Ribosome accumulation during early phase resistance training

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**Running title:** Ribosome biogenesis in early phase resistace training

# Abstract

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# Introduction

# Methods

## Study overview

## Muscle strength and thickness

Muscle strength was assessed as maximal knee extension isokinetic (90° sec-1) and isometric (60° angle, fully extended leg 0°) torque. After a brief warm-up (5-min cycling, RPE 12-14), participants were seated and secured in the individually adjusted dynamometer. The test protocol consisted of unilateral knee-extensions. Participants were instructed to gradually increase their effort during three warm-up repetitions (50, 60 and, 70% of subjective maximal effort). After a 30-sec restitution period participants were instructed to perform three repetitions with maximal effort in the concentric phase. Sixty seconds after the isokinetic test the lever automatically moved to a 60° angle and participants were instructed to push against the lever enough to see feedback from the visual feedback system. After an additional 15-sec restitution period, participants were instructed to push against the lever with maximal effort. During the same assessment session, participants remained seated in the dynamometer for meassurement from both legs. The first measurement was alternated between legs and sessions. For statistical treatment of the data, all successful attempts were used. Successful attempts were decided based on participants (…). The last strength assessment at baseline was performed at least **seven** days prior to the first biopsy sampling. At least one strength was performed on separate days with two sessions allowed perform on the same day with a short rest between sessions. Post training assessments were performed approximately 48 hours after the last session and after seven days of no training.

Muscle thickness was assessed using (…) ultra sound.

## Muscle biopsy sampling

Muscle specimens were sampled bilaterally from *m. vastus laterlis* under local anesthesia (lidocaion %, Manufacturer, Oslo, Norway) using a disposable needle (12-14 gauge, Universal plus, Medax, Italy), operated with a spring loaded device (Bard Magnum). Two to four passes were made to get sufficient material. One to two aliquots of the samples were quickly dissected free from connective and fat tissue, weighed and frozen in isopentan chilled to -80°C and stored at -80°C until further processing. At baseline, after twelve weeks of training and, after the de-training period, a further aliquot from each sample was selected for immunohistochemistry analysis and subsequently immersed in 4% formalin for fixation.

## RNA and protein extraction

Frozen muscle tissue was homogenized in 1 ml of Trizol (ThermoFisher Scientific, Oslo, Norway) spiked with 0.04 ng of an external, non-mammalian, RNA spike-in (Lambda, Takara Bioscience). Mechanical disruption of the samples was achieved using Zirconium Oxide Beads (0.5 mm, Next Advance, Inc., New York, USA) and a bead mill (Bullet blender, Next Advance). Chloroform (200 μl) was prior to centrifugation (12000 g, 15 min at 4°C) to achieve phase separation. Four houndred fifty μl of the upper aqueous phase was transferred to a fresh tube and 500 μl of isopropanol was added to precipitate the RNA. After a 10 min incubation at room temperature, samples were centrifuged (12000 g, 10 min at 4°C), after which a pellet formed. The pellet was washed three times in chilled 75% ethanol with centrifugation between each wash (7500 g, 5 min at 4°C). After the final wash all ethanol was removed and the pellet was eluted in 0.1X Tris-EDTA buffer. RNA concentration and purity was assessed by spectrophotometry. All samples had 260/280 ratios > 1.95.

Protein was extracted from Trizol preparations according to manufacturers instructions and (**???**) with modifications. The remaining aqueous phase was removed and DNA was precipitated by the addition of XXX μl of absolute ethanol followed by gentle centrifugation (2000 g, 5 min at room temperature). An aliquot of the phenol-ethanol phase, corresponding to ~1.75 mg of tissue, was transferred to to a fresh tube. After addition of at least two volumes of isopropanol and incubation (10 min at room temperature), samples were centrifuged (7500 g, 10 min 4°C) and a pellet formed. The pellet was washed three times in 95% ethanol with each wash separated by centrifugation (5000g, 5 min at room temperature). After the last wash all liquid was removed and 45 μl of Kopec buffer was added (4% SDS, 300 mM Tris …, pH; containing protease and phosphatase inhibitors). Pellets were incubated at 50°C for three hours after which the majority of samples were dissolved. Any undissolved material was sedimented by centrifugation (10000 g, 10 min at room temperature). Protein concentrations were measured (Pierce Detergent Compatible Bradford Assay, ThermoFisher Scientific). Sample were normalized in Laemmli buffer (Bio-Rad), boiled (95°C, 5 min) and stored at -20°C before later use.

## Quantitative polymerase chain reaction (qPCR)

## Immunoblotting

## Immunohistochemistry

## Statistics and data analysis

# Results

# Discussion

# References