PHYSIOLOGICAL ADAPTATIONS TO RESISTANCE TRAINING IN RATS SELECTIVELY BRED FOR LOW AND HIGH RESPONSE TO AEROBIC EXERCISE TRAINING

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Running title: Resistance training with high and low responder rats

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New Findings:

1. What is the central question of this study?

Can phenotypic traits associated with low response to one mode of training be extrapolated to other exercise-inducible phenotypes? The present study investigated whether the low responder rats to endurance training are also low responders for resistance training.

2. What is the main finding and its importance?

By resistance training, high responder rats to aerobic exercise training (HRT) improved more maximal strength compared to the low responder rats (LRT). However, greater gains in strength in HRT was not accompanied with muscle hypertrophy suggesting that these responses observed could be mainly of neural origin.

ABSTRACT

The purpose of this study was to determine whether rats selectively bred for low and high response to aerobic exercise training cosegregate for differences in muscle adaptations to ladder climb resistance training. Five high responder (HRT) and five low responder (LRT) rats completed the resistance training while six HRT and six LRT rats served as sedentary controls. Before and after the 6-week intervention, body composition was determined by DXA. Before tissue harvesting, right triceps surae muscles were loaded by electrical stimulation. Muscle fiber cross-sectional areas, nuclei per cell, phosphorylation status of selected signaling proteins of mTOR and Smad pathways, and muscle protein, DNA, and RNA concentrations were determined for the right gastrocnemius muscle. Daily protein synthesis rate was determined by deuterium oxide (D_2O) method from the left quadriceps femoris muscle. Tissue weights of leg and arm muscles were measured. In response to resistance training, maximal carrying capacity was greater in HRT (~3.3 times per body mass) than LRT (~2.5 times body mass), indicating greater improvements of strength in HRT. However, muscle hypertrophy that could be related to greater strength gains in HRT was not observed. Furthermore, noteworthy changes within the experimental groups or differences between groups were not observed in the present measures. Lack of hypertrophic muscular adaptations despite considerable increases in muscular strength suggest that adaptations to the present ladder climb training in HRT and LRT rats were largely induced by neural adaptations.

1. INTRODUCTION

Resistance training (RES) is widely recommended as a part of physical activity guidelines for the improvement of functional capacity and cognitive function, and for the management and prevention of several chronic degenerative diseases (Steele *et al*. 2017). Compared to humans, animal RES models permit specific control of environmental conditions while nutritional intakes can be regulated and monitored. Animal studies enable harvesting of several tissue types as well as experimental manipulations, such as pharmacological interventions, that are not possible to implement with humans (Cholewa *et al*. 2014). Thus, an animal model of RES that closely resembles characteristics of physical activity for humans is of utmost importance.

Various experimental models have been utilized in rats aiming to mimic human responses to RES (Lowe & Alway 2002). One of those models is weighted ladder climbing in which rats climb a vertical ladder (~80° incline) with progressively increased weights affixed to the base of the tail over the course of several weeks of RES. With ladder climbing RES, the loading parameters such as volume, intensity and frequency can be planned and RES adaptation in climbing performance can be determined by assessing the maximal load the rats can carry. Furthermore, to avoid extra stress, there is no need for external motivators such as food reward or negative reinforcements (e.g. electric shock or food deprivation) to execute the climbing task (Hornberger & Farrar 2004; Strickland & Smith 2016).

Considerable inter-individual differences in the responsiveness to aerobic training have been observed in highly standardized training programs in humans (Bouchard & Rankinen 2001) and in animals (Koch *et al*. 2013). Similarly, large inter-individual variability has been observed in muscle strength and size gains by chronic RES in humans (Ahtiainen *et al*. 2016; Hubal *et al*. 2005). While individuality in responses to RES is acknowledged, investigating determinants of this phenomena is gaining widespread popularity (Bamman *et al*. 2007;

89 Davidsen *et al*. 2011; Mobley *et al*. 2018; Ogasawara *et al*. 2016; Petrella et a., 2013).
90 However, whether the individual responsiveness to aerobic training and RES are similar
91 between each other is currently largely unknown. We recently utilized ladder-climbing RES
92 with rats selectively bred for high (HRT, high-response trainer) and low (LRT, low-response
93 trainer) response to aerobic exercise training (Nokia *et al*. 2016) and observed a greater
94 increase in strength (i.e. the maximal amount of weight the rats were able to carried) in the
95 HRT compared to LRT rats. Based on that observation, we hypothesized that HRT would
96 demonstrate larger skeletal muscle adaptations to ladder climbing RES compared to LRT
97 rats.

98

99 **2. MATERIALS AND METHODS**

100 **Ethical Approval**

101 All the experimental procedures were implemented in accordance with the directive
102 2010/63/EU of the European Parliament and approved by the National Animal Experiment
103 Board, Finland (Permit number ESAVI-2010-07989/Ym-23). This work complies with the
104 animal ethics checklist outlined by Experimental Physiology. Animals received humane care
105 and every attempt was made to reduce animal suffering and discomfort. At the end of the
106 experiments, animals were quickly euthanized with a rising concentration of CO_2, and killed
107 by cardiac puncture.

108 **Animals**

109 The animals used in the study were adult male LRT (n=12) and HRT (n=12) rats,
110 representing the 18th generation of these rat lines developed by selective breeding (Koch *et*
111 *al*. 2013). Upon arrival at the University of Jyväskylä, the rats were allowed to acclimate for
112 4–5 weeks. After this, when the rats were ~6 months old, they were tested for their response

to aerobic exercise training. All rats were subjected to an 8-week exercise regimen, during which they were trained on a motorized treadmill three times a week as previously described (Koch *et al*. 2013). Maximal running capacity was tested before and after the training period to determine the phenotype for response to aerobic training. Following the aerobic training period, the rats were randomly divided to resistance training (HRT-RES, n=6; LRT-RES, n=6) or sedentary (HRT-CONT, n=6; LRT-CONT, n=6) control groups. One month after completion of the aerobic exercise training, the RES group was subjected to 6-week resistance training while the CONT groups were not subjected to any physical exercise and spent the entire time in their home cage (Tecniplast 1354, Italy; size: 595mm× 380mm× 200 mm).

All animals were single housed and had free access to tap water and standard pelleted rodent food (R36; Lantmännen, Kimstad, Sweden). Room temperature and humidity were maintained at 21 ± 2°C and 50 ± 10%, respectively. Body mass and chow consumption was monitored weekly. During the entire resistance training intervention, the average daily chow consumption normalized to body mass was significantly greater ($p < 0.05$) in LRT-CONT (0.0505 (0.0038) g/g) compared to HRT-RES (0.0412 (0.0043) g/g). The rats were maintained on a 12 h–12 h light–dark cycle, with lights on at 08.00 h. All procedures were conducted during the light portion of the cycle.

Resistance training

The 6-week resistance training (RES) protocol was a modification of previous study by Hornberger & Farrar (2004). The HRT and LRT rats of RES group were familiarized with a custom-made vertical ladder (height × width: 90 cm × 15 cm, 2 cm separation between steps, 85 degrees incline) on three occasions during the first week. On the first day, the rats climbed without an extra load. On the next 2 days, a load pouch containing lead weights

corresponding to <50% of the rat's body weight was fixed to the proximal part of the tail with double-sided tape and a Velcro strap. One rat in LRT and one in HRT group refused to climb acceptably during the familiarization and were excluded from the study. Next, the rats (LRT, n=5; HRT, n=5) began a progressive RES three times a week (Monday, Wednesday and Friday). The first load was 75% of the body weight of a rat and thereafter the load was increased in 30 g increments for each climb until the rat could no longer reach the top of the ladder. The highest load the rat successfully carried to the top of the ladder was considered as the maximal carrying capacity for that session. Subsequent training sessions consisted of nine trials. During the first three climbs, 50, 75 and 90% of the previous maximal load was used. Then the load was increased by 30 g until a new maximal load was reached. Three trials were then attempted with this new maximal load. Between the climbing trials, the rats were allowed to rest for 90 s in an open chamber (length × width × height: 30 cm × 15 cm × 11 cm) located at the top of the ladder. Note that the rats were not punished or rewarded to motivate them to climb; only occasionally a gentle push to the backside of the rat was applied to start the climb.

We found that with very high loads rats mostly refused to climb from the bottom of the ladder and started to climb down to the floor. Therefore, the rats were placed to the higher position on the ladder whereof the rats started to climb to the top due to their inquisitive nature. Thus, the actual climbing height was approximately 60 cm and the rats performed approximately 5-6 repetitive muscle actions per limb in one climb before reaching the top of the ladder. Due to voluntary nature of the present training method, rats refused to exercise once or twice out of 18 sessions during the 6-week RES period.

Body composition

Whole-body DXA scans (LUNAR Prodigy, GE Medical systems, WI, U.S.) were performed before and after the RES intervention. The rats were anesthetized in an induction chamber with 3-4% isoflurane (Isoba vet., Intervet/Shering-Plough, Uxbridge, UK). Anesthetized rats were placed on the centerline of scanning bed in the prone position. Throughout the measurement, anesthesia was maintained by a gas inhalation through a facemask continuously supplied with 1-2% isoflurane. The facemask was connected to open-circuit gas anesthesia equipment (Harvard Apparatus with MSS-Vaporizer, Kent, UK). Before the measurements, calibration of DXA scanning equipment was done according to the manufacturer's guidelines. The small-animal mode of the enCORE software (GE Healthcare, v. 14.10.022) was used to obtain fat and lean mass content in total body. Moreover, lean mass of the right leg was determined by manually adjusting cut positions for region of interest (ROI) within the area encompassing the thigh and shank muscles.

Acute loading by muscle stimulation procedure

To induce equal loading to muscle tissue for each rat in HRT-RES and LRT-RES, muscle twitches were elicited through electrical stimulation of triceps surae muscle complex 3 - 4 days following the last RES session. The measurement setup was modified from the protocol designed by Torvinen *et al*. (2012) that stimulates specifically the gastrocnemius muscle. The rats were anesthetized (as in DXA measurements), and placed in a custom-built dynamometer designed for non-invasive functional investigation of the right triceps surae muscle. The dynamometer allowed isometric and dynamic measurements in which range and rate of movement can be adjusted. The dynamometer had a built-in strain-gauge sensor and two transcutaneous electrodes to elicit and measure twitch responses under isometric or dynamic conditions. The right lower hindlimb was shaved and conductive electrolyte gel was applied at the area of attachment of electrodes. The foot was positioned and fixed on the pedal and isometric force was measured at 90 degrees of knee and ankle angle.

Isometric maximal twitch (i.e. recruitment curve) was elicited through double twitch (DT) technique (electrical stimulation length 1ms, interval 10ms) with a rest period between trials of 30-45s. Intensity was increased with 1mA steps until maximal DT intensity was reached. The force signal from the strain-gauge sensor was amplified, converted to digital signals by a 32-bit analog to digital converter (Power 1401, CED Ltd., Cambridge, U.K.), and processed using dedicated software (Signal software, CED Ltd.). Maximal torque and maximal rate of torque development were analyzed. The dynamic stimulation trial was utilized with 30% of maximal DT intensity by the single twitch technique (stimulus length 1ms with 100Hz) with 60 - 120 degrees of ankle angle movement. Stimulation was applied 20s continuously per set of 10 repetitions (eccentric 1s - concentric 1s). Three sets were performed with 1min rest period between each set. Force and movement of the footpad (angle) were analyzed throughout the stimulation period. Isometric maximal twitch (1ms stimulus, 100Hz for 1s) was applied immediately after each set of dynamic contractions to examine acute fatigue. Following the entire loading protocol, maximal isometric torque decreased to 24 ± 6 % and 25 ± 8 %, and maximal rate of torque development decreased to 36 ± 16 % and 31 ± 16 % from the pre-loading level in HRT-RES and LRT-RES, respectively, with no statistically significant differences between the groups. Immediately after the stimulation test, the rats were anesthetized by exposure to CO_2, the thoracic cavity was opened, and death was verified by cardiac puncture in the right ventricle.

Blood count

Blood samples were collected into K-EDTA tubes via cardiac puncture at necropsy. The blood samples were immediately analyzed using an automated KoneLab device (Thermo Scientific, Vantaa, Finland) for the content of white blood cells (WBC), content of red blood cells (RBC), concentration of hemoglobin (HGB), hematocrit (HCT), mean red cell volume (MCV), mean cell hemoglobin content (MCH), content of platelets (PLT), relative content of

lymphocytes (LYMPH), absolute and relative content of the mixture of monocytes, basophils, and eosinophils (MXD), absolute and relative content of neutrophils (NEUT), and red cell distribution width (RDW_CV).

Muscle tissue Processing

At necropsy, selected hind limb (gastrocnemius, soleus, plantaris, flexor hallucis longus, extensor digitorum longus and quadriceps femoris) and forelimb (triceps, biceps) muscles were immediately removed, weighed, and frozen in liquid nitrogen. The muscle weights are reported as average weights of the left and right side. For immunohistochemistry, the proximal part of right gastrocnemius muscle was mounted in an O.C.T. embedding medium (Tissue Tek, Sakura Finetek Europe) with vertical orientation of muscle fibers and snap-frozen in isopentane cooled with liquid nitrogen. The remaining part of gastrocnemius and the other muscle samples were snap-frozen in liquid nitrogen and stored at -80 °C for further analysis.

Muscle immunohistochemistry

Cross-sections (8 µm) were cut on a cryostat microtome (Leica CM3000, Leica Biosystems, Nussloch GmbH, Germany) at -24°C. Cross-sections were immunohistochemically stained with dystrophin antibody (1:660, ab15277, rabbit polyclonal, Abcam, Cambridge, UK or 1:200, NCL-DYS2, mouse monoclonal, Novocastra, Leica Biosystems, Nussloch, Germany) for visualization of borders of muscle fibers, combined with slow myosin heavy chain antibody for counting the type 1 fiber proportion (1:100, BA-F8, mouse monoclonal, Developmental Studies Hybridoma Bank).

All dilutions were made in PBS. Sections were washed for 5 minutes in PBS, permeabilized in 0.2% Triton X-100 (Sigma-Aldrich, St. Louis, Missouri, USA) for 10 minutes, blocked with 5% goat serum (Gibco, Thermo Fisher Scientific Inc.) for 30 minutes at room

234 temperature and incubated overnight with primary antibody dilution in 1% goat serum at 4
235 C°. After washing the slides for 10 minutes in PBS the sections were incubated for 60
236 minutes in dark with Alexa Fluor® 488 or 555 goat anti—mouse IgG and goat anti-rabbit
237 IgG secondary antibody (Molecular Probes, Thermo Fisher Scientific Inc.) diluted 1:233 in
238 1% PBS at room temperature. After washing the fluorochrome-stained sections for 10
239 minutes in PBS, the slides were mounted and nuclei were stained with ProLong® Diamond
240 Antifade Mountant with DAPI mounting medium (P36971, Life Technologies).

241 Sections were color imaged with an UPlanFI 10x/0.30 objective, mounted on an Olympus
242 BX-50 fluorescent microscope (Olympus, Japan), using a ColoView III camera and AnalySIS
243 software (Soft Imaging Systems GmbH, Germany). The average fiber number in randomly
244 selected fields of high quality was 1284 ± 634 fibers per section. Fiber size, distribution of
245 Type I and Type II fibers, and a number of nuclei per fiber were analyzed using ImageJ (U.S.
246 National Institutes of Health, Bethesda, MD, USA) (NIH) and Matlab (The MathWorks, Inc.,
247 Natick, Massachusetts, US). Since only 0.52 % of all the fibers were identified as Type I, the
248 results are presented as Type I and II fibers combined. One sample of HRT-RES group was
249 excluded from the analyses due to low sample quality.

250 **Western immunoblot analyses**

251 The part (~50 mg) of the lateral portion of the right gastrocnemius muscle was hand-
252 homogenized in ice-cold buffer with proper inhibitors: 20 mM HEPES (pH 7.4), 1 mM
253 EDTA, 5 mM EGTA, 10 mM MgCl2, 100 mM b-glycerophosphate, 1 mM Na3VO4, 2 mM
254 DTT, 1 % Triton X-100, 0.2 % sodium deoxycholate, 30 mg/mL leupeptin, 30 mg/mL
255 aprotinin, 60 mg/mL PMSF, and 1 % phosphatase inhibitor cocktail (P 2850; Sigma, St
256 Louis, Missouri, USA). Total protein content was determined using the bicinchoninic acid

257 protein assay (Pierce Biotechnology, Rockford, IL) with KoneLab device (Thermo Scientific,
258 Vantaa, Finland).

259 Muscle homogenates containing 50 μg of protein were solubilized in Laemmli sample buffer
260 and heated at 95°C to denature proteins. Proteins were separated by SDS-Page using 4–20%
261 Criterion gradient gels (Bio-Rad Laboratories, Richmond, CA) and transferred to
262 nitrocellulose membranes. The uniformity of the protein loading was confirmed by staining
263 the membrane with Ponceau S. After blocking (Odyssey Blocking Buffer (PBS), LI-COR
264 Biosciences, Lincoln, NE, USA), the membranes were probed overnight at 4°C with
265 following primary antibodies (Rabbit IgG) to determinate differences in phosphorylation
266 status between HRT-RES and LRT-RES: mTOR (Ser2448), AS160 (Thr642), PKCζ/λ
267 (Thr410/403), p70S6K (Thr389), AMPKα (Thr172), Akt1 (Ser473), Smad3 (Ser423/425),
268 Smad2 (Ser245/250/255), p38 MAPK (Thr180/Tyr182), p44/42 MAPK (Erk1/2)
269 (Thr202/Tyr204), S6 Ribosomal Protein (Ser240/244), 4E-BP1 (Thr37/46), SAPK/JNK
270 (Thr183/Tyr185), PLD1 (Thr147), FAK (Tyr576/577) and CaMKII (Thr286) (all diluted at a
271 ratio 1:1000). Also α-Tubulin (Mouse IgG) was analyzed as a loading control (1:3000
272 dilution). Odyssey anti-rabbit IRDye 800CW and anti-mouse IRDye 680RD (LI-COR
273 Biosciences, Lincoln, NE, USA) were used as secondary antibodies (1:15000 dilution). The
274 blots were scanned and quantified by using Odyssey CLX Infrared Imager of Li-COR and
275 manufacturer's software. If re-probing was needed, the membranes were incubated for 10 min
276 in 0.2MNaOH at RT, washed with TBS and re-probed with appropriate antibodies.
277 Immunoblots of PLD1 (Thr147) and FAK (Tyr576/577) were too faint to be analyzed. All
278 samples and results were normalized to α-Tubulin and Ponceau S. All antibodies were
279 purchased from Cell Signaling Technology (Danvers, MA, USA).

280 **Muscle fractionation and determination of protein bound alanine enrichment**

Myofibrillar, sarcoplasmic, collagen and mitochondrial protein synthesis rates were measured from the left quadriceps muscle by deuterium oxide (D_2O) method (Brook *et al*. 2017). From the control rats, five HRT-CONT and four LRT-CONT were included to the experiment. At the start of the RES experiment, the rats were provided with 7.2ml/kg D_2O (i.g.). Thereafter, animals were provided with free access to drinking water enriched with 2% (v/v) of D_2O throughout the RES period. Muscle myofibrillar, collagen, sarcoplasmic and mitochondrial proteins were extracted by homogenizing ~50 mg of muscle in ice-cold homogenization buffer pH7.5 (Tris-HCL 50mM, EDTA 1mM, EGTA 1mM, β-glycerophosphate 10mM, NaF 50mM) containing a protease inhibitor tablet (Roche) and sodium orthavanadate 0.5mM, rotated for 10 min. The supernatant containing sarcoplasmic proteins was collected after centrifugation at 13,000 g for 5 min. After washing, the remaining pellet was dounce homogenized in mitochondrial extraction buffer (MOPS 20mM, KCl 110mM and EGTA 1mM) and centrifuged at 1000 g for 5 min to pellet myofibrillar and collagen fractions. The supernatant containing mitochondria was removed and pelleted by centrifugation at 17,000 g. Myofibrillar proteins were extracted from myofibrillar and collagen fractions by solubilizing in 0.3M NaOH and separated from the insoluble collagen by centrifugation, with myofibrillar proteins precipitated using 1 M perchloric acid (PCA). Myofibrillar, collagen, mitochondrial and sarcoplasmic protein-bound AA were released using acid hydrolysis by incubating in 0.1M HCl in Dowex H+ resin slurry overnight before being washed and eluted from the resin with 2M NH4OH and evaporated to dryness. Resulting AA were derivatized to their N-methoxycarbonyl methyl esters and alanine enrichment determined by gas chromatography tandem mass spectrometry (TSQ 8000 Thermo Finnigan, Thermo Scientific, Hemel Hempstead, UK) alongside a standard curve of known DL-Alanine-2,3,3,3-d4 enrichment to validate measurement accuracy of the machine.

Body water enrichment and determination of fractional synthetic rate

306 Body water enrichment was determined from blood samples collected at necropsy and used
307 to represent the average enrichment throughout. 100 µl of plasma was incubated with 2 µl of
308 10 M NaOH and 1 µl of acetone for 24 h at room temperature. Following incubation, the
309 acetone was extracted into 200 µl of n-heptane, and 0.5 µl of the heptane phase was injected
310 into the GC-MS/MS for analysis. A standard curve of known D_2O enrichment was run
311 alongside the samples for calculation of enrichment. Fractional synthetic rate (FSR) was
312 calculated from the incorporation of deuterium-labeled alanine into protein, using the
313 enrichment of body water [corrected for the mean number of deuterium moieties incorporated
314 per alanine (3.7)] as the surrogate precursor labeling. The equation used was
315

$$FSR = -Ln\left(\frac{1 - \left[\frac{(APEala)}{(APEp)}\right]}{t}\right)$$

316

317 where APEala equals deuterium enrichment of protein-bound alanine, APEp indicates mean
318 precursor enrichment over the time period, and t is time.

319 **Skeletal muscle protein, DNA, and RNA concentrations**

320 To determine muscle protein, DNA, and RNA concentrations (i.e. translational
321 efficiency/capacity), ~15 mg of the medial portion of the right gastrocnemius muscle tissue
322 of HRT-RES and LRT-RES was pulverized in liquid nitrogen and homogenized in 1ml 0.2 M
323 PCA. After centrifugation at 4^{o}C at 11,000 rpm for 8 min to form a pellet and washing with
324 1ml 0.2M PCA (washing repeated twice), the pellet was resuspended in 800µl 0.3M NaOH,
325 and incubated at 37°C for 2 x 20 min to dissolve the pellet. The samples were gently vortexed
326 before, in between and after the incubations. Total protein concentration was analyzed as
327 described above (see western immunoblot analyses). Thereafter, proteins were precipitated

with 400µl 1M PCA before centrifugation at 4°C at 5'000 rpm for 5 min. Next, 300µl 0.2M PCA was added to supernatant of each sample and centrifuged at 4°C at 5'000 rpm for 5 min before removal of the supernatant for quantification of RNA by NanoDrop Lite Spectrophotometer (Thermo Scientific). The remaining pellet was resuspended in 1ml 2M PCA and incubated at 70°C for 1 h before centrifugation at 4°C at 5'000 rpm for 5 min. Next, 300µl 2M PCA was added to supernatant of each sample and centrifuged at 4°C at 5'000 rpm for 5 min before removal of the supernatant for quantification of DNA by NanoDrop Lite Spectrophotometer (Thermo Scientific).

Muscle Fibre Contractility

Female HRT (n = 6) and LRT (n = 6) rats of 13^{th} generation were previously used to study the contractility of permeabilized muscle fibres following 8-week of phenotyping aerobic training (i.e. age of ~8-months) as previously described (Mendias *et al*. 2011; Mendias *et al*. 2015). Briefly, bundles of fibres that were approximately 5 mm in length and 0.5 mm in diameter were dissected from the deep aspect of the tibialis anterior muscle. Bundles were then placed in skinning solution for 30 min to permeabilize sarcolemmal membranes, and then in storage solution for 16 h at 4°C. Bundles were then stored at -80°C. On the day of fibre contractility testing, bundles were thawed on ice, and individual fibres were plucked from bundles using fine mirror-finished forceps. Fibres were then placed in a chamber containing relaxing solution and secured at one end to a servomotor (Aurora Scientific) and the other end to a force transducer (Aurora Scientific) using two ties of 10-0 monofilament nylon suture at each fibre end. The length of the fibre was adjusted to obtain a sarcomere length of 2.5µm, as assessed with a laser diffraction measurement system. The average fibre CSA was calculated assuming an elliptical cross-section, with diameters measured at five positions along the fibre from high-magnification images at two different views (top and side). Maximum fibre isometric force (F_o) was elicited by submerging the fibre in a solution

353 containing a super-physiological concentration of calcium. Specific force of fibres (sF_o) was
354 determined by dividing F_o by fibre CSA. Fibres were categorized as fast or slow by
355 examining their force response to rapid, constant-velocity shortening contraction. Ten fast
356 fibres were tested from each tibialis anterior muscle from both groups.

357 **Statistics**

358 Statistical analyses were carried out using IBM SPSS Statistics version 24 software (SPSS
359 Inc., Chicago, IL, USA). The non-parametric tests were used since each experimental group
360 consisted of a low number of rats. For pairwise comparisons, a Mann-Whitney U Test was
361 used to evaluate differences between the groups and a Wilcoxon Signed-Ranks Test was used
362 to evaluate changes within the groups. A Friedman Test was applied for repeated measures
363 within the groups and a Kruskal-Wallis H Test for comparisons between the multiple groups.
364 Post hoc analysis was conducted with a Bonferroni correction. The Spearman's rank
365 correlation coefficient was utilized to examine associations between the variables. $p \leq 0.05$
366 was considered as statistically significant. The data are expressed as mean and standard
367 deviation (SD).

368

369 **3. RESULTS**

370 Before the RES intervention, the adaptive response in running capacity to 8-week aerobic
371 training period was 7 (5) % (Z=-2.023, p=0.043) in the LRT-RES (n=5) while in the HRT-
372 RES (n=5) the response of 30 (16) % (Z=-2.023, p=0.043) was significantly greater (U=0.0,
373 p=0.009).

374 Following the 6-week RES intervention, the maximal extra weight carried up during a single
375 climb was significantly greater in HRT compared to LRT (U=1.0, p=0.016); the maximal

carried load normalized to the total body mass of the animal was 3.27 (0.43) in HRT and 2.49 (0.25) in LRT (Figure 1).

Insert Figure 1 here

Selected observations of RES intervention: Determined by DXA, total body fat increased in all groups except in HRT-RES during the intervention (Table 1). The Pre-values or changes during the intervention in the total body fat mass, total body lean mass or leg lean mass did not differ between the groups. When data of HRT-RES and LRT-RES were combined and compared with the combined data of HRT-CONT and LRT-CONT, changes during the RES period in the total body lean mass determined by DXA were greater in sedentary controls than resistance trained rats ($U=98.0$, $p=0.011$). Following the intervention, soleus muscle wet tissue weight related to body weight was smaller in HRT-RES than in HRT-CONT ($X^2(3)=8.134$, $p=0.049$) (Table 2). Other statistically significant and relevant associations, changes within the experimental groups or differences between the groups were not observed in body composition, blood analyses (Table 3), skeletal muscle protein, DNA or RNA concentrations, immunohistochemical and immunoblot analyses (Table 4) or protein synthesis investigated in this study.

Insert Tables 1 - 4 here

Insert Figure 2 here beside the table 2

For permeabilized muscle fibre contractility experiments, there was no difference in fibre CSA (6776 ± 860 vs. 7303 ± 1038 μm^2, $U=12.0$, $p=0.394$), F_o (0.75 ± 0.11 vs. 0.77 ± 0.07 mN, $U=14.0$, $p=0.589$), or sF_o (111.0 ± 14.1 vs. 106.8 ± 17.4 kPa, $U=23.0$, $p=0.485$) in HRT and LRT rats, respectively.

4. DISCUSSION

In the present study, ladder climbing resistance training induced only minimal physiological responses in male HRT and LRT rats in comparison with their non-trained counterparts, whether compared by groups separately or by HRT and LRT groups combined. While muscular strength (i.e. load carrying capacity) in both HRT and LRT ladder climbing groups improved remarkably, morphological adaptations in skeletal muscles were absent.

An open scientific question is whether phenotypic traits associated with responsiveness to one mode of training can be extrapolated to other exercise-inducible phenotypes (i.e. intra-individual variability). The present HRT/LRT rat model has been developed by divergent artificial selective breeding for low and high adaptation response to aerobic exercise training in a genetically heterogeneous stock of rats. The underlying theory is that a set of modifier genes, which cause the variation in adaptation capacity and other phenotypic endpoints (such as cardiac output or oxygen utilization within exercising skeletal muscle), will segregate with adaptation for oxidative capacity in the LRT and HRT rats (Koch *et al*. 2013).

Interestingly, significant difference occurred between HRT and LRT in RES-induced strength gains in the present study. This finding suggest that individual responsiveness to aerobic and resistance exercise training are somewhat similar, at least with regards to running capacity and strength gains, respectively. In previous observations, single muscle fibre contractility in vitro did not differ between HRT and LRT rats indicating that other factors than intrinsic muscle fibre contractile characteristics explain the training adaptations. However, no differences were observed between the trained HRT and LRT rats in any muscular or systemic level variable measured in this study.

In previous studies using a ladder-climbing model for resistance training (unconditioned male or female Sprague Dawley, Wistar or Fisher 344 rats of different ages), the muscle

423 hypertrophy has been observed in some studies in muscle weight (~11-23%) (Duncan *et al*.
424 1998; Gil & Kim 2015; Harris *et al*. 2010; Hornberger & Farrar 2004; Jung *et al*. 2015; Lee
425 *et al*. 2004; Lee *et al*. 2016; Luciano *et al*. 2017; Molanouri Shamsi *et al*. 2016) or muscle
426 fiber CSA (~20-88%) (Begue *et al*. 2013; Cassilhas *et al*. 2012; Jung *et al*. 2015; Peixinho-
427 Pena *et al*. 2012; Prestes *et al*. 2012) but in some studies hypertrophy has been absent (de
428 Sousa Neto *et al*. 2017; Deschenes *et al*. 2000; Deschenes *et al*. 2015; Kim *et al*. 2012;
429 Neves *et al*. 2016; Safarzade & Talebi-Garakani 2014; Souza *et al*. 2014) when compared to
430 non-training controls. The high variation between the studies in loading protocols and
431 examined skeletal muscles prevents conclusions of muscle-specific dose-response
432 relationship to RES. Nevertheless, it could be speculated that ladder climbing RES in rats
433 requires relatively high training volume, and consequently lower intensity (i.e. extra carrying
434 load), in order to induce statistically significant morphological changes in most of the trained
435 muscles.

436 In the present study, we focused on resistance training responses especially in
437 m.gastrocnemius since it was electrically stimulated before collecting the skeletal muscle
438 samples and it is a commonly studied muscle for endurance training adaptations. Because we
439 examined in detail only few selected skeletal muscles, the training responses in other muscles
440 cannot be verified. However, when considering the findings of the previous studies and the
441 present experiment, muscle size responses may explain only marginally strength gains in
442 ladder climbing RES model. Thus, neural responses (i.e. motor learning) may be important
443 mechanism in training adaptations. We have previously studied adult hippocampal
444 neurogenesis (AHN) with the present rats and, unlike in aerobic training, we found no effect
445 of RES on AHN (Nokia *et al*. 2016). Therefore, ladder climbing RES seem to include other
446 adaptations in central nervous system than AHN.

447 The ladder climbing RES model with rats, or mice, has several strengths. The training is

448 relatively stress free to animals and allows precise monitoring of the loading parameters (i.e.
449 external load, number of climbs, climbing distance and duration, and recovery periods) and
450 records of performance throughout the intervention. However, there are also some limitations
451 that should be taken into consideration. The climbing consist of mainly concentric muscle
452 actions and the lacking of eccentric component may hinder muscle hypertrophy. The
453 climbing training can be considered a whole body workout but muscle mass gains appears to
454 be typically modest and localized only in a few loaded muscles. Therefore, adaptations in
455 body composition will be minor and e.g. investigations of health benefits of training in
456 systemic level may be challenging, as also indicated by the data of the present study.
457 Nevertheless, the ladder climbing RES model, when carried out with the present loading
458 protocol, is applicable to induce great maximal strength adaptations while hypertrophy is
459 minor or non-existing. Thus, the present RES model allows studying gains in muscle function
460 (i.e. strength) without significant hypertrophic response in skeletal muscles.

461 Although the present HRT rats got stronger by the RES, drawing conclusions of the present
462 findings to the physical training outcomes in humans should be done with caution. Changes
463 in strength following RES may be induced by both morphological adaptations in skeletal
464 muscles and neural factors (Balshaw *et al*. 2017). The mechanisms underpinning individual
465 variation in neural responses to RES are largely unknown but some physiological factors
466 have been identified to be associated with individual RES-induced changes in skeletal muscle
467 size in humans, such as ribosome biogenesis (Mobley *et al*. 2018; Stec *et al*. 2016), activity
468 of growth and remodelling related genes (Bamman *et al*. 2007; Davidsen *et al*. 2011;
469 Thalacker-Mercer *et al*. 2013), satellite cell activity (Petrella *et al*. 2008), and activation of
470 signaling pathways regulating protein synthesis (Mayhew *et al*. 2011; Mitchell *et al*. 2013).
471 Although not verified by research, it is likely that these factors are specific to RES-induced
472 skeletal muscle adaptations while aerobic training adaptations might be driven predominantly

473 by other determinants, such as cardiorespiratory function. Thus, it could be suggested that
474 skeletal muscle adaptations associated with responsiveness to one mode of training may not
475 be entirely extrapolated to other exercise modes but further research in this area is warranted.

476 In conclusion, HRT rats were capable to carry heavier loads in ladder climbing when
477 compared to LRT rats, which is in line with their responsiveness to aerobic training.
478 However, muscular adaptations did not differ between the HRT and LRT rats in the present
479 study indicating that other factors than studied here, for example neural system adaptations,
480 may explain their divergent adaptations of muscular strength to the present RES.

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CONFLICT OF INTEREST

No conflicts of interest, financial or otherwise, are declared by the authors.

504

505 **AUTHOR CONTRIBUTIONS**

506 J.P.A., S.L., C.L.M., P.J.A., L.G.K., S.L.B. and H.K. conceived and designed research;
507 J.P.A., S.L., I.L. and C.L.M. performed experiments; J.P.A., S.L., I.L., M.S., J.K.I., V.F.,
508 C.L.M., M.S.B., K.S. and P.J.A. analyzed data; J.P.A., S.L., V.F., C.L.M., M.S.B., K.S.,
509 P.J.A., L.G.K., S.L.B., and H.K. interpreted results of experiments; J.P.A. prepared figures;
510 J.P.A. drafted manuscript; S.L., I.L., M.S., J.K.I., V.F., C.L.M., M.S.B., P.J.A., L.G.K.,
511 S.L.B. and H.K. edited and revised manuscript; All authors approved final version of
512 manuscript.

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676

677 **Table 1. Body mass and total body fat and lean mass and right leg lean mass determined by**
678 **DXA before and after the 6-week ladder climbing resistance training (RES) or control (CONT)**
679 **period in high (HRT) and low (LRT) responder rats to aerobic exercise training. Values are**
680 **expressed as mean (SD). * Statistically significant (p≤0.05) change from the Pre-measurement**
681 **within the group.**

		HRT-RES	LRT-RES	HRT-CONT	LRT-CONT	*P*-value
Body mass (g)	Pre	382.8 (28.8)	407.2 (48.7)	378.5 (38.3)	386.5 (39.1)	0.658
	Post	376.6 (33.0)	394.0 (39.5)	410.5 (40.6)	419.7 (43.1)	
Fat mass (g)	Pre	71.6 (21.7)	66.2 (12.9)	67.5 (18.7)	67.7 (8.0)	0.941
	Post	90.2 (33.0)	81.6 (16.5)*	93.3 (20.2)*	96.5 (17.3)*	
Lean mass (g)	Pre	281.4 (37.7)	298.0 (43.5)	272.2 (32.9)	281.7 (35.8)	0.690
	Post	248.4 (29.5)	271.4 (22.4)	278.0 (29.3)	284.5 (50.1)	
Leg lean mass (g)	Pre	25.2 (4.3)	24.0 (2.8)	22.2 (3.3)	24.2 (2.8)	0.721
	Post	22.8 (5.1)	23.2 (2.3)	22.8 (3.7)	24.7 (4.5)	

682

683 **Table 2. Muscle wet tissue weight related to body weight (mean of right and left side), type I and**
684 **II cross-sectional areas (CSA), nuclei per cell, and muscle protein, DNA and RNA**
685 **concentrations in right gastrocnemius muscle, and fractional synthetic rate of muscle proteins in**
686 **left quadriceps muscle following 6-week ladder climbing resistance training (RES) or control**
687 **(CONT) period in high (HRT) and low (LRT) responder rats to aerobic exercise training.**
688 **Values are expressed as mean (SD). * Statistically significant (p≤0.05) difference compared to**
689 **the HRT-RES group.**

	HRT-RES	LRT-RES	HRT-CONT	LRT-CONT	*P*-value
Muscle wet tissue weight (mg/g)					
Gastrocnemius	4.39 (0.45)	4.82 (0.71)	5.03 (0.56)	4.96 (0.70)	0.426
Soleus	0.34 (0.03)	0.42 (0.04)	0.40 (0.05)	0.44 (0.07)*	0.043
Plantaris	0.94 (0.11)	1.02 (0.12)	0.98 (0.11)	0.97 (0.14)	0.666
FHL	1.37 (0.19)	1.49 (0.14)	1.51 (0.25)	1.46 (0.18)	0.694
MQF	7.74 (0.78)	8.20 (0.75)	8.61 (0.98)	8.78 (1.16)	0.239
EDL	0.42 (0.02)	0.46 (0.06)	0.45 (0.05)	0.45 (0.06)	0.606
Triceps	3.53 (0.39)	4.09 (0.25)	4.20 (0.49)	4.01 (0.61)	0.117
Biceps	0.66 (0.05)	0.68 (0.08)	0.69 (0.11)	0.64 (0.11)	0.810
Muscle fiber characteristics					
Type I & II CSA (μm^2)	4427 (494)	3988 (594)	4536 (519)	4045 (982)	0.254
Nuclei per cell	3.0 (0.9)	3.0 (0.5)	3.2 (0.7)	4.2 (1.6)	0.491
Muscle protein synthesis rate (%/day)					
Myofibrillar	2.6 (0.4)	2.6 (0.3)	2.7 (0.9)	3.2 (0.7)	0.556
Sarcoplasmic	3.8 (0.5)	3.5 (0.3)	3.4 (0.8)	3.5 (1.1)	0.540
Mitochondrial	3.1 (0.6)	2.8 (0.5)	2.7 (0.8)	2.9 (0.5)	0.927
Collagen	1.1 (0.4)	1.1 (0.7)	1.1 (0.5)	1.1 (0.7)	0,996
Muscle protein, DNA, and RNA concentrations					
Protein (μg/mg)	217.0 (38.2)	177.7 (38.3)			0.175
RNA (μg/mg)	1.53 (0.39)	1.45 (0.32)			0.602
DNA (μg/mg)	2.08 (0.30)	2.00 (0.33)			0.602

690 FHL, flexor hallucis longus; MQF, quadriceps femoris; EDL, extensor digitorum longus;
691 CSA, cross-sectional area

692 **Table 3. Hematological values (Mean, SD) following 6-week ladder climbing resistance training**
693 **(RES) or control (CONT) period in high (HRT) and low (LRT) responder rats to aerobic**
694 **exercise training**

	HRT-RES	LRT-RES	HRT-CONT	LRT-CONT	*P*-value
WBC (x 10^9/l)	8.8 (2.2)	6.5 (1.0)	12.2 (1.4) *	9.5 (1.0)	0.005
RBC (x 10^{12}/l)	9.4 (0.3)	9.2 (0.6)	9.4 (0.3)	9.2 (0.6)	0.801
HGB (g/l)	156.3 (5.0)	152.6 (9.6)	154.4 (2.1)	154.2 (4.7)	0.586
HCT (%)	51.3 (2.2)	50.4 (3.4)	51.0 (1.0)	50.8 (2.2)	0.745
MCV (fl)	54.8 (1.3)	54.6 (0.5)	54.0 (1.4)	55.8 (1.5)	0.188
MCH (pg)	16.7 (0.2)	16.6 (0.1)	16.4 (0.6)	16.9 (0.7)	0.567
PLT (x 10^9/l)	673.8 (187.0)	829.0 (40.5)	761.6 (63.9)	774.6 (92.8)	0.286
LYMPH (%)	79.9 (9.4)	73.4 (6.6)	85.0 (5.9)	70.7 (12.2)	0.084
LYMPH (x 10^9/l)	7.1 (2.1)	4.8 (1.0)	10.3 (0.8) *	6.8 (1.7)	0.005
RDW_SD (fl)	30.9 (0.9)	31.1 (0.8)	31.9 (2.0)	31.7 (2.0)	0.967
RDW_CV (%)	16.7 (1.4)	16.8 (1.2)	17.9 (2.0)	16.6 (1.2)	0.595

695 WBC, white blood cell count; RBC, red blood cell count; HGB, concentration of hemoglobin; HCT,
696 hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; PLT, content of
697 platelets; LYMPH, content of lymphocytes; RDW, red cell distribution width. Content of the mixture
698 of monocytes, basophils, and eosinophils (MXD), and content of neutrophils (NEUT) were below the
699 limit of detection.
700 * Significantly greater compared to LRT-RES.
701

Table 4. Phosphorylation levels (Mean, SD) of selected signaling proteins in electrically stimulated m.gastrocnemius muscle following 6-week ladder climbing resistance training (RES) or control (CONT) period in high (HRT) and low (LRT) responder rats to aerobic exercise training

Signaling protein phosphorylation (au)	HRT-RES	LRT-RES	*P*-value
Akt1 (Ser473)	3.75 (1.91)	1.79 (1.48)	0.151
mTOR (Ser2448)	4.86 (1.14)	7.46 (4.86)	1.000
p70S6K (Thr389)	6.22 (2.33)	6.76 (4.81)	0.421
S6 Ribosomal Protein (Ser240/244)	1.26 (0.62)	1.33 (1.32)	0.421
4E-BP1 (Thr37/46)	7.37 (4.76)	36.80 (23.22)	0.841
p44/42 MAPK (Erk 1/2) (Thr202/Tyr204)	7.82 (4.87)	12.13 (7.31)	0.151
p38 MAPK (Thr180/Tyr182)	2.06 (1.23)	2.18 (1.03)	0.421
AMPKα (Thr172)	9.17 (3.03)	9.75 (3.61)	0.690
CaMKII (Thr286)	4.16 (2.73)	4.48 (1.77)	0.841
PKCζ/λ (Thr410/403)	1.75 (0.97)	3.88 (2.15)	0.151
AS160 (Thr642)	9.41 (36.49)	34.67 (28.53)	0.056
SAPK/JNK (Thr183/Tyr185)	2.36 (0.83)	2.07 (0.39)	0.841
Smad2 (Ser245/250/255)	3.32 (1.80)	2.08 (1.23)	0.690
Smad3 (Ser423/425)	1.04 (0.78)	1.29 (0.40)	0.548

Akt, AKT8 virus oncogene cellular homolog; mTOR, mechanistic target of rapamycin; S6K, Ribosomal protein S6 kinase; 4E-BP, eIF4E binding protein; MAPK, mitogen-activated protein kinase; AMPK, AMP-activated protein kinase; CaMK, Calcium/calmodulin-dependent kinase; PKCζ/λ, atypical protein kinase C zeta/lambda; AS160, Akt substrate of 160 kDa; SAPK/JNK, stress-activated protein kinase/Jun N-terminal kinase; Smad, contraction of Sma and Mad. Phosphorylation status of Phospholipase D1 (PLD1 Thr147) and Focal adhesion kinase (FAK Tyr576/577) were below the limits of detection.

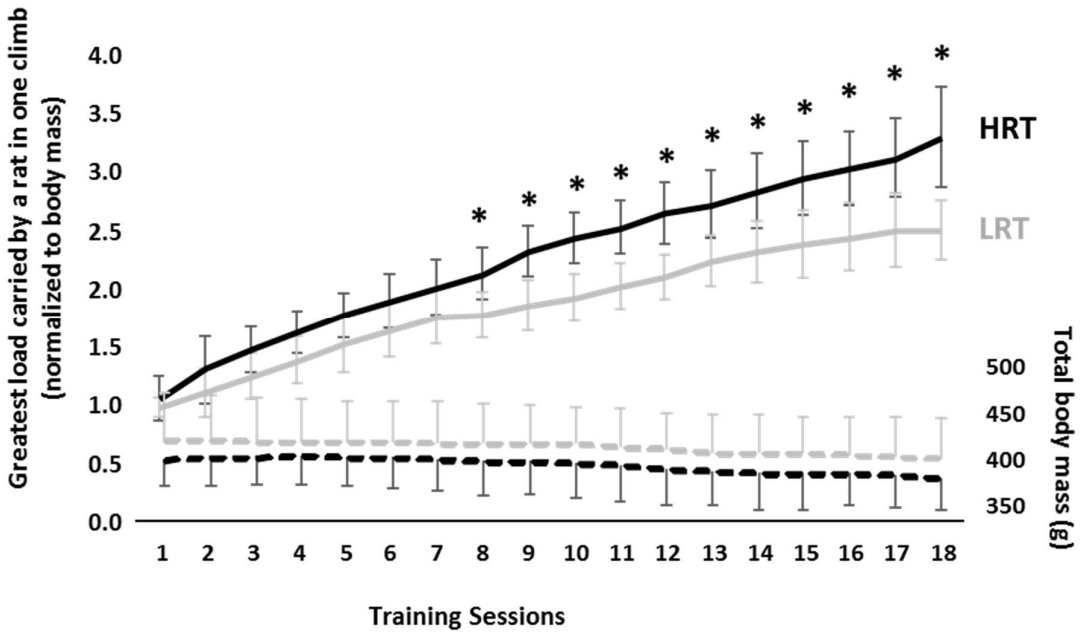
714 **FIGURE TEXTS**

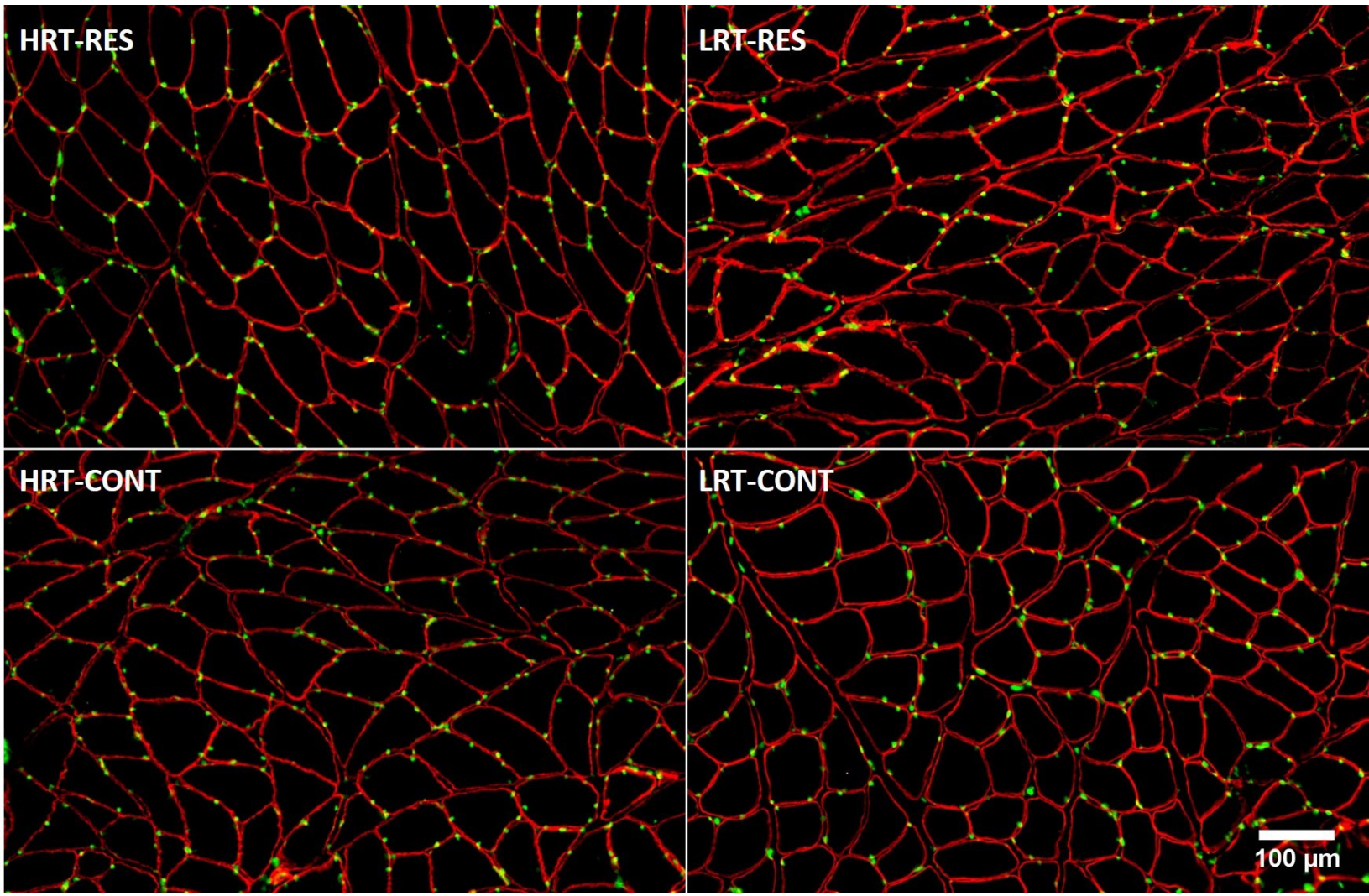
715 Figure 1. Maximal carrying capacity (solid lines) and body mass (dotted lines) per training
716 session. Values are expressed as mean (SD). Black lines, high responders to aerobic training
717 (HRT, n=5); Grey lines, low responders to aerobic training (LRT, n=5). * Statistically
718 significant ($p \leq 0.05$) differences between the groups.

719

720 Figure 2. The representative image of immunohistological analyses of muscle fiber cross-
721 sectional areas. All fibers presented in the image are type II fibers. Scale bar measures 100
722 μm. HRT, high responders to aerobic training; LRT, low responders to aerobic training; RES,
723 resistance trained rats; CONT, non-trained controls.

FIGURE 1


Greatest load carried by a rat in one climb (normalized to body mass)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HRT
LRT
500
450
400
350
Total body mass (g)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Training Sessions


HRT-RES
LRT-RES
HRT-CONT
LRT-CONT
100 µm