incubation, washing and ECL incubation were performed at room temp, on a tipping board. Primary antibody incubation was performed at 4°C on a tipping board. Chemiluminescence signals were quantified using Image Studio Lite (LI-COR Biotechnology, Lincoln, NE, USA), and total protein content was quantified using ImageJ (RUEDENETAL2017), whereas total protein content was defined as mean grey value of the whole well with between-well values subtracted as background.

### Data handling and statistical analysis

A priori power calculations showed that 20 participants would grant a statistical power of 80% (= 0.05), with an expected drop out of 20%. This power calculation was based on an assumption that the effects of glucose ingestion on ribosome biogenesis may equate to the effects of increasing RT volume from low to moderate (HAMMARSTROM20). . Raw data was imported an analyzed in R via R studios (SOURCE). Legs were categorized as either glucose or placebo in accordance with the randomization. Before quantitative analyses, total RNA was normalized by wet muscle weight, qPCR values were normalized by wet muscle weight and an external reference gene (Lambda), while quantified protein signals from western blot were normalized by pool signals and total protein signal per gel . Total RNA and qPCR values were analyzed by comparing post to pre log-fold change score differences. This was achieved by modelling change as response with baseline and supplement (glucose/placebo) as explanatory factors, and mean-centered baseline values as a covariate in a linear mixed effects model, then calculating the estimated marginal means . Plasma glucose and c-peptide, training volume, and the strength index were analyzed by multiple time-point log-fold change score comparisons, using the same linear mixed effects model with time added as explanatory factor . The linear mixed effects model was designed with the lmer function of the lme4 package (BATES14) in R studios, using the lmerTest function to procure p-values. The relationship between Log-transformation and mean-centering baseline values was done to control for heteroscedasticity and regression to the mean, respectively. Log-transformed values were reverse-transformed for figure illustration. Values are presented as mean change + 95% CL unless otherwise stated, alpha-levels was set to α = 0.05.

## Results

There were no difference in mean change of total training session volume between exercising with placebo or glucose, with an increase of 18 and 16% from session 1 to session 6, respectively (Tab 2; Mean change, 2: *p* = 0.345, 3: *p* = 0.896, 4: *p* = 0.683, 5: *p* = 0.472, 6: *p* = 0.887). Both exercising with glucose and placebo led to significant increases in mean total training session volume at session four, five and six, compared to session 1 (Tab 2; Mean change, 2: *p* = 0.6568, 3: *p* = 0.3417, 4: *p* = 0.008, 5: *p* = 0.002, 6: *p* = 4.5^{-4}). In regard to the dietary intervention, there were no difference in mean macro nutrient intake (protein, fat, carbohydrates) or total calorie intake between glucose and placebo on pairwise consecutive days (*p* > 0.05).

Glucose ingestion before and after RT led to significant increases in plasma levels of glucose by 38% immediately before RT (Fig 1B, 0 min: *p* = 0.00000), by 31% during RT (1B, 15 min: *p* = 0.00000) and by 32% immediately after RT (1B, 30 min: *p* = 0.00000), compared to placebo ingestion before and after RT. Two hours after the session, RT with glucose measured 8% lower plasma glucose levels compared to RT with placebo (1B, 270min: *p* = 0.03). Similarly, levels of c-peptide increased significantly more in exercises with glucose compared to placebo, by 95% immediately before (Fig 1C, 0 min: *p* = 0.00000) and 87% after RT (1C, 30 min: *p* = 0.00000).

After 5 RT sessions (Fig 1D, Post 5RT), there was a significant difference in strength between glucose and placebo, where placebo decreased strength 7% more than glucose (1D, *p* = 0.039). At the remaining time points, there were no significant differences in mean change between exercising with glucose and placebo (1D, Post 2RT: *p* = 0.514, Post 4RT: *p* = 0.735, 30min post 6RT: *p* = 0.178, 2h post 6RT: *p* = 0.245, 23h post 6RT: *p* = 0.96). Both RT with glucose and placebo led to significantly reduced strength post fifth session compared to baseline, by 11 and 18% respectively (1D, Post 5RT: *p* = 0.00001). Comparisons of the acute data gathered from post fifth session until and including 23hrs post sixth session showed a significant mean increase in strength of 5-9% after RT with glucose and placebo 30min after sixth RT session (1D, 30min post 6RT: *p* = 0.01) and two hours after sixth RT session (1D, 2h post 6RT: *p* = 0.004). Twenty-three hours after the last RT, strength was unchanged compared to after fifth RT session (23h post 6RT: *p* = 0.117).

### Markers of ribosome biogenesis

#### Total RNA and ribosomal RNA

Resistance training with glucose did not induce a higher accumulation of total RNA (fig 2A, *p* = 0.499) or rRNA (fig 2B, 47S: *p* = 0.502, 18S: *p* = 0.585, 28S: *p* = 0.74, 5.8S: *p* = 0.935, 5S: *p* = 0.79) compared to RT with placebo. From baseline to post-intervention, there was a mean increase in levels of total RNA by 26 and 22% after RT with glucose and placebo, respectively (*p* < 0.05, compared to baseline). A robust accumulation was also observed in ribosomal RNA expression in both RT with glucose and placebo, with mean increases between 34-43% (GLU) and 33-41% (PLAC) (*p* < 0.05, compared to baseline) in the four rRNA’s. The expression of the 47S pre-rRNA increased by 37 and 59% with glucose and placebo respectively, where only RT with placebo increased significantly from baseline to post (fig 45, *p* = 0.04).

#### Protein

Levels of c-Myc increased by 51% more after RT with placebo compared to RT with glucose, a significant difference (Fig 3A: *p* = 0.027). Levels of UBF and rpS6 increased by 42 and 33% more in placebo compared to glucose, however, this was not a significant difference (Fig 3A: UBF: *p* = 0.185, rpS6: *p* = 0.178). The increase in both RT with glucose and placebo for all measured proteins was significant at post compared to baseline (*p* < 0.05). Further, we found a linear relationship between UBF and total RNA, where an increase of 1 SD unit of UBF equated to a 13% increase in total RNA (*p* = 0.002).

## Discussion

The main findings of the present study were that resistance training with glucose for five sessions did not affect markers of ribosome biogenesis, measured as total RNA, rRNA and rDNA transcription initiation associated proteins, compared to RT with placebo. Similarly, RT with glucose did not affect markers of skeletal muscle functionality such as muscular performance and -recovery, total session volume, and training intensity, compared to RT with placebo. There are studies suggesting that high vs. low glucose treatment/glucose starvation impacts ribosome biogenesis in yeast, rodent and human non-muscle cells (KIM13;MARIAPPAN11;TANAKA15;ZHAI12), however, this effect does not seem evident when total daily glucose intake is matched.

To our knowledge, no previous study has investigated the effects of glucose compared to placebo ingestion with RT on ribosome biogenesis in human skeletal muscle cells, making our hypotheses exploratory and comparisons not straight forwards. The current model of ribosome biogenesis suggests that changes in the accumulation of total RNA and rRNA early after the onset of an RT period predict long-term RT adaptations (FIGUEIREDO19; HAMMARSTROM20; HAMMARSTROM21).Therefore, we assumed 2 weeks to be a suitable time frame for a moderate RT volume to elicit changes in ribosome biogenesis, as similar observations have been made previously (FIGUEIREDO16; HAMMARSTROM20; HAMMARSTROM21). In Hammarström et al. (2020), participants that increased ribosome biogenesis after two weeks (5 RT sessions) of RT, also responded well after the entire intervention (12 weeks, 31 sessions). Figueiredo and colleagues (2016) also observed markedly acute effects, where 47S pre-rRNA increased after a single RT session, passing baseline after 4hrs, peaking at 24hrs and remaining elevated until 48hrs post-exercise. The four mature rRNA did not measurably increase until after multiple sessions (FIGUEIREDO16). As such, increases in total RNA and rRNA observed in the present study are in line with previous observations (FIGUEIREDO16; HAMMARSTROM20; H21?), and also evident i moderately trained individuals, compared to the untrained or RT-untrained individuals of previous studies (FIGUEIREDO16;HAMMARSTROM20;H21?).

Further, the accumulation of total RNA and rRNA is also suggested to reach a plateau after approximately 5 RT-sessions, thus, in addition to the demanding RT- and dietary protocol and the exploratory nature of the hypotheses, we did not conduct a more long-term intervention. COMMENT ON PROTEIN DATA HERE?

As we did not observe any effect of ingesting glucose before and after RT compared to placebo in this design, there is little to suggest that glucose *per se* has any additional effect on ribosome biogenesis, as long as the daily energy intake is sufficient to support cellular energy levels required to drive this process. Looking at previous observations from *in vitro* studies of rodents, yeast, and non-muscle cell cultures (MARIAPPAN11; KIM13; TANAKA18; ZHAI12; HOPPE09; MURAYAMA08), one interesting notion would be how high glucose vs. low glucose or glucose starvation would affect ribosome biogenesis over time in human skeletal muscle cells. Time being of the essence in such a perspective, as previous *in vitro* studies have utilized cells with higher physiological rate than human skeletal muscle cells (KIM13;MARIAPPAN11;TANAKA15;ZHAI12).

Indeed, our findings suggest that glucose ingestion *per se* before and after RT does not induce changes in the internal physiological milieu of human skeletal muscle cells with anabolic effects. It has been theorised that glucose may convey an anabolic signal through insulin, and thus operate as a signal mediator affecting upstream pathways of ribosome biogenesis (KUSNADI15; TANAKA18). Such an anabolic signal could then possibly increase PIC assembly, and consequently Pol I recruitment, initiating rDNA transcription and subsequent rRNA maturation, leading to increased ribosome content (FIGUEIREDO19; TANAKA18). As significantly higher blood levels of c-peptide and glucose were observed in RT with glucose compared to placebo in the present study, without any difference in ribosome biogenesis, there is little to suggest that either insulin or glucose has any direct acute effects.

Another theory is that glucose ingestion leads to higher cellular energy levels compared to placebo, stimulating energy-sensitive pathways such as PIH1, mTORC1, ERK1/2, AMPK and SIRT1 in a dose-response relationship (KIM13;MARIAPPAN11;TANAKA15;ZHAI12). The mTORC1, along with ERK1/2 and PIH1 up-regulates PIC assembly during high-energy conditions, while AMPK and possibly SIRT1 represses PIC assembly during low-energy conditions, as observed in rodent (KILDE), yeast (KILDE), and non-muscle cells (KILDE) *in vitro*. Specifically, high glucose treatment augmented 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-BRAUN17; MARIAPPAN11; ZHAI12). In addition to UBF and SL-1, assembly of the PIC requires TIF-IA and Pol I recruitment, induced by amino acid intake in an mTOR-S6K-dependent manner and possibly by direct c-Myc activation (KUSNADI15). As such, increasing energy supply and nutrient availability should in line with these theories potently stimulate PIC assembly and rDNA transcription. Importantly, these pathways were not directly investigated in the present study. However, downstream targets (UBF, c-Myc, rpS6?) remained unaffected by glucose compared to placebo ingestion, suggesting there is not a dose-response relationship between muscle cellular energy levels and ribosome biogenesis during an RT-intervention (Not sure if I can say this).

A weakness in the present study, is the lack of data on intracellular glucose levels in the muscle cells. Thus, it cannot be said for sure whether there were differences between glucose and placebo or not.

A possible explanation for the lack of effect of glucose compared to placebo, is that both treatments may have exercised with sufficient levels of nutrients and energy, meaning that mTORC1 had sufficient nutrient availability and, thus was not inhibited by AMPK due to energy deficiency (HOPPE09; KUSNADI15). Indeed, mTORC1 is characterised as energy-sensitive (DENNIS01), and therefore it might have been directly affected by high vs. low-glucose conditions (MARIAPPAN11). However, previous studies suggest that mTORC1 is not sensitive enough to register levels of ATP, rather depending on signals from AMPK to assess ATP levels (KIM10). This would arguably make AMPK the primary regulator of the potential impact of glucose on ribosome biogenesis, and thus, glucose has no direct effect on mTORC1 signalling (REFERENCES?). Importantly, intramuscular glycogen content was not measured in the present study, therefore we cannot determine whether cellular energy levels in fact were equal or not, or if there is reason to assume that AMPK-dependent mTORC1 signalling was different in the two treatments. Potentially, such data could give higher resolution to our data. Along with the robust increases in proxy markers of ribosome biogenesis, the equal macronutrient intake between treatments on pairwise consecutive days and plasma glucose levels not indicating energy deficiency during RT, potent mTORC1 stimulation without AMPK-dependent inhibition may be a plausible explanation. Together with previous studies on this topic (MARIAPPAN11; ZHAI12), there are currently scarce observations to suggest glucose carries a direct anabolic signal. As such, physiological levels of plasma glucose during RT, combined with a sufficient daily macronutrient intake, seem potent enough to supply the energy needed for ribosome biogenesis. The lacking differences between treatments in the biological data are further supported by our observations on functional data.

Overall, glucose did not affect the isometric or isokinetic peak torque at 240 d/s, or muscular recovery following the sixth and last RT session, measured as temporal changes in isometric and isokinetic peak torque. Generally, the mean isometric and isokinetic peak torque slightly decreased during the intervention, especially between session 4 and 6, and regained after the intervention. Further, there were no differences between glucose and placebo in total session volume or training intensity. Both exercise with glucose and placebo yielded higher total session volumes at higher percentage of 1RM at the last session compared to baseline, as expected from earlier reports on the moderate-volume RT protocol (KRIEGER09; RHEA03; SCHOENFELD17), in addition to a more recent study implementing the unilateral protocol (HAMMARSTROM20+21/22). Interestingly, we did observe a significant difference between treatments in mean change of isokinetic peak torque at 60 d/s before the sixth RT session, where exercise with glucose decreased peak torque 9% less compared to placebo. However, as none of the other measurements indicated any difference between treatments, a true effect of glucose seems unlikely. Taken together, these results may add to previous observations with no differences between co-ingestion of carbohydrate and protein compared to protein alone, in regard to skeletal muscular recovery following RT (KOOPMAN07; STAPLES11). Collectively, the functional data point towards an exhaustive, yet growth-inducing RT protocol, especially considering the biological data. As a side note, an interesting observation was made by another master’s thesis on this project, finding significantly lower MuRF protein levels at post with glucose treatment compared to placebo. This may suggest an effect of glucose on protein degradation (WILKES09), however, whether this contributes to growth or simply protein turnover has been a topic of debate (FIGUEIREDO19).

Despite being a thoroughly standardised design, a limitation may be posed through lack of participants. We initially reach our minimum limit for statistical power, i.e. 16 participants, however, three dropped out during the intervention, possibly impacting our power. Coupled with the fact that no previous study could guide our power calculations, this, of course, introduces some degree of uncertainty regarding our results. However, we’ve reported little to suggest any true acute effect of RT with glucose ingestion compared to placebo. Moreover, the study was specifically designed to investigate differences in pre-post changes within participants, and thus also quite limited to this perspective. Hence, the effects of time observed exclusively serve as supplementary to the efficiency of the design, without a negative control group to either confirm or refute them. If further investigations are to be done on this topic, these analyses should include more participants and optimally a measure of intramuscular glycogen levels.

In conclusion, resistance exercise training combined with glucose ingestion does not acutely augment ribosome biogenesis following 2 weeks of moderate-volume resistance training in moderately trained young male and female adults. Neither does it acutely affect muscular performance in isometric and isokinetic knee extension peak torque, nor enhance post resistance training recovery within 24hrs.

## References

1. **Hammarström D**, **Øfsteng S**, **Koll L**, **Hanestadhaugen M**, **Hollan I**, **Apró W**, **Whist JE**, **Blomstrand E**, **Rønnestad BR**, **Ellefsen S**. Benefits of higher resistance-training volume are related to ribosome biogenesis. *J Physiol* 598: 543–565, 2020. doi: [10.1113/JP278455](https://doi.org/10.1113/JP278455).

2. **Moss T**, **Stefanovsky VY**. Promotion and regulation of ribosomal transcription in eukaryotes by RNA polymerase I. *Progress in nucleic acid research and molecular biology* 50: 25–66, 1995.

3. **Hillier T**, **Long W**, **Jahn L**, **Wei L**, **Barrett EJ**. Physiological Hyperinsulinemia Stimulates P70 S6k Phosphorylation in Human Skeletal Muscle 1. *The Journal of Clinical Endocrinology & Metabolism* 85: 4900–4904, 2000. doi: [10.1210/jcem.85.12.7036](https://doi.org/10.1210/jcem.85.12.7036).

4. **Mori T**, **Ato S**, **Knudsen JR**, **Henriquez-Olguin C**, **Li Z**, **Wakabayashi K**, **Suginohara T**, **Higashida K**, **Tamura Y**, **Nakazato K**, **Jensen TE**, **Ogasawara R**. C-Myc overexpression increases ribosome biogenesis and protein synthesis independent of mTORC1 activation in mouse skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism* 321: E551–E559, 2021. doi: [10.1152/ajpendo.00164.2021](https://doi.org/10.1152/ajpendo.00164.2021).

5. **West DW**, **Baehr LM**, **Marcotte GR**, **Chason CM**, **Tolento L**, **Gomes AV**, **Bodine SC**, **Baar K**. Acute resistance exercise activates rapamycin-sensitive and -insensitive mechanisms that control translational activity and capacity in skeletal muscle. *J Physiol* 594: 453–68, 2016. doi: [10.1113/JP271365](https://doi.org/10.1113/JP271365).

6. **Poortinga G**, **Wall M**, **Sanij E**, **Siwicki K**, **Ellul J**, **Brown D**, **Holloway TP**, **Hannan RD**, **McArthur GA**. C-MYC coordinately regulates ribosomal gene chromatin remodeling and Pol I availability during granulocyte differentiation. *Nucleic acids research* 39: 3267–81, 2011. doi: [10.1093/nar/gkq1205](https://doi.org/10.1093/nar/gkq1205).

7. **Mariappan MM**, **D’Silva K**, **Lee MJ**, **Sataranatarajan K**, **Barnes JL**, **Choudhury GG**, **Kasinath BS**. Ribosomal biogenesis induction by high glucose requires activation of upstream binding factor in kidney glomerular epithelial cells. *American Journal of Physiology-Renal Physiology* 300: F219–F230, 2011. doi: [10.1152/ajprenal.00207.2010](https://doi.org/10.1152/ajprenal.00207.2010).

8. **Zhai N**, **Zhao Z**, **Cheng M**, **Di Y**, **Yan H**, **Cao C**, **Dai H**, **Zhang Y**, **Shen Y**. Human PIH1 associates with histone H4 to mediate the glucose-dependent enhancement of pre-rRNA synthesis. *Journal of Molecular Cell Biology* 4: 231–241, 2012. doi: [10.1093/jmcb/mjs003](https://doi.org/10.1093/jmcb/mjs003).

## Figure/table text

**Table 1:** Baseline characteristics. Age = mean age in years, Height = mean height in cm, Weight and Lean mass = mean in kg, Iso 60/240 = mean unilateral isokinetic knee extension peak torque in Nm at 60 d/s and 240 d/s, Isom = mean unilateral isometric knee extension peak torque in Nm.

**Table 2:** Mean total session volume and mean change in total session volume from session 1 to session 6.

**Figure 1:** A) An overview of the experimental design with 12 days of concomitant dietary intervention and resistance training (RT), preceded by 7 days involving familiarization. Between days -7 and -1, participants were familiarized to the RT exercises via 1RM leg press and knee extension testing, and to the strength tests via Humac Norm dynamometer (days -7 and -5). Before baseline testing, the participants were randomly allocated to exercise one leg with GLU (glucose) and the other with PLA (placebo), in a unilateral, alternating fashion. Further, non-dominant/dominant + GLU/PLA, and onset with GLU or PLA was also randomized, i.e. the figure illustrates an example where the participant was randomized to start RT with GLU. Biopsies were taken from m. vastus lateralis at baseline (Day 1 leg 1, Day 2 leg 2), and after five RT sessions (Day 11 leg 1, Day 12 leg 2). Blood for measurement of plasma glucose and -c-peptide was sampled at baseline (Day 1), and during post testing (Day 11 leg 1, Day 12 leg 2), via finger draws and venous blood samples. Skeletal muscle strength was measured as peak torque in unilateral isometric and isokinetic (at 60 and 240 d/s) knee extension before, multiple times during, and after five and six session. A total of three participants dropped out of the intervention, either due to reported muscular discomforts of heavy RT, or sickness in their family. B and C) Changes in plasma glucose (B, mmol/L) and c-peptide levels (C, pmol/L). Glucose levels in blood was measured via finger draws 120 (-120), 90 (-90), and 30min (-30) before RT, immediately before RT (0), during RT (15), immediately after RT (30) and 2hrs after RT (120). C-peptide levels were measured simultaneously to these finger draws, except for 90min before and during RT. D) Changes in muscular strength measured as isometric and isokinetic peak torque (60 and 240 d/s) via Humac Norm Dynamometer, conducted at baseline (A: Day -1), after two and four RT sessions (A: Day 4 and 8 leg 1, Day 5 and 9 leg 2), after five RT sessions/before the 6th session (Day 11 leg 1, Day 12 leg 2), as well as 30min, 2hrs and 23hrs after the 6th RT session (A: Day 11/12 leg 1, Day 12/13 leg 2). The index was calculated by normalizing peak torque values to the highest peak torque value at each respective speed, and then summarized and used in change score calculations. Values are presented as changes in estimated marginal means ± 95% CI. \* = *p* < 0.05 between groups. Glucose n = 13, placebo n = 13.

**Figure 2:** Changes in total RNA and ribosomal RNA. A) Total RNA, B) 47S pre-rRNA, 18S rRNA, 28S rRNA, 5.8S rRNA, 5S rRNA. Baseline = Day 1 leg 1/ Day 2 leg 2, Post = Day 11 leg 1, Day 12 leg 2. Total RNA and rRNA were analyzed in duplicates, with two duplicates per biopsy (two muscle tissue pieces per time point), and normalized to ng x mg wet muscle weight for total RNA and external reference gene (Lambda) for rRNA. Total RNA and rRNA changes were calculated as log-fold change score per mg wet muscle weight. Mean change scores of the duplicates were calculated and transformed to the log-scale before modelling, then reverse-transformed for figure illustration. Values are estimated marginal means fold change per leg per supplement ± 95% CI. Glucose n = 13, placebo n = 13.

**Figure 3:** Changes in levels of protein. A) Mean UBF, c-Myc and rpS6 (AU, arbitrary units) at baseline and post + representative western blots of the respective proteins. Baseline = Day 1 leg 1, Day 2 leg 2, post = Day 11 leg 1, Day 12 leg 2. GLU = glucose, PLA = placebo. Protein samples were analyzed in two duplicates per biopsy per time point, loaded on separate gels in an inverted order, e.g. from gel 3 to 6, as shown by the duplicates (1 and 2). Change in protein levels were calculated as log-fold change scores normalized by pools (pool of all protein samples per gel). Mean log-change scores of the duplicates were calculated before modelling and reverse-transformed for figure illustration. Values are estimated marginal means fold change per leg per supplement ± 95% CI. \* = *p* < 0.05 between groups. Glucose n = 13, placebo n = 13. B) Representative total protein stain blot. C) Linear relationship between total RNA (ng x mg) and UBF levels (SD units), with time added as a covariate. Total RNA was normalized by wet muscle weight, and UBF was normalized by pools per gel, and total protein per lane factor. Values are presented as log-transformed means.