# Elevated muscle mass accompanied by transcriptional and nuclear alterations several months following cessation of resistance-type training in rats

## Introduction

Loading-induced hypertrophy with transcriptional upregulation has been observed concomitant with nuclei accretion in various studies regarding both humans and rodents. Bruusgaard et al. in 2010 pioneered work utilizing a model of synergist ablation to cause hypertrophy followed by denervation-induced atrophy to demonstrate that load-induced gains in myonuclei could be long-lasting after the termination of such exposure. This finding was consistent with the idea of enduring myonuclear accretion as a form of "muscle memory" allowing enhanced muscle adaptation following a period of detraining. Subsequent research groups further investigated this possibility in the context of exercise utilizing rodents and various loaded exercise paradigms such as weighted wheel running and ladder climbing. This research yielded a spectrum of results. While these studies were instrumental in highlighting the variation in outcomes possible following load-induced hypertrophy in general, they were limited in their direct relatedness to resistance training – the predominate form of exposure utilized to induce hypertrophy. Our research group has established and repeatedly validated a rat research model to investigate resistance-type training. The model is based on using a dynamometer to precisely expose dorsiflexor muscles of rats to 8 sets of 10 repetitions (per set) of stretch-shortening contractions (SSCs) – a consecutive sequence of isometric, lengthening, and shortening contractions. Training with this exposure 3 sessions per week for one month results in increases in muscle mass and performance. This is accompanied by an increase in muscle fiber area accompanied by a proportional increase in myonuclei number and a rise in overall transcriptional output as measured by total RNA levels. The purpose of the present study was to determine to what extent alterations in performance, muscle mass, nuclei number, and transcriptional output persist several months following the termination of this relevant and valid model of resistance-type training.

**Material Collection**

Experimental animal and overview of design

- 3 month old male Fischer Brown Norway hybrid rats (F344 X BN F1) were obtained from the National Institutes of Aging colony.
- All animal procedures were done in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and approved by the Animal Care and Use Committee at the National Institute for Occupational Safety and Health in Morgantown, WV.
- For the trained group, 3-month-old rats were exposed to SSC training for one month, thereupon reaching 4 months of age.
- At 7 months of age, performance testing was completed for both groups of rats – rats that underwent the training and rats which were never trained. After performance testing and while still anesthetized (isoflurane gas 2-3% by volume), all animals were euthanized by pentobarbital (100-300 mg/kg body weight) intraperitoneal injection followed by exsanguination.

SSC training

- Each rat was anesthetized with isoflurane gas (2-3% by volume), placed supine on a heated table, the left knee was secured in 90º flexion, and the left foot was secured to a fixture containing a load cell.
- Platinum electrodes were placed subcutaneously at the region of the common peroneal nerve for activation of dorsiflexor muscles at 4-V magnitude, 0.2-ms pulse duration, and 120-Hz frequency, optimal settings for maximal contraction.
- Exposure to SSCs consisted of 8 sets with 2-minute intervals between sets and 10 SSCs per set with 2-second intervals between SSCs. For each SSC, the dorsiflexor muscles were maximally activated, while the ankle was set to 90º for 100 ms (i.e. isometric phase), then rotated to 140º at

60º/s (i.e. stretch phase), returned to 90º at the same velocity (i.e. shorten phase), and lastly, deactivated 300 ms later.

- Each rat was exposed to this protocol 3 times per week (i.e. Monday, Wednesday, and Friday) for 4 weeks. Performance measures (i.e. torque values for the isometric and stretch phases and work values for the stretch and shorten phases) for the first SSC of each of the sessions during the first and last week of training were averaged to determine initial and final training values, respectively.
- At 3 months following the cessation of training (i.e. 7 months of age), muscles were exposed to a SSC to assess performance and compared with that of muscles from age-matched (i.e. 7 month old) nontrained rats. Immediately following this assessment, both right and left tibialis anterior (TA) muscles were surgically removed, weighed, and the tibia lengths recorded.

Total RNA and mRNA analysis

- A ~65 mg portion of frozen TA muscle tissue was homogenized with a Mini-BeadBeater 8 (Biospec) while in a vial of 1 ml of TRIzol with 1.0 mm zirconia beads (BioSpec, Cat#11079110zx).
- The RNAqueous phenol-free total RNA Isolation Kit (Ambion, Cat# AM1912) was used to isolate RNA and total RNA concentration was quantified (NanoDrop 2000c, Thermo Fisher Scientific, Waltham, MA).
- The cDNA was synthesized utilizing 0.5 μg of RNA and the $RT^2$ First Strand Kit (Qiagen, Cat# 330401).
- The expression of genes relevant to growth and energy sensing was investigated using the $RT^2$ Profiler$^{TM}$ PCR Array for mTOR signaling (Qiagen, Cat# PARN-098Z) per manufacturer's instructions.

Immunofluorescence

- The mid-belly of each TA muscle was covered with tissue freezing media and immersed in isopentane (-160 °C). This tissue was then cryosectioned at 12 μm thickness.
- Sections were fixed in HistoChoice (Sigma-Aldrich; H2904) for 45 minutes, washed (3 x 5 minutes in PBS), washed (3 x 5 minutes in PBS), and then blocked with 5% goat serum in 0.4% Triton X-100 in PBS for 1 hour.
- A primary polyclonal antibody for laminin (Sigma-Aldrich; L9393; 1:50) was applied for 1 hour. Sections were washed (3 x 5 minutes in PBS) and secondary antibody (donkey anti-rabbit IgG Cy3 at 1:100 in PBS with 0.4% Triton X-100) was applied for 30 minutes.
- After 3 x 5 minute washes in PBS, sections were mounted with Prolong™ Gold Antifade Reagent (Thermo Fisher Scientific; P36931) with 4′, 6-diamidino-2-phenylindole (DAPI).
- At 2 mm offset from either side of the midpoint, 5 equally spaced fields (at 20X magnification) were imaged for a total of 10 images. Image analysis utilized ImageJ (version 1.46, National Institutes of Health, USA). Each muscle fiber (118 ± 2 fibers per section) was traced to determine muscle fiber size.
- Number of nuclei within each muscle fiber was counted to assess number of nuclei per muscle fiber.
- Muscle fiber area per nucleus was determined for each muscle fiber by dividing the muscle fiber area by the number of nuclei per fiber.
- ImageJ functions of watershed and particle analysis were utilized for each DAPI image to yield nuclei size and circularity of all the nuclei (2626 ± 70 nuclei per section) in the muscle tissue for the regions imaged. The index of circularity was determined by the equation $4\pi(\text{area}/\text{perimeter}^2)$ with a perfect circle as a value of 1 and increasing elongation as the value decreases.

## Citations

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