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Physiological adaptations to resistance training in rats selectively bred for low and high response to aerobic exercise training

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Abstract

The purpose of this study was to determine whether rats selectively bred for low and high response to aerobic exercise training co-segregate for differences in muscle adaptations to ladder-climbing 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 control animals. Before and after the 6 week intervention, body composition was determined by dual energy X-ray absorptiometry. Before tissue harvesting, the right triceps surae muscles were loaded by electrical stimulation. Muscle fibre cross-sectional areas, nuclei per cell, phosphorylation status of selected signalling proteins of mTOR and Smad pathways, and muscle protein, DNA and RNA concentrations were determined for the right gastrocnemius muscle. The daily protein synthesis rate was determined by the deuterium oxide method from the left quadriceps femoris muscle. Tissue weights of fore- and hindlimb muscles were measured. In response to resistance training, maximal carrying capacity was greater in HRT (~3.3 times 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. The lack of hypertrophic muscular adaptations despite considerable increases in muscular strength suggest that adaptations to the present ladder-climbing training in HRT and LRT rats were largely induced by neural adaptations.

KEYWORDS

fibre contractility, muscle hypertrophy, muscle stimulation, protein synthesis

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 with humans, animal RES models permit specific control of environmental conditions, while nutritional intakes can be regulated and monitored. Animal studies enable the harvesting of several tissue types and 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 used 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 deg 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 programmes in humans (Bouchard & Rankinen, 2001) and in animals (Koch, Pollott, & Britton, 2013). Likewise, large inter-individual variability has been observed in muscle strength and size gains after chronic RES in humans (Ahtiainen et al., 2016; Hubal et al., 2005). Although individuality in responses to RES is acknowledged, investigating the determinants of this phenomenon is gaining widespread popularity (Bamman, Petrella, Kim, Mayhew, & Cross, 2007; Davidsen et al., 2011; Mobley et al., 2018; Ogasawara et al., 2016; Thalacker-Mercer et al. 2013). However, whether the individual responsiveness to aerobic training and RES are similar between training modalities is largely unknown. We recently used ladder-climbing RES with rats selectively bred for high (HRT, high-response trainer) and low (LRT, low-response trainer) response to aerobic exercise training (Nokia et al., 2016) and observed a greater increase in strength (i.e. the maximal amount of weight the rats were able to carry) in the HRT compared with the LRT rats. Based on that observation, we hypothesized that HRT rats would demonstrate larger skeletal muscle adaptations to ladder-climbing RES compared with LRT rats.

New Findings

- **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 rats that are low responders to endurance training are also low responders to resistance training.
- **What is the main finding and its importance?**
  After resistance training, rats that are high responders to aerobic exercise training improved more in maximal strength compared with low-responder rats. However, the greater gain in strength in high-responder rats was not accompanied by muscle hypertrophy, suggesting that the responses observed could be mainly neural in origin.

2 | METHODS

2.1 | Ethical approval

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

2.2 | Animals

The animals used in the study were adult male LRT (n = 12) and HRT (n = 12) rats, representing the 18th generation of these rat lines developed by selective breeding (Koch et al., 2013). Upon arrival at the University of Jyväskylä, the rats were allowed to acclimate for 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. After the aerobic training period, the rats were randomly divided into resistance training (HRT-RES, n = 6; LRT-RES, n = 6) or sedentary control (HRT-CONT, n = 6; LRT-CONT, n = 6) groups. One month after completion of the aerobic exercise training, the RES group was subjected to 6 weeks of resistance training, whereas the CONT groups were not subjected to any physical exercise and spent the entire time in their home cage (Tecniplast 1354, Italy; size: 595 mm × 380 mm × 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 were monitored weekly. During the entire resistance training intervention, the average daily chow consumption normalized to body mass was significantly greater ($P \leq 0.05$) in LRT-CONT [mean (SD): 0.0505 (0.0038) g g^{-1}] compared with HRT-RES [0.0412 (0.0043) g g^{-1}]. 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.

2.3 | 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 the RES group were familiarized with a custom-made vertical ladder (height × width: 90 cm × 15 cm, with 2 cm separation between steps and 85 deg 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 the LRT and one in the 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 back-side 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 at the higher position on the ladder, whereupon the rats started to climb to the top owing to their inquisitive nature. Thus, the climbing height was ∼60 cm and the rats performed approximately five or six repetitive muscle actions per limb in one climb before reaching the top of the ladder. Owing 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.

2.4 | Body composition

Whole-body dual energy X-ray absorptiometry (DXA) scans (LUNAR Prodigy; GE Medical systems, Madison, WI, USA) were performed before and after the RES intervention. The rats were anaesthetized in an induction chamber with 3–4% isoflurane (Isoba vet.; Intervet/Shering-Plough, Uxbridge, UK). Anaesthetized rats were placed on the centreline of scanning bed in the prone position. Throughout the measurement, anaesthesia was maintained by gas inhalation through a facemask continuously supplied with 1–2% isoflurane. The facemask was connected to open-circuit gas anaesthesia 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 the region of interest within the area encompassing the thigh and shank muscles.

2.5 | Acute loading by muscle stimulation procedure

To induce equal loading to muscle tissue for each rat in HRT-RES and LRT-RES groups, muscle twitches were elicited through electrical stimulation of the triceps surae muscle complex 3–4 days after the last RES session. The measurement set-up was modified from the protocol designed by Torvinen et al. (2012) that stimulates the gastrocnemius muscle specifically. The rats were anaesthetized (as for 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 could be adjusted. The dynamometer had a built-in strain-gauge sensor and two transcutaneous electrodes to elicit and measure twitch responses in 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 deg of knee and ankle angle.

Isometric maximal twitch (i.e. recruitment curve) was elicited through double-twitch (DT) technique (electrical stimulation duration 1 ms, interval 10 ms), with a rest period between trials of 30–45 s. The inntensity was increased in 1 mA 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, UK) and processed using dedicated software (Signal software; CED Ltd). The maximal torque and maximal rate of torque development were analysed. The dynamic stimulation trial was used with 30% of maximal DT intensity by the single-twitch technique (stimulus duration 1 ms, with 100 Hz) with 60–120 deg of ankle angle movement. Stimulation was applied for 20 s continuously per set of 10 repetitions (eccentric 1 s–concentric 1 s). Three sets were performed with 1 min rest period between each set. Force and movement of the footpad (angle) were analysed throughout the stimulation period. Isometric maximal twitch (1 ms stimulus, 100 Hz for 1 s) was applied immediately after each set of dynamic contractions to examine acute fatigue. After 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 preloading level in HRT-RES and LRT-RES, respectively, with no statistically significant differences between the groups. Immediately after the stimulation test, the rats were rendered unconscious by exposure to CO_2, the thoracic cavity was opened, and death was verified by cardiac puncture of the right ventricle.

2.6 | Blood count

Blood samples were collected into K-EDTA tubes via cardiac puncture at the end of the experiment. The blood samples were analysed immediately using an automated KoneLab device (Thermo Scientific, Vantaa, Finland) for the content of white blood cells, content of red

blood cells, concentration of haemoglobin, haematocrit, mean red cell volume, mean cell haemoglobin content, content of platelets, relative content of lymphocytes, absolute and relative content of the mixture of monocytes, basophils and eosinophils, absolute and relative content of neutrophils and red cell distribution width.

2.7 | Muscle tissue processing

Post mortem, selected hindlimb (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 OCT embedding medium (Tissue Tek, Sakura Finetek Europe) with vertical orientation of muscle fibres 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.

2.8 | Muscle immunohistochemistry

Cross-sections (8 μm) were cut on a cryostat microtome (Leica CM3000; Leica Biosystems, Nußloch, 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) for visualization of borders of muscle fibres, combined with slow myosin heavy chain antibody for counting the proportion of type I fibres (1:100, BA-F8, mouse monoclonal, Developmental Studies Hybridoma Bank).

All dilutions were made in PBS. Sections were washed for 5 min in PBS, permeabilized in 0.2% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA) for 10 min, blocked with 5% goat serum (Gibco, Thermo Fisher Scientific Inc.) for 30 min at room temperature and incubated overnight with primary antibody dilution in 1% goat serum at 4°C. After washing the slides for 10 min, in PBS, the sections were incubated for 60 min in the dark with Alexa Fluor 488 or 555 goat anti-mouse IgG and goat anti-rabbit IgG secondary antibody (Molecular Probes, Thermo Fisher Scientific Inc.) diluted 1:233 in 1% PBS at room temperature. After washing the fluorochrome-stained sections for 10 min in PBS, the slides were mounted, and nuclei were stained with ProLong Diamond Antifade Mountant with DAPI mounting medium (P36971; Life Technologies).

Sections were colour imaged with an UPlanFI 10×/0.30 objective, mounted on an Olympus BX-50 fluorescent microscope (Olympus, Japan), using a ColoView III camera and AnalySIS software (Soft Imaging Systems GmbH, Germany). The average fibre number in randomly selected fields of high quality was 1284 ± 634 fibres per section. Fibre size, distribution of type I and type II fibres, and the number of nuclei per fibre were analysed using ImageJ [US National Institutes of Health (NIH), Bethesda, MD, USA] and Matlab (The MathWorks, Inc., Natick, MA, USA). Given that only 0.52% of all the fibres were identified as type I, the results are presented as type I and II fibres combined. One sample from the HRT-RES group was excluded from the analyses owig to low sample quality.

2.9 | Western immunoblot analyses

A part (∼50 mg) of the lateral portion of the right gastrocnemius muscle was hand-homogenized in ice-cold buffer with proper inhibitors: 20 mM Hepes (pH 7.4), 1 mM EDTA, 5 mM EGTA, 10 mM $MgCl_2$, 100 mM β-glycerophosphate, 1 mM Na_3VO_4, 2 mM DTT, 1% Triton X-100, 0.2% sodium deoxycholate, 30 mg ml^{-1} leupeptin, 30 mg ml^{-1} aprotinin, 60 mg ml^{-1} PMSF and 1% phosphatase inhibitor cocktail (P 2850; Sigma, St Louis, MO, USA). Total protein content was determined using the bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL, USA) with a KoneLab device (Thermo Scientific, Vantaa, Finland).

Muscle homogenates containing 50 μg of protein were solubilized in Laemmli sample buffer and heated at 95°C to denature proteins. Proteins were separated by SDS-PAGE using 4–20% Criterion gradient gels (Bio-Rad Laboratories, Richmond, CA, USA) and transferred to nitrocellulose membranes. The uniformity of the protein loading was confirmed by staining the membrane with Ponceau S. After blocking [Odyssey Blocking Buffer (PBS), LI-COR Biosciences, Lincoln, NE, USA], the membranes were probed overnight at 4°C with the following primary antibodies (rabbit IgG) to determinate differences in phosphorylation status between HRT-RES and LRT-RES: mTOR (Ser2448) #2971, AS160 (Thr642) #4288, PKCζ/λ (Thr410/403) #9378, p70S6K (Thr389) #9234, AMPKα (Thr172) #2531, Akt1 (Ser473) #9271, Smad3 (Ser423/425) #9520, Smad2 (Ser245/250/255) #3104, p38 MAPK (Thr180/Tyr182) #9211, p44/42 MAPK (Erk1/2) (Thr202/Tyr204) #9101, S6 ribosomal protein (Ser240/244) #2215, 4E-BP1 (Thr37/46) #9459, SAPK/JNK (Thr183/Tyr185) #4668, PLD1 (Thr147) #3831, FAK (Tyr576/577) #3281 and CaMKII (Thr286) #3361 (all diluted at a ratio 1:1000). Also, α-tubulin (mouse IgG) #3873 was analysed as a loading control (1:3000 dilution). Odyssey anti-rabbit IRDye 800CW and anti-mouse IRDye 680RD (LI-COR Biosciences) were used as secondary antibodies (1:15000 dilution). The blots were scanned and quantified by using an Odyssey CLX Infrared Imager of Li-COR and the manufacturer's software. If reprobing was needed, the membranes were incubated for 10 min in 0.2 mM NaOH at room temperature, washed with Tris buffered saline (TBS) and reprobed with appropriate antibodies. Immunoblots of PLD1 (Thr147) and FAK (Tyr576/577) were too faint to be analysed. All samples and results were normalized to α-tubulin and Ponceau S. All antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

2.10 | 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 the deuterium oxide (D_2O) method (Brook, Wilkinson, Atherton, & Smith, 2017). From the control rats, five HRT-CONT and four LRT-CONT animals were included in the experiment. At the start

of the RES experiment, the rats were provided with 7.2 ml kg^{-1} D_2O by intragastric (ig) administration. 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 pH 7.5 (50 mM Tris–HCl, 1 mM EDTA, 1 mM EGTA, 10 mM β-glycerophosphate and 50 mM NaF) containing a protease inhibitor tablet (Roche) and 0.5 mM sodium orthavanadate, 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 (20 mM Mops, 110 mM KCl and 1 mM EGTA) and centrifuged at 1000*g* for 5 min to pellet the 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 0.3 M 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 amino acids were released using acid hydrolysis by incubating in 0.1 M HCl in Dowex H^+ resin slurry overnight before being washed and eluted from the resin with 2 M NH_4OH and evaporated to dryness. Resulting amino acids were derivatized to their *N*-methoxycarbonyl methyl esters, and alanine enrichment was determined by gas chromatography tandem mass spectrometry (GC-MS/MS; 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 the accuracy of measurement by the machine.

2.11 | Body water enrichment and determination of fractional synthetic rate

Body water enrichment was determined from blood samples collected post mortem and used to represent the average enrichment throughout. One hundred microlitres of plasma was incubated with 2 μl of 10 M NaOH and 1 μl of acetone for 24 h at room temperature. After incubation, the acetone was extracted into 200 μl of n-heptane, and 0.5 μl of the heptane phase was injected into the GC-MS/MS for analysis. A standard curve of known D_2O enrichment was run alongside the samples for calculation of enrichment. Fractional synthetic rate (FSR) was calculated from the incorporation of deuterium-labelled alanine into protein, using the enrichment of body water [corrected for the mean number of deuterium moieties incorporated per alanine (3.7)] as the surrogate precursor labelling. The equation used was as follows:

$$\mathrm{FSR} = -\ln\left\{1 - \left[\frac{(\mathrm{APEala})}{(\mathrm{APEp})}\right] \Big/ t\right\}$$

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

2.12 | Skeletal muscle protein, DNA and RNA concentrations

To determine muscle protein, DNA and RNA concentrations (i.e. translational efficiency/capacity), ~15 mg of the medial portion of the right gastrocnemius muscle tissue of HRT-RES and LRT-RES animals was pulverized in liquid nitrogen and homogenized in 1 ml 0.2 M PCA. After centrifugation at 4°C at 11,600 g for 8 min to form a pellet and washing with 1 ml 0.2 M PCA (washing repeated twice), the pellet was resuspended in 800 μl 0.3 M NaOH and incubated at 37°C for 2 × 20 min to dissolve the pellet. The samples were gently vortexed before, in between and after the incubations. Total protein concentration was analysed as described above (see western immunoblot analyses). Thereafter, proteins were precipitated with 400 μl 1 M PCA before centrifugation at 4°C at 2400 g for 5 min. Next, 300 μl of 0.2 M PCA was added to the supernatant of each sample and centrifuged at 4°C at 2400 g for 5 min before removal of the supernatant for quantification of RNA by NanoDrop Lite Spectrophotometer (Thermo Scientific). The remaining pellet was resuspended in 1 ml of 2 M PCA and incubated at 70°C for 1 h before centrifugation at 4°C at 2400 g for 5 min. Next, 300 μl of 2 M PCA was added to the supernatant of each sample and centrifuged at 4°C at 2400 g for 5 min before removal of the supernatant for quantification of DNA by NanoDrop Lite Spectrophotometer (Thermo Scientific).

2.13 | Muscle fibre contractility

Female HRT ($n = 6$) and LRT ($n = 6$) rats of the 13th generation were previously used to study the contractility of permeabilized muscle fibres after 8 weeks of phenotyping aerobic training (i.e. age of ~8 months) as previously described (Mendias et al., 2015; Mendias, Kayupov, Bradley, Brooks, & Claflin, 2011). Briefly, bundles of fibres that were ~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, Aurora, ON, Canada) and the other end to a force transducer (Aurora Scientific, Aurora, ON, Canada) 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 2.5 μm, as assessed with a laser diffraction measurement system. The average fibre cross-sectional area (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). The maximal fibre isometric force (F_o) was elicited by submerging the fibre in a solution containing a supraphysiological concentration of calcium. Specific force of fibres (sF_o) was determined by dividing F_o by fibre CSA. Fibres were categorized as fast or slow by examining their force response to rapid, constant-velocity shortening contraction. Ten fast fibres were tested from each tibialis anterior muscle from both groups.

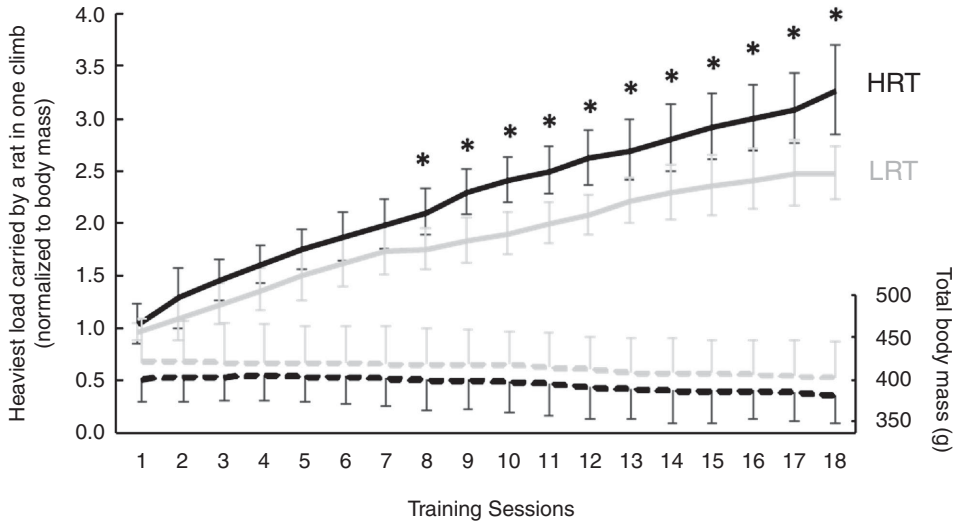

FIGURE 1 Maximal carrying capacity (continuous lines) and body mass (dashed lines) per training session. Values are expressed as the mean (SD). Black lines, high responders to aerobic training (HRT, $n = 5$); grey lines, low responders to aerobic training (LRT, $n = 5$). *Statistically significant ($P \leq 0.05$) differences between the groups

2.14 | Statistics

Statistical analyses were carried out using IBM SPSS Statistics version 24 software (SPSS Inc., Chicago, IL, USA). Non-parametric tests were used because each experimental group consisted of a low number of rats. For pairwise comparisons, a Mann–Whitney *U* test was used to evaluate differences between the groups, and a Wilcoxon signed-ranks test was used to evaluate changes within the groups. A Friedman test was applied for repeated measures within the groups and a Kruskal–Wallis H test for comparisons between the multiple groups. *Post hoc* analysis was conducted with a Bonferroni correction. Spearman's rank correlation coefficient was used to examine associations between the variables. A value of $P \leq 0.05$ was considered as statistically significant. The data are expressed as means and SD.

3 | RESULTS

Before the RES intervention, the adaptive response in running capacity to the 8 week aerobic training period was 7 (5)% ($Z = -2.023$, $P = 0.043$) in the LRT-RES group ($n = 5$), whereas in the HRT-RES group ($n = 5$) the response of 30 (16)% ($Z = -2.023$, $P = 0.043$) was significantly greater ($U = 0.0$, $P = 0.009$).

After the 6 week RES intervention, the maximal extra weight carried up during a single climb was significantly greater in HRT compared with LRT rats ($U = 1.0$, $P = 0.016$); 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 rats (Figure 1).

Selected observations of RES intervention are as follows. 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 from the HRT-RES and LRT-RES animals were combined and compared with the combined data of HRT-CONT and LRT-CONT animals, changes during the RES period in the total body lean mass determined by DXA were greater in sedentary control animals than in resistance-trained rats ($U = 98.0$, $P = 0.011$). After the intervention, soleus muscle wet tissue weight related to body weight was smaller in HRT-RES than in HRT-CONT rats ($\chi^2(3) = 8.134$, $P = 0.049$; Table 2). Other statistically significant and relevant associations, changes within the experimental groups or differences between the groups were not observed in protein synthesis rate, skeletal muscle protein, DNA or RNA concentrations, or immunohistochemical analyses (Table 2, Figure 2), body composition and blood analyses (Table 3) or immunoblot analyses (Table 4) in the present study.

For permeabilized muscle fibre contractility experiments, there was no difference in fibre CSA (6776 ± 860 *versus* 7303 ± 1038 μm^2, $U = 12.0$, $P = 0.394$), F_o (0.75 ± 0.11 *versus* 0.77 ± 0.07 mN, $U = 14.0$, $P = 0.589$) or sF_o (111.0 ± 14.1 *versus* 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. The muscular strength (i.e. load-carrying capacity) in both HRT and LRT ladder-climbing groups improved remarkably, but 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

TABLE 1 Body mass, total body fat and lean mass and right leg lean mass determined by dual energy X-ray absorptiometry before and after the 6 week ladder-climbing resistance training (RES) or control (CONT) period in rats that are high (HRT) or low (LRT) responders to aerobic exercise training

		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)	

Values are expressed as the mean (SD). *Statistically significant ($P \leq 0.05$) change from the 'Pre' measurement within the group.

TABLE 2 Muscle wet tissue weight related to body weight (mean of right and left side), type I and II cross-sectional areas (CSA), nuclei per cell, and muscle protein, DNA and RNA concentrations in the right gastrocnemius muscle, and fractional synthetic rate of muscle proteins in the left quadriceps muscle after the 6 week ladder-climbing resistance training (RES) or control (CONT) period in rats that are high (HRT) or low (LRT) responders to aerobic exercise training

	HRT-RES	LRT-RES	HRT-CONT	LRT-CONT	*P* value
Muscle wet tissue weight (mg g^{-1})					
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 fibre characteristics					
Type I and 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^{-1})					
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 ($\mu g\ mg^{-1}$)	217.0 (38.2)	177.7 (38.3)	—	—	0.175
RNA ($\mu g\ mg^{-1}$)	1.53 (0.39)	1.45 (0.32)	—	—	0.602
DNA ($\mu g\ mg^{-1}$)	2.08 (0.30)	2.00 (0.33)	—	—	0.602

Values are expressed as the mean (SD). *Statistically significant ($P \leq 0.05$) difference compared with the HRT-RES group. Abbreviations: CSA, cross-sectional area; EDL, extensor digitorum longus; FHL, flexor hallucis longus; and MQF, quadriceps femoris.

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 rats in RES-induced strength gains in the present study. This finding suggests that individual responsiveness to aerobic and resistance exercise training are somewhat similar, at least with regard to running capacity and strength gains, respectively. In this study, single muscle fibre contractility *in vitro* did not differ between HRT and LRT rats, indicating that factors other 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 the present study.

In previous studies using a ladder-climbing model for resistance training (unconditioned male or female Sprague–Dawley, Wistar

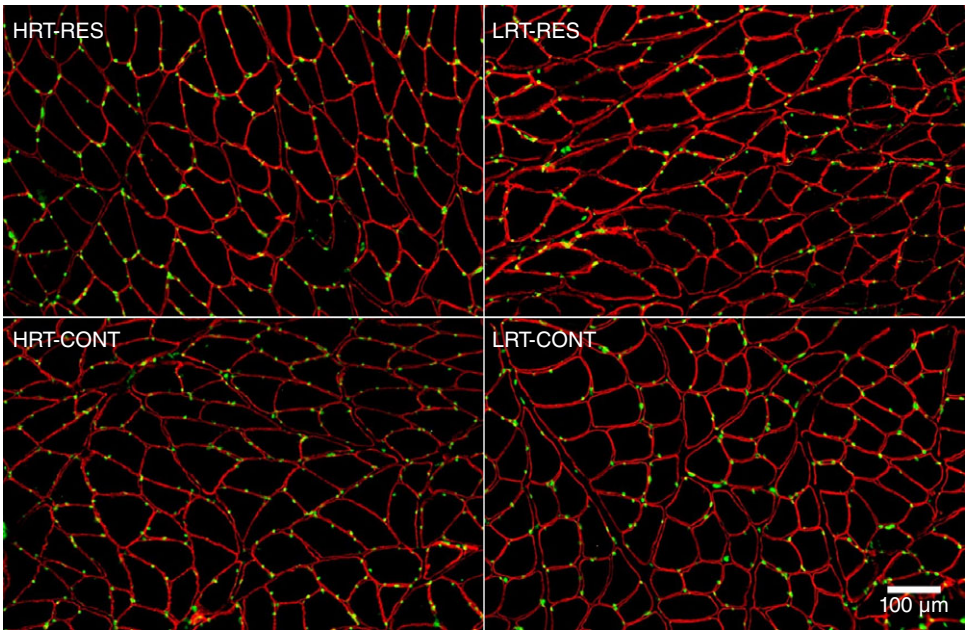

FIGURE 2 Representative image of immunohistological analyses of muscle fibre cross-sectional areas. All fibres presented in the image are type II fibres. Scale bar: 100 μm. Abbreviations: CONT, non-trained control animals; HRT, high responders to aerobic training; LRT, low responders to aerobic training; and RES, resistance-trained rats

TABLE 3 Haematological values [mean (SD)] after the 6 week ladder-climbing resistance training (RES) or control (CONT) period in rats that are high (HRT) and low (LRT) responders to aerobic exercise training

	HRT-RES	LRT-RES	HRT-CONT	LRT-CONT	*P* value
WBC ($\times 10^9$ l^{-1})	8.8 (2.2)	6.5 (1.0)	12.2 (1.4)*	9.5 (1.0)	0.005
RBC ($\times 10^{12}$ l^{-1})	9.4 (0.3)	9.2 (0.6)	9.4 (0.3)	9.2 (0.6)	0.801
HGB (g l^{-1})	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 ($\times 10^9$ l^{-1})	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 ($\times 10^9$ l^{-1})	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

Abbreviations: HCT, haematocrit; HGB, concentration of haemoglobin; LYMPH, content of lymphocytes; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; PLT, content of platelets; RDW, red cell distribution width; RBC, red blood cell count; and WBC, white blood cell count. The content of the mixture of monocytes, basophils and eosinophils (MXD) and the content of neutrophils (NEUT) were below the limit of detection. *Significantly ($P \leq 0.05$) greater compared with LRT-RES.

or Fisher 344 rats of different ages), muscle hypertrophy has been observed in some studies in muscle weight (~11–23%; Duncan, Williams, & Lynch, 1998; Gil & Kim, 2015; Harris, Slack, Prestosa, & Hryvniak, 2010; Hornberger & Farrar, 2004; Jung et al., 2015; Lee, Barton, Sweeney, & Farrar, 2004; Lee, Hong, & Kim, 2016; Luciano et al., 2017; Molanouri Shamsi, Mahdavi, Quinn, Gharakhanlou, & Isanegad, 2016) or muscle fibre CSA (~20–88%; Begue et al., 2013; Cassilhas et al., 2012; Jung et al., 2015; Peixinho-Pena et al., 2012; Prestes et al. 2012), but in some studies hypertrophy has been absent (de Sousa Neto et al., 2017; Deschenes et al., 2000; Deschenes, Sherman, Roby, Glass, & Harris, 2015; Kim et al., 2012; Neves et al., 2016; Safarzade & Talebi-Garakani 2014; Souza et al. 2014) when compared with non-training control rats. The high variation between the studies in loading protocols and examined skeletal muscles prevents conclusions of a muscle-specific dose–response relationship to RES. Nevertheless, it could be speculated that ladder-climbing RES in rats requires a relatively high training volume, and consequently lower intensity (i.e. extra carrying load), in order to induce statistically significant morphological changes in most of the trained muscles.

In the present study, we focused on resistance training responses especially in the gastrocnemius muscle, because it was electrically stimulated before collecting the skeletal muscle samples and it is a

TABLE 4 Phosphorylation levels [mean (SD)] of selected signalling proteins in electrically stimulated gastrocnemius muscle after the 6 week ladder-climbing resistance training (RES) or control (CONT) period in rats that are high (HRT) or low (LRT) responders to aerobic exercise training

Signalling protein phosphorylation (a.u.)	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

Abbreviations: Akt, AKT8 virus oncogene cellular homologue; AMPK, AMP-activated protein kinase; AS160, Akt substrate of 160 kDa; CaMK, calcium/calmodulin-dependent kinase; 4E-BP, eIF4E binding protein; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; PKCζ/λ, atypical protein kinase C zeta/lambda; S6K, ribosomal protein S6 kinase; SAPK/JNK, stress-activated protein kinase/Jun N-terminal kinase; and Smad, contraction of Sma and Mad. The phosphorylation status of phospholipase D1 (PLD1 Thr147) and focal adhesion kinase (FAK Tyr576/577) was below the limits of detection.

muscle commonly studied for endurance training adaptations. Given that we examined in detail only few selected skeletal muscles, the training responses in other muscles cannot be verified. However, when considering the findings of the previous studies and the present experiment, muscle size responses may explain only marginally the strength gains in the ladder-climbing RES model. Thus, neural responses (i.e. motor learning) may be an important mechanism in training adaptations. We have previously studied adult hippocampal neurogenesis with the present rats and, unlike aerobic training, we found no effect of RES on adult hippocampal neurogenesis (Nokia et al., 2016). Therefore, ladder-climbing RES seems to include adaptations in the CNS other than adult hippocampal neurogenesis.

The ladder-climbing RES model with rats or mice has several strengths. The training is relatively stress free to the animals and allows precise monitoring of the loading parameters (i.e. external load, number of climbs, climbing distance and duration, and recovery periods) and records of performance throughout the intervention. However, there are also some limitations that should be taken into consideration. The climbing consists mainly of concentric muscle actions, and the lack of an eccentric component might hinder muscle hypertrophy. The climbing training can be considered a whole-body workout, but muscle mass gain appears typically to be modest and localized in only a few loaded muscles. Therefore, adaptations in body composition will be minor and thus investigations of the health benefits of training at the systemic level might be challenging, as also indicated by the data of the present study. Nevertheless, the ladder-climbing RES model, when carried out with the present loading protocol, is applicable to induce great maximal strength adaptations, while hypertrophy is minor or non-existent. Thus, the present RES model allows the study of gains in muscle function (i.e. strength) without a significant hypertrophic response in the skeletal muscles.

Although the present HRT rats became stronger as a consequence of the RES, extrapolating the conclusions from the present findings to physical training outcomes in humans should be done with caution. Changes in strength after RES may be induced by both morphological adaptations in skeletal muscles and neural factors (Balshaw et al., 2017). The mechanisms underpinning individual variation in neural responses to RES are largely unknown, but some physiological factors have been identified to be associated with individual RES-induced changes in skeletal muscle size in humans, such as ribosome biogenesis (Mobley et al., 2018; Stec et al., 2016), the activity of genes related to growth and remodelling (Bamman et al., 2007; Davidsen et al., 2011; Thalacker-Mercer et al., 2013), satellite cell activity (Petrella, Kim, Mayhew, Cross, & Bamman, 2008), and the activation of signalling pathways regulating protein synthesis (Mayhew, Hornberger, Lincoln, & Bamman, 2011; Mitchell et al., 2013). Although not verified by research, it is likely that these factors are specific to RES-induced skeletal muscle adaptations, whereas aerobic training adaptations might be driven predominantly by other determinants, such as cardiorespiratory function. Thus, it could be suggested that skeletal muscle adaptations associated with responsiveness to one mode of training might not be extrapolated entirely to other exercise modes, but further research in this area is warranted.

In conclusion, HRT rats were capable of carrying heavier loads in ladder climbing when compared with LRT rats, which is in line with their responsiveness to aerobic training. However, muscular adaptations did not differ between the HRT and LRT rats in the present study, indicating that factors other than those studied here, for example neural system adaptations, might

explain their divergent adaptations of muscular strength to the present RES.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

J.P.A., S.L., C.L.M., P.J.A., L.G.K., S.L.B. and H.K. conceived and designed the research. J.P.A., S.L., I.L. and C.L.M. performed the experiments. J.P.A., S.L., I.L., M.S., J.K.I., V.F., C.L.M., M.S.B., K.S. and P.J.A. analysed the data. J.P.A., S.L., V.F., C.L.M., M.S.B., K.S., P.J.A., L.G.K., S.L.B. and H.K. interpreted the results of experiments. J.P.A. prepared the figures. J.P.A. drafted the manuscript. S.L., I.L., M.S., J.K.I., V.F., C.L.M., M.S.B., P.J.A., L.G.K., S.L.B. and H.K. edited and revised the manuscript. All authors approved the final version of manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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