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 (**figueiredo\_genetic\_2021?**, **thalacker-mercer\_cluster\_2013?**). 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 (**hammarstrom\_benefits\_2020?**, **krieger\_single\_2009?**, **schoenfeld\_dose-response\_2017?**). For instance, although changes in training volume generally induces greater benefits of RT, not all individuals benefit from such modifications (**hammarstrom\_benefits\_2020?**). 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 (**cermak\_protein\_2012?**, **lanhers\_creatine\_2015?**, **lanhers\_creatine\_2017?**, **morton\_systematic\_2018?**). 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 (**mul\_exercise\_2015?**, **sebata\_control\_2018?**). Furthermore, energy availability is a decisive factor in *de novo* synthesis of ribosomes (**kusnadi\_regulation\_2015?**, **moss\_housekeeper\_2007?**, **sebata\_control\_2018?**) which in turn determines muscle growth by increasing the muscles translational capacity (**figueiredo\_regulation\_2019?**, **hammarstrom\_benefits\_2020?**, **sebata\_control\_2018?**, **stec\_ribosome\_2016?**, **von\_walden\_ribosome\_2019?**). 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 (**moss\_promotion\_1995?**). Multiple signaling pathways converge to regulate rRNA transcription, including the mammalian target of rapamycin (mTORC1) signal-transduction pathway and c-Myc (**kusnadi\_regulation\_2015?**, **von\_walden\_ribosome\_2019?**, **sebata\_control\_2018?**, **west\_acute\_2016?**, **mori\_c-myc\_2021?**). mTOR receives input from growth factors, hormones, mechanical loading, and nutrients to balance protein synthesis through multiple mechanisms based on cellular energy levels (**hoppe\_amp-activated\_2009?**). Importantly, mTOR and its downstream target S6K1 serve as a mediator from insulin signaling in skeletal muscle(**hillier\_physiological\_2000?**). 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 (**figueiredo\_regulation\_2019?**, **von\_walden\_ribosome\_2019?**). Parallel to mTORC1 (**mori\_c-myc\_2021?**, **west\_acute\_2016?**), c-Myc increases ribosomal biogenesis directly and through transcriptional control of the upstream binding factor (UBF) (**sanij\_ubf\_2008?**, **poortinga\_c-myc\_2011?**). 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(**mariappan\_ribosomal\_2011?**). Independently from UBF and mTORC1, high glucose was shown to lead to chromatin remodeling, which promotes rRNA transcription in cell cultures(**zhai\_human\_2012?**). 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. There has been multiple studies the last years suggesting translational capacity to be as important as, if not more, translational efficiency (**hammarstrom\_benefits\_2020?**, **hammarstrom\_ribosome\_2022?**, **figueiredo\_revisiting\_2019?**). Previously, *in vitro* studies has shown UBF and c-Myc to be a important factors in the PIC, c-Myc as a general transcription factor and UBF as a specific transcription factor to rDNA transcription initiation (**mariappan\_ribosomal\_2011?**, **von\_walden\_ribosome\_2019?**). Coupled with the recent observations by Hammarström et al. (**hammarstrom\_benefits\_2020?**, **hammarstrom\_ribosome\_2022?**), understanding underlying factors regulating and affecting ribosome biogenesis seem key to further our understanding in optimization of RT to individual phenotypes.

## 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 one leg with glucose and one leg with 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 on pairwise consecutive days. To further standardize this, participants also recorded and repeated 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. Lastly, training volume (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 42% 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 19 and 23% 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 heavy resistance training with glucose did not affect markers of ribosome biogenesis, measured as total RNA, rRNA and rDNA transcription initiation associated protein, compared to RT with placebo. Similarly, RT with glucose in general did not affect markers of skeletal muscle functionality such as strength and recovery, or total training session volume. At one time point, RT with glucose led to less reduction in strength compared to placebo, and levels of plasma glucose and c-peptide were significantly higher before, during and after the RT sessions with glucose. Further, there were no differences in daily macronutrient intake on pairwise consecutive days. This would suggest the effects of high vs. low glucose/glucose starvation treatment on ribosome biogenesis or rDNA transcription initiation observed in previous *in vitro* studies (**mariappan\_ribosomal\_2011?**, **tanaka\_mild\_2015?**) are not translatable to acute effects in human skeletal muscle *in vivo*. Notably, the study was specifically design to investigate differences in pre-post changes within-participants, and thus also quite limited to this perspective. Hence, the observed effects of time serve as supplementary to the efficiency of the design, without a negative control group to either confirm or refute them.

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, making our hypotheses exploratory and comparisons not straight forwards. Mariappan and colleagues (**mariappan\_ribosomal\_2011?**) investigated the effect of high glucose treatment on ribosome biogenesis in glomerular epithelial cells (GECs) from diabetic (type 1 and 2) mice, observing a UBF-dependent augmentation of ribosome biogenesis. In a study from 2015, Tanaka et al. (**tanaka\_mild\_2015?**) observed that mild glucose starvation reduced rRNA transcription in human breast cancer cells. These findings do imply an important role for glucose in the initiation of rDNA transcription, the rate-limiting step of ribosome biogenesis (**mariappan\_ribosomal\_2011?**, **tanaka\_mild\_2015?**). In the present study however, we did not find observations indicative of glucose having an effect acute effect on rDNA transcription. Thus, glucose more likely affects rDNA transcription through energy availability (**hoppe\_amp-activated\_2009?**, **kusnadi\_regulation\_2015?**, **mariappan\_ribosomal\_2011?**, **tanaka\_mild\_2015?**) Notably, kidney cells from mice and human breast cancer cells are likely to have a different physiological rate compared to human skeletal muscle cells, thus, our two-week intervention are likely not directly comparable to these findings, as was expected.

Nevertheless, the increases observed in total RNA and rRNA after only five sessions of RT in moderately RT-trained participants do concur with recent findings after acute (**figueiredo\_impact\_2016?**) and short-term RT (**hammarstrom\_benefits\_2020?**, **hammarstrom\_ribosome\_2022?**) in previously RT-untrained participants. Therefore, the time frame and training volume chosen for this design was indeed adequate to stimulate rDNA transcription and subsequently ribosome biogenesis by proxy. Further, the within-participant design along with the standardized conditions for testing and biological sampling decreased are day-to-day/test-to-test variation, effectively increasing the reliability of measurements (**HALPERIN?**, **THIESE?**, **MACINNIS?**). Importantly, both Mariappan (**mariappan\_ribosomal\_2011?**) and Tanaka (**tanaka\_mild\_2015?**) compared high glucose treatment to either low glucose treatment or glucose starvation. In the present study however, we compared RT with either protein and glucose or protein and placebo. The participants fasted from 2200hrs the preceding day until the morning of each training day, receiving only the prescribed treatment until 2.5 hrs after RT. Thus, except from approximately 0700hrs-1230hrs, the participants ingested an equal amount of carbohydrates, protein, fat and calories on pairwise days. As such, there were no differences in baseline levels of plasma glucose prior to the daily intervention, as by design, since we were interested in investigating if there were direct effects of glucose as a signalling molecule, based on the observations of Tanaka et al. (**tanaka\_mild\_2015?**) and Mariappan et al. (**mariappan\_ribosomal\_2011?**). Further, the significant difference in levels of plasma glucose and c-peptide measured before, during and after session six, confirms that the treatment during RT with glucose indeed was sufficient. Despite of this, we found no difference in markers of ribosome biogenesis between RT with glucose and RT with placebo, this does suggest that there are no additional acute effects of exercising with glucose in this regard, when total macro nutrient intake is standardized.

Prior to the present study, we was theorized that glucose may convey an anabolic signal, either directly or through insulin, and thus operate as a signal mediator affecting upstream pathways of ribosome biogenesis based on previous observations (**kusnadi\_regulation\_2015?**, **sebata\_control\_2018?**). This theoretical anabolic signal could possibly stimulate PIC assembly and Pol I recruitment to the rDNA promoter, initiating rDNA transcription and subsequent rRNA maturation, and lastly increased ribosome content of the skeletal muscle cell (**figueiredo\_regulation\_2019?**, **sebata\_control\_2018?**). Thus, we hypothesized that if glucose directly or indirectly via insulin can stimulate initiation of rDNA transcription, five RT sessions with glucose will elicit this effect compared to five RT sessions with placebo. This time frame has previously shown to be sufficient to measure changes in markers of ribosome biogenesis (**hammarstrom\_benefits\_2020?**, **hammarstrom\_ribosome\_2022?**, **figueiredo\_impact\_2016?**). As no such effect was observable in the present study, this hypothesis was rejected. Further, glucose ingestion affects cellular energy levels; thus another theory is that high glucose treatment would stimulate energy-sensitive pathways such as PIH1, mTORC1, ERK1/2, AMPK and SIRT1 in a dose-response relationship (**kim\_nutrient\_2013?**, **mariappan\_ribosomal\_2011?**, **tanaka\_mild\_2015?**, **zhai\_human\_2012?**). Mammalian target of rapamycin complex 1, along with ERK1/2 and PIH1 upregulates PIC assembly during high-energy conditions, while AMPK and possibly SIRT1 represses assembly of the PIC during low-energy conditions, as observed in rodents , yeast , and human non-muscle cells *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-1A and chromatin regulation suppression (**mariappan\_ribosomal\_2011?**). In addition to UBF and SL-1, assembly of the PIC requires TIF-1A and Pol I recruitment, induced by amino acid intake in an mTOR-S6K-dependent manner and possibly by direct c-Myc activation (**kusnadi\_regulation\_2015?**). As such, increasing energy supply by RT with glucose vs. RT with placebo and nutrient availability should in line with these observations potently stimulate PIC assembly and rDNA transcription. Though we did not investigate these pathways directly, the downstream targets UBF and rpS6 did not differ in mean change between treatments. As the participants in the present study fasted until the daily training was completed, and matched their daily energy intake on pairwise days, this suggests that as long as the daily energy requirements are met, ingesting glucose with RT does not stimulate further gains in anabolic responses. Notably, the daily RT protocol was quite short (~30min), which may have reduced the effectiveness of the energy addition through glucose. In this regard, it would have been interesting to measure the intramuscular glycogen storages, to see how the RT sessions impacted intracellular energy levels. Unfortunately, such analyses were not conducted in the present study. Regrettably, this is a weakness of the design, as we have not measured directly what happened to the excess plasma glucose. Nevertheless, the absent effect of increasing energy availability during RT suggest that, there is no dose-response relationship between acutely increasing myocellular energy levels before and after RT and ribosome biogenesis.

Interestingly, we did observe a linear relationship between total RNA and UBF, suggesting ribosome biogenesis to be dependent on UBF activation, in line with observations in Mariappan et al. (2011). This was not evident with total RNA and c-Myc or rpS6, which we observed no linear relationship between. The upstream binding factor (UBF) has indeed previously been described as a master regulator of rDNA transcription, based on findings from cell cultures (**figueiredo\_regulation\_2019?**, **kusnadi\_regulation\_2015?**, **russell\_rna-polymerase-i-directed\_2005?**). However, it has only recently been found in a linear relationship with total RNA content in human skeletal muscle cells after a short period of RT (**hammarstrom\_ribosome\_2022?**), which the observation of the present study supports. As such, UBF also seem to respond to mechanical loading in human muscle cells in line with responses seen in cell cultures and synergist ablation models (**mariappan\_ribosomal\_2011?**, **von\_walden\_mechanical\_2012?**), in addition to the previously reported response to hormonal, nutritional, and cellular energy signals (**figueiredo\_regulation\_2019?**, **kusnadi\_regulation\_2015?**, **russell\_rna-polymerase-i-directed\_2005?**). While c-Myc and rpS6 did not increase linearly with accumulation of total RNA, the cellular content of c-Myc and rpS6 increased from baseline to post with both treatments. The transcription initiation factor c-Myc has been implicated as important to ribosome biogenesis (**von\_walden\_ribosome\_2019?**, **figueiredo\_regulation\_2019?**), though its role is likely as a general transcription factor rather than specific, compared to UBF. Further, the ribosomal protein S6 (rpS6) has previously been shown to correlate strongly with rRNA content after synergist ablation (**nakada\_correlation\_2016?**, **chaillou\_hypoxia\_2012?**), and is suggested as a reliable measurement of ribosome biogenesis (**nakada\_correlation\_2016?**). Together, this may explain why the between treatment difference in c-Myc content did not induce differences in total RNA or rRNA content, as the specific transcription factor UBF and the rpS6 increased equally with both treatments. Notably, the difference between c-Myc in glucose and placebo may have caused responses in pathways not measured in the present study.

The results from the strength tests do indicate that the participants might have been fatigued during the intervention, as they exercised every day for 12 days straight, with strength testing in-between. In this perspective, we expected glucose to increase energy availability and thus, muscle performance during strength testing compared to placebo. However, this was not evident, as the mean response in strength as an index of peak torque at all speeds decreased with both treatments from baseline to post five and six sessions. Notably, RT with glucose reduced strength significantly less than RT with placebo post five sessions, which may indeed have been an effect of increased energy availability, seeing as glucose is the preferred energy source during strenuous activity (**mul\_exercise\_2015?**). Arguably, this might imply that RT with placebo experienced a higher stress response to the RT program, thus reduced muscle performance in strength tests. This could also explain the differences between treatments in content of the stress responsive c-Myc , meaning higher stress exerted during RT with placebo compared to RT with glucose inducing higher accumulation of c-Myc. If this was the case, it does seem inconclusive that strength did not differ between treatments at earlier or later time points. In addition, there were no differences in training volume per session between treatments, therefore, the mechanical loading should have been equal. Another potential explanation to this observation might be …. (bifasisk restitusjon?). Lastly, these inconclusive observations may also reflect our limited sample size, despite using a within-subjects design. Initially, we met our minimum limit of 16 participants however, three dropped out during the intervention, leaving us slightly underpowered according to the a priori calculation.

In conclusion, resistance exercise training combined with glucose ingestion does not acutely augment ribosome biogenesis following two weeks of heavy resistance training in moderately trained young male and female adults. Neither does it acutely enhance muscular performance or recovery withing 23hrs, measured as unilateral isometric and isokinetic knee extension peak torque. The observations in total RNA, rRNA and rDNA transcription initiation associated proteins confirm the key role of increased UBF content in ribosome biogenesis in moderately trained human skeletal muscle following resistance training, previously observed in RT-untrained participants, rodents or *in vitro* . If future investigations are to be made on this topic, a greater sample size coupled with a negative control group and analyses of the intramuscular glycogen storage may provide higher resolution results.

## 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).

## 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. . 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.