

High-Speed Treadmill Running Reduces Systemic Inflammation But Fails as a Secondary Intervention For Peripheral Musculoskeletal Discomfort

Tianqi Tenchi Gao Smith

Auburn University

Ann E Barr-Gillespie

Pacific University College of Health Professions

Michelle Y Harris

Lewis Katz School of Medicine at Temple University

Mamta Amin

Lewis Katz School of Medicine at Temple University

Sean Gallagher

Auburn University

Mary Frances Barbe (✉ mary.barbe@temple.edu)

Lewis Katz School of Medicine at Temple University <https://orcid.org/0000-0002-5235-9803>

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Abstract

Background: Musculoskeletal disorders can result from prolonged repetitive and/or forceful movements. It has been shown that performance of highly repetitive tasks increases serum cytokines, nerve inflammation and sensorimotor declines in a rat model. This study investigated the effectiveness of flat treadmill running in preventing these responses. Methods: Thirteen young adult female Sprague-Dawley rats were trained to perform a high force task for 5 weeks (15 min/day, 5 days/week). All trained rats went on to perform a high repetition, high force reaching and lever-pulling task for 10 weeks (10-week HRHF; 2 hrs/day in four 30 min sessions, 3 days/week). Five task rats were randomly chosen to run on a flat treadmill (TM) for the last 6 weeks of task performance (10-week HRHF+TM; ramping up to 23 m/min, 1hr/day, 5 days/week). Results were compared to 10 control rats. Voluntary task and reflexive sensorimotor behavioral outcomes were assessed and compared. Serum was assayed for inflammatory cytokines, median nerves were assayed for CD68+ macrophages and extraneural thickening, flexor tendons were assayed for any pathological changes. Results: Treadmill running attenuated HRHF task-induced increases in serum TNF-alpha, IL-1beta, CXCL2/MIP2 and IL-10 levels. HRHF task-induced increases in flexor digitorum epitendon cellularity improved with treadmill running. However, several voluntary task performance outcomes, specifically voluntary grasp force, percent successful reaches, and voluntary task participation, worsened with treadmill running. The treadmill intervention failed to rescue HRHF task-induced declines in reflexive grip strength and forepaw mechanical sensitivity, and increases in CD68+ macrophages and extraneural fibrosis within and around median nerves. Conclusions: The treadmill intervention attenuated systemic inflammation and moderate tendinosis, but did not improve task performance or sensorimotor behaviors most likely because this exercise intervention continued to load involved injured forelimbs and worsened median nerve inflammation and fibrosis.

Background

Musculoskeletal Disorders (MSDs), also commonly referred to as Cumulative Trauma Disorders and Repetitive Strain Injuries are prevalent at workplaces worldwide. According to the 2018 report from the Bureau of Labor Statistics, musculoskeletal disorders accounted for 34% of all nonfatal occupational injuries and illnesses involving days away from work in the manufacturing sector during 2017 [1]. MSDs are thought to be a result of one or a combination of risk factors, including physical risk factors (e.g., forceful exertions, repetitive tasks, awkward posture, vibration, heat or chemical exposure), temporal aspects (e.g., work-rest scheduling and work pace), psychosocial risk factors (e.g., low job control, insufficient rest, time pressure, monotonous work, low support from management and coworkers), and individual factors (e.g., age, gender, BMI, smoking), and more [2, 3]. Musculoskeletal Health is a Cross-Sector Council focus of the National Institute for Occupational Safety and Health

(NIOSH) National Occupational Research Agenda, and musculoskeletal disorder prevention has remained a prioritized goal for the NORA Manufacturing Sector Council [4]. The impact of MSDs is tangible and significant, and may include workers' negative health outcomes, such as chronic pain, anxiety, insomnia, social dysfunction and depression [5, 6].

The current widely accepted biopsychosocial approach in treating MSD-related pain and disability aims at rehabilitation rather than cure, taking into consideration the complex and dynamic interaction among the physiological, psychological and social factors associated with pain and disability. This approach differs from the traditional biomedical reductionist approach, which targets tissue pathology, and which may include surgery or pharmacological blocking of pain pathways, with little emphasis on managing continued exposure to risk factors following return to work. Such a treatment approach may be effective in acute cases, even with continued exposure to risk factors in the workplace. However, a preventive strategy is more likely to lead to favorable long-term health outcomes. Prevention can be primary, secondary or tertiary [7]. Primary prevention targets uninjured workers using interventional methods, such as ergonomic job design or stretching programs at the work site designed to avoid injury. Secondary prevention typically takes place within six months after an injury and before the condition turns chronic, and is therapy-driven. The goal of secondary prevention interventions, including job-modification programs, is to aid patients' return to work as soon as possible. Tertiary prevention is geared toward treatment of patients with established chronic pain and/or disabilities. Tertiary prevention interventions, such as vocational rehabilitation, include intensive and sometimes individualized treatments with the hopes of preventing permanent loss of productivity and ongoing disability [7-10]

There remains a crucial need for definitive understanding of MSD injury and recovery mechanisms at different stages to inform development of effective prevention of MSDs at all levels [11]. The current general understanding of MSD development and progression is that when micro-trauma forms in tissues due to one or more risk factors, tissue either heals and returns to homeostasis during a sub-acute phase in which local inflammatory factors (e.g., cytokines) are released. The sub-acute phase may end at this point via healing (although scar

tissue may persist), or may be exacerbated to a chronic inflammation and/or fibrosis phase [12, 13].

Cytokines and chemokines are protein molecules that are released by a wide range of cells from the immune system. They communicate with cells that function in the immune response and signal cell movement to inflammation, infection and trauma sites [14]. Some cytokines are released from skeletal muscles in response to exertion or muscle injury [15]. Macrophages that have infiltrated injured tissues also release cytokines through a series of orchestrated pathways [16]. Interestingly, neural macrophage infiltration is linked to injury-induced mechanical sensitivity of axons [17]. Cytokines have been used in both human and animal studies of upper extremity work-related MSDs as serum biomarkers of underlying tissue inflammation and in identification of different pathological stages of injury, inflammation and repair [12, 13, 18, 19].

We used our previously developed, unique rat model of prolonged exposure to a voluntary repetitive task of high repetition, high force reaching and lever-pulling in order to induce exposure-dependent tissue injury and inflammation and associated pain-related behaviors [20, 21]. Our previous work with this model has led us to hypothesize that tissue specific inflammation decreases tissue tolerance, which in turn can exacerbate tissue damage [22]. We have also found that serum cytokine levels correlate significantly and positively with tissue cytokine levels [12]. Studies from other labs have shown that aerobic exercises were effective in recovering motor performance by reducing systemic inflammation and pain associated with MSDs [23-26]. Therefore, here, as a secondary prevention intervention to the tissue damage incurred by the high repetition, high force lever pulling task, we used flat treadmill running in order to examine its effect in reducing systemic inflammation and pain-related behaviors as suggested from the literature [12, 17, 27-29]. We hypothesized that the treadmill intervention would attenuate systemic and tissue inflammation and sensorimotor declines.

Methods

Animals

This experiment was approved by the Institutional Animal Care and Use Committee and was compliant with NIH guidelines for the humane care and use of laboratory animals. All Sprague-Dawley rats were procured at 4-7 weeks of age from Charles Rivers (King of Prussia, Pennsylvania), housed and handled until they reached young adulthood (2.5 months of age at the onset of the experiment). Animals were housed individually in standard rat cages (ventilated and with hardwood chip rodent bedding) in an AAALAC-accredited animal facility with a 12-hour light: 12-hour dark cycle with free access to water. All rats were handled at least 3 times per week to reduce investigator-induced stressors. Female rats were procured to eliminate sex as a potential confounder, and because females are reported to be more prone to work-related MSDs [30].

All rats were food-restricted to body weights of no more than 10% less than age-matched normal controls so as to be motivated for food reinforcement provided upon successful completion of the prescribed lever-pulling task. The normal control rats were used for weight comparison purposes only, and were not included in the study. All rats were weighed twice per week, provided regular rat chow daily (PicoLab Rodent Diet #5053, Lab Diet, Durham, NC), in addition to food reward pellets (a mix of Banana sucrose and Chocolate dustless precision pellets; # F0024 and #F0299, 45 mg, Bio-Serv, Flemington, NJ), and allowed to gain weight over the course of the experiment, since they were young adult rats at onset of experiments. Control rats that did not perform the task were provided similar amounts of food reward pellets as task rats, in order to be similarly food restricted. All rats were handled at least twice per week and provided cage enrichment toys that included chew bones, tunnels and paper twists (Diamond Twists, Teklad #7979C.CS, Envigo, South Easton, MA). Rats were inspected weekly and postmortem for illnesses and tumors that could contribute to elevation of serum cytokine; none were observed. To reduce illness-related confounders, additional sentinel rats were examined for presence of illnesses as part of regular veterinary care (none were detected).

Twenty-three young adult female Sprague-Dawley rats were used in this study. Task rats were randomly chosen. Thirteen task rats first trained to perform a high-force task for 5 weeks (15 min/day, 5 days/week) to learn the high force task with no specific reach rate, as previously described [20]. Eight trained rats were randomly chosen to perform a high repetition, high force reaching and lever pulling task for 10 weeks without any intervention (10-week HRHF; 2 hrs/day in four 30 min sessions, 3 days/week), as previously described and depicted [27] and as described further below. The five remaining trained rats performed the lever pulling task for 10 weeks and ran on a treadmill during their last 6 weeks of task performance (10-week HRHF+TM; 1 hr/day, 5 days/week), as described further below. Results were compared to 10 food restricted only control rats (FRC) that went through no training or task performance. FRC rats were euthanized at week 10 as indicated later in the Serum and Tissue Analysis section with a terminal dose of sodium pentobarbital (120 mg/kg of body weight), at matched time points as the 10-week HRHF and 10-week HRHF+TM rats.

Behavioral Apparatus

A total of 16 operant rodent chambers were utilized. Standard open field boxes were placed within larger sound dampening boxes (Med Associates, St. Albans, VT). The boxes were integrated with custom-designed force apparatuses. The force lever bar, which task rats were trained to reach and pull on, was a metal bar of 1.5 mm in diameter, placed 2.5 cm outside of each operant chamber wall at the rats' shoulder height [21]. The lever bar was attached to a miniature tension-compression load cell (Model LSB200, Futek Advanced Sensor Technology, Irvine, CA) connected with a strain-gauge amplifier (Model CSG110, Futek). The load cell signal was low pass filtered at 50 Hz and was sampled digitally at 100 Hz by the Force Lever activity software (ENV-118 M, Product Number SOF-808, Med Associates) that allowed the investigator to select the force level exertion threshold at which the rat received the food reward. A successful lever-pull occurred when the rat recognized the cue provided by the auditory indicator (Med Associates) and pulled on the lever bar at the target force threshold, based on a predetermined percentage of maximum isometric force, within a 90 - 500 ms cueing

period. If the lever bar were pulled in the correct time frame to the correct force threshold, a reward light would turn on indicating the dispensing of a 45 mg food pellet (Bioserve, NJ) into a trough at floor height [31].

Training, Task Regimen and Treadmill Running

All rats were handled and acclimated every day for 1 week upon arrival. A subset of rats was randomly selected as food restricted control rats (FRC, n=10). These FRC rats remained sedentary for the duration of the experiment with weekly handling, and grip strength and von Frey sensitivity testing. The remaining 13 rats were trained to reach and pull a lever bar at a force threshold of 60% of the average of all rats' mean maximum pulling force (MPF, 1.18 Newtons) for 10 min/day, 5 days/week, for 5 weeks. Trained rats were then randomly assigned to either a 10-week HRHF (n=8) or a 10-week HRHF+TM group (n=5). These two task groups performed the HRHF task for 2 h/day and 3 days/week for 10 weeks [20]. The daily task was performed in four 30-min sessions, separated by 1.5 h in order to prevent satiation. If the rats could maintain the HRHF 60% MPF pulling force target for between 90 msec and 500 msec after the auditory cue was delivered, a food reward would be delivered and the pull was considered as successful.

The task rats randomly assigned for the treadmill intervention regimen performed flat treadmill running in the last 6 weeks of task performance. These rats ran on the treadmill (Columbus Instruments) for 1 hour/day, 5 days/week, ramping up to 23 m/min in the last 20 min on each day. Electric shock was not utilized to avoid stressing the rats. To be clear, the 10-week HRHF+TM group performed the HRHF task for 10 weeks, in addition to performing the treadmill exercise program in the last 6 weeks of the task.

Voluntary task performance outcomes

HRHF voluntary task reach outcomes were recorded continuously during each task session and later extracted into Excel [31]. For this study, grasp force, grasp time, reach rate, percent success rate, and duration of voluntary participation per task session, were assessed by the Force Lever computer program in 10-week HRHF and 10-week HRHF+TM groups on the last day of task week 10, using previously described methods [31, 32]. These data could not be generated for FRC rats, as they did not perform the task.

Briefly defined, grasp force (in Newtons) was the mean recordable force of all reaches per day; grasp time (in seconds) was the mean time the rat spent exerting force on the lever bar over the total number of pulls per day; reach rate was the mean number of reaches per minute (including partial and full pulls on the lever bar) per day; success rate was the percent of successful reaches that resulted in a food reward per day out of all recordable reaches; and the duration of voluntary task participation per day was the amount of time (out of 120 min per day) that the rat spent participating in the task rather than sitting in the chamber not pulling. Grasp time and grasp force were calculated using the interval which started when a reach was detected on the lever bar and ended when the force fell below 2.5% of the minimum required force [31].

Reflexive grip strength and forepaw mechanical sensitivity testing

Reflexive grip strength was measured in both forelimbs of all rats using a rat grip strength tester (Stoelting, Wood Dale, IL). The test was repeated 5 times per side. Maximum grip strength of the limbs used to reach was reported for all rats after food restriction, at the end of task week 10 for the 10-week HRHF and 10-week HRHF+TM rats, and at matched time points for FRC rats.

The “up-down” von Frey testing method was used for forepaw sensory testing of all rats, bilaterally, at similar time points as for grip strength [21]. Monofilaments (North Coast Medical, Morgan Hill, CA) of different diameters were used to elicit a forepaw withdrawal

reflex. The force (in grams) of the smallest-sized filament eliciting a withdrawal reflex was recorded as the withdrawal threshold.

The person that carried out these assays was blinded to the rats' group assignments. Data for only the limbs used to reach were reported.

Serum and Tissue Analyses

All animals were euthanized and tissues collected at 36 hours after the final task session was completed in task week 10, in order to avoid possible serum cytokine fluctuations induced by exercise. All animals were deeply anesthetized with a terminal dose of sodium pentobarbital (120 mg/kg of body weight). Blood was then collected from all rats using cardiac puncture with a 23-gauge needle. The blood was immediately centrifuged at 1000g at 4° C. Serum (the supernatant) was extracted and stored at -80° C until assayed. Custom rat multiplex ELISA kits were used to assay serum, in duplicate, for 6 cytokine and chemokines: (1) CXCL2/MIP2, a macrophage and mast cell secreted, wound-healing signaling inflammatory chemokine; (2 & 3) IL-1alpha and IL-1beta, each pro-inflammatory cytokines; (4) IL-6, a proteic cytokine with both pro-inflammatory and anti-inflammatory properties; (5) IL-10, an anti-inflammatory cytokine; and (6) TNF-alpha, a potent pro-inflammatory cytokine. Array sensitivity of the serum analytes were: 1.5 pg/ml for IL-1alpha, 6.2 pg/ml for IL-1beta, 6 pg/ml for IL-6, 0.8 pg/ml for IL-10, and 3.1 pg/ml for TNF-alpha.

All animals were perfused intracardially with 4% paraformaldehyde in 0.1M phosphate buffer using a perfusion pump, before collection of forearm tissues for later histological analyses. The forelimb soft tissue mass was removed from bones *en bloc*, fixed in formalin for 3 days, equilibrated in 10% and then 30% sucrose in 0.1M phosphate buffer for 2 days each, before being cryosectioned into 12-micrometer thick longitudinal sections and mounted onto positively charged slides.

Subsets of cryosections containing the median nerve at the level of the wrist and mid-

forepaw were immunostained for CD68, a marker of phagocytic macrophages, using an antibody directed against CD68 (1:500 dilution in phosphate buffered saline (PBS), Abcam, Massachusetts, United States). After 15 minutes of 0.5% pepsin antigen retrieval at room temperature, sections were incubated for 20 minutes in 4% goat serum in PBS and then incubated with the anti-CD68 at a 1:250 dilution in PBS at 4° C overnight. The next day, sections on slides were washed 3 x 15 min each, and then incubated with the secondary antibody, AffiniPure F(ab)2 fragment, conjugated to a red fluorescent cyanine dye (Cy3; Jackson ImmunoResearch, West Grove, PA) at a dilution of 1:100 at room temperature for 2 hours. When cover-slipping, DAPI was used as a nuclear counterstain. Numbers of CD68+ cells per mm² in the median nerve at the level of the wrist and in the mid-forepaw were quantified using previously described methods [33] in three to four non-adjacent sections per nerve, and per rat. Nerves were quantified in the 10 FRC rats' forelimbs, and each reach limb of the eight 10-week HRHF rats and five 10-week HRHF+TM rats. This quantification was performed in 3-4 sections/nerve after batch staining by one individual who was blinded to group assignment.

Epineurium and extraneural connective tissue thickening was quantified in hematoxylin and eosin stained slides containing branches of the median nerve at the level of the wrist using a digital camera (*Retiga 4000R QImaging Firewire Camera, Surry, BC Canada*) interfaced with an image analysis system (Life Science, Bioquant Image Analysis Corporation, Nashville, TN). An irregular region of interest (ROI) cursor of 75 micrometers in size was used to outline the median nerve within the epineurium, and then again at micrometers external to that outline [34]. Then a Videocount Area Array option of the software was utilized (defined as the number of pixels in a field that met a user-defined color threshold of staining) to quantify the number of pixels containing dense pink stained connective tissue within the chosen region of interest, relative to the total number of pixels in that region [28]. This quantification was performed in 3-4 sections/nerve by one individual who was blinded to group assignment.

Subsets containing flexor digitorum tendon sections were stained with hematoxylin and eosin. Tendons were scored using a semi-quantitative method, the modified Bonar scale, using previously described methods [35]. Briefly, using a scale from 0 to 3, 0 represented a normal histological appearance in the epitendon and endotendon (that is, an elongated cell shape,

collagen fibers that were aligned with tenocyte cell shape, and even distribution of cells), while 3 represented advanced pathological changes (e.g., rounded cell shape, wavy fibers, and dense distribution of cells). Tendons were quantified in the 10 FRC rats' forelimbs, and each reach limb of the eight 10-week HRHF rats and five 10-week HRHF+TM rats. The person who performed the scoring was blinded to group assignment.

Statistical Analyses

A power analysis from past work was performed and showed a minimum of 5/group was needed [17, 36]. Results are reported as mean and standard error of the mean (SEM). Unpaired t-tests were used to compare voluntary reach outcomes at week 10 between the 10-week HRHF and 10-week HRHF+TM groups. One-way ANOVAs were used to compare differences in grip strength and forepaw mechanical sensitivity between the three groups (FRC, 10-week HRHF and 10-week HRHF+TM) using the maximum grip strength and the smallest-sized filament eliciting a withdrawal reflex, for each individual animal's reach limb. One-way ANOVAs were used to compare differences in serum cytokines, numbers of macrophages in the median nerve, and various tendon counts between the three groups (FRC, 10-week HRHF and 10-week HRHF+TM) using replicate data from individual animals for each analyte. ANOVAs were followed by Tukey's Honestly Significant Difference (HSD) tests for multiple comparisons; adjusted p values are reported. All statistical analyses and data visualization were conducted with the aid of GraphPad Prism 8.0.2. Type I error rates were set at 0.05 for all statistical tests.

Results

Weights

Task and control rats gained equivalently from 269 ± 4.47 to 327 ± 10.38 grams by the end of the experiment (data not shown), as reported previously in our model [37, 38].

Serum Levels of Inflammatory Cytokines Are Reduced by Treadmill Exercise

Results of Tukey HSD tests indicated that serum levels of two key inflammatory cytokines and a chemokine, TNF α , IL-1 β and CXCL2/MIP2, respectively, were increased in 10-week HRHF animals, compared to FRC and 10-week HRHF+TM rats ($p < 0.05$ each, Fig. 1A-C). Furthermore, serum levels of an anti-inflammatory cytokine, IL-10, were also higher in 10-week HRHF animals, compared to FRC and 10-week HRHF+TM rats in Tukey HSD tests ($p < 0.05$ each, Fig. 1D). No significant differences were observed between groups in serum levels of IL-1 α or IL-6 (data not shown).

Voluntary Task Performance Declines in Treadmill Intervention Group

In task week 10, the grasp force of 10-week HRHF+TM rats was reduced, compared to 10-week HRHF rats (Fig. 2A). In contrast, grasp time did not differ between the two task groups (Fig. 2B), nor did the number of reaches per minute (all partial and full pulls were considered; Fig. 2C). The proportion of successful reaches was low in general in each task group (Fig. 2D), yet was even lower in the 10-week HRHF+TM rats, compared to 10-week HRHF rats (Fig. 2D), as was the duration of voluntary task performance per day (Fig. 2E).

Treadmill intervention did not rescue task-induced grip strength declines or forepaw mechanical sensitivity, perhaps because of median nerve inflammation

The size of monofilament needed to induce a forepaw withdrawal response, also called the withdrawal threshold, was significantly lower in 10-week HRHF and 10-week HRHF+TM groups, compared to FRC rats (Fig. 3A), indicative of increased forepaw mechanical sensitivity (also known as allodynia). This was likely due to median nerve inflammatory changes observed as increased CD68+ macrophages in median nerve branches at the level of the wrist and in the forepaw in 10-week HRHF rats and 10-week HRHF+TM, compared to FRC rats (Fig. 3B,C).

Reflexive grip strength declined significantly in 10-week HRHF rats, compared to FRC rat levels. It recovered slightly in 10-week HRHF+TM rats, although remained significantly lower than FRC rat levels (Fig. 3D).

As shown in Figure 4, only a thin layer of epineurium (outer dense connective tissue surrounding nerves) was seen around median nerve branches at wrist level of FRC rats (Fig. 4A). However, this dense connective tissue was thicker around median nerve branches in 10-week HRHF rats (Fig. 4B) and 10-week HRHF+TM rats (Fig. 4C-E), and expanded into surrounding typically loose areolar connective tissue, indicative of extraneural fibrosis. Unlike the other groups, in 10-week HRHF+TM rats, median nerve branches appeared tethered by this dense extraneural connective tissues to lumbrical muscles (Fig. 4C) and tendon slips (Fig. 4D). They also were often engulfed in similar thick connective tissue in the forepaw region (Fig. 4E). Quantification of this thickened extraneural connective tissue confirmed these observations (Fig. 4F).

Flexor digitorum tendon changes

When quantified using a Bonar scale, cellularity in the distal flexor digitorum epitendon region was significantly higher in 10-week HRHF rats than in FRC rats ($p < 0.05$, Fig. 5A). This cellularity recovered enough in the 10-week HRHF+TM rats to no longer be significantly different from the FRC rats (Fig. 5A). Cellularity was not different in intramuscular regions of the 10-week HRHF flexor digitorum tendons, relative to the other groups (Fig. 5B). Also, there were only moderate, non-significant changes in cell shape, collagen fibril organization or other tendon characteristics between the groups as shown in Figures 5A-E (data not shown).

Discussion

The treadmill running intervention attenuated HRHF task-induced increases in serum levels of several inflammatory cytokines and chemokines, an expected finding that is similar to those of several other groups showing that exercise interventions can lower systemic inflammation [23-26]. HRHF task-induced increases in the distal flexor digitorum epitendon

cellularity improved slightly with treadmill running. However, unexpectedly, several voluntary task performance outcomes, specifically voluntary grasp force, percent successful reaches, and voluntary task participation, worsened with treadmill running. The treadmill intervention failed to rescue HRHF task-induced declines in reflexive grip strength and forepaw mechanical sensitivity. There were also significant increases in CD68+ macrophages and extraneural fibrosis within and around median nerves, respectively. Thus, the treadmill intervention attenuated systemic inflammation, but did not improve task performance or sensorimotor behaviors likely because this exercise intervention continued to load the involved injured forelimbs and worsened median nerve inflammation and fibrosis.

The ability to perform this voluntary task is known to decline over time in rats performing this high repetition, high force task for up to 18 weeks [12, 20, 21]. Similarly, in this study, despite a high number of total reaches per minute, a number that included both partial and full pulls on the lever bar (the target reach rate was 4 reaches per minute), the percent success rate was low in both task groups (10-week HRHF and 10-week HRHF+TM). Task rats also showed low levels of voluntary participation in each task session and neither group was able to meet the grasp force target of 60% of their voluntary maximum pulling force in most sessions. We have previously observed improved task performance outcomes after: 1) daily ibuprofen treatment of rats performing the same HRHF task for 12 weeks (although those improvements were not sustained for the full 6 weeks of treatment) [32]; 2) ergonomic task reduction intervention in which rats were moved from the HRHF task after week 4 to a low repetition, low force task that they continued to perform for another 6 weeks [31]; and 3) modelled manual therapy provided 5 days/week simultaneously with performance of the HRHF task for 3 or 12 weeks [17, 28]. However, here, the treadmill running intervention further reduced reach task performance. Voluntary grasp force, success rate, and duration of task performance were each decreased in the 10-week HRHF+TM rats, compared to the 10-week HRHF rats, suggestive of further discomfort when performing the task.

Reflexive type sensorimotor assays (monofilament forepaw testing for mechanical forepaw sensitivity and reflexive grip strength testing using a rat grip strength meter) showed similar indices of discomfort in both task groups, compared to control levels. Lowered withdrawal

thresholds, indicative of mechanical sensitivity (allodynia), was evident in the 10-week HRHF rats, and lower still in the 10-week HRHF+TM group. Reflexive grip strength was also significantly decreased in both 10-week HRHF and 10-week HRHF+TM groups, compared to the control group. We have previously shown declines in grip strength correlated with both local muscle and systemic levels of inflammatory cytokines [39, 40], matching studies showing that injection of muscles with inflammatory cytokines induces myalgia (muscle inflammation and discomfort) [41, 42]. Grip strength can also decline as a consequence of median nerve dysfunction seen in this model as median nerve demyelination and decreased nerve velocity [17, 21, 28, 33], and injury-induced autophagy and skeletal muscle atrophy following long term exposure to a high repetition, negligible force food pellet retrieval task [43].

We speculate that the treadmill running was too fast for animals in which local tissue injury was developing in forelimb tissues as a consequence of the repetitive task, because the treadmill running exercise was loading the same tissues (as rats are quadrupeds). This might explain the difference between our findings and prior studies showing that regular physical exercise lowers inflammatory responses [25, 29], and that treadmill running specifically promotes axonal regeneration after peripheral nerve injury [44], and expansion of satellite cell pool and muscle fiber formation [45, 46], a change that should increase grip strength. Our hypothesis that both the task and treadmill running level chosen here was too intense is supported by observations of significantly elevated numbers of activated macrophages and extraneural fibrosis that appeared to be tethering the nerve to adjacent tissues after 10 weeks of task performance, and even further with the combined task performance and treadmill running. We recently reported that nerve injury in the form of aberrant ectopic nerve firing is present by week 3 of the HRHF task [17]. It is therefore likely that the nerve injury also worsened with the combined effects of the rigorous lever pulling and intense treadmill regimens.

There are several limitations associated with this study. This is a study of female quadruped animals. The force transducers used in our current lever pulling chambers were chosen for their sensitivity to the range of pulling strength of the female rats used. Expanding to include male rats, that are both larger and stronger than female rats, would have required both

different force transducers and maximum pulling force ranges, making direct comparisons difficult. The treadmill running loaded the forelimbs that were developing task-induced tissue injuries during the treadmill intervention period. Treadmill interventions with different levels of intensity, including considerations of speed and duration, need to be studied in order to observe whether there is a range of treadmill intervention intensity that not only reduces pain and discomfort but also is remedial to task performance.

Conclusion

The HRHF task negatively affected task performance outcomes and grip strength, caused increased mechanical sensitivity, and provoked nerve inflammation and extraneural fibrosis. The treadmill running intervention did not remedy but worsened these responses, although it successfully reduced systemic levels of pro-inflammatory cytokines and chemokines. These findings raise awareness that the selection of aerobic exercises for secondary prevention aimed to reduce the systemic inflammatory response must also take into account the potential for such exercises to exacerbate local tissue exposure, thereby paradoxically worsening tissue injury and the risk of long-term disability.

Declarations

Abbreviations:

CD68 = cluster of differentiation 68, a protein highly expressed by cells of the monocyte lineage (phagocytic macrophages)

CXCL2/MIP2 = C-X-C Motif Chemokine Ligand 2 / macrophage inflammatory protein 2

FRC = food restricted control rats

HRHF = high repetition high force

HSD = honestly significant difference

IL-10 = interleukin 10

IL-1beta = interleukin 1 beta

Min = minutes

m/min = meters/minute

MPF = maximum pulling force

MSDs= musculoskeletal disorders

NIH = National Institute of Health

NIOSH = National Institute of Occupational Safety and Health

NORA = National Occupational Research Agenda

Pg/ml = picograms/milliliter

ROI = region of interest

TM = treadmill

TNFalpha = tumor necrosis factor alpha

Ethics approval and consent to participate: This experiment was approved by the Institutional Animal Care and Use Committee and was compliant with NIH guidelines for the humane care and use of laboratory animals.

Consent for publication: All authors gave approval of the final submitted version.

Availability of data and material: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests: The submitted content has not been previously published and is not currently under consideration for publication in any other scientific journal. We have no conflicts of interest to disclose.

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Figures

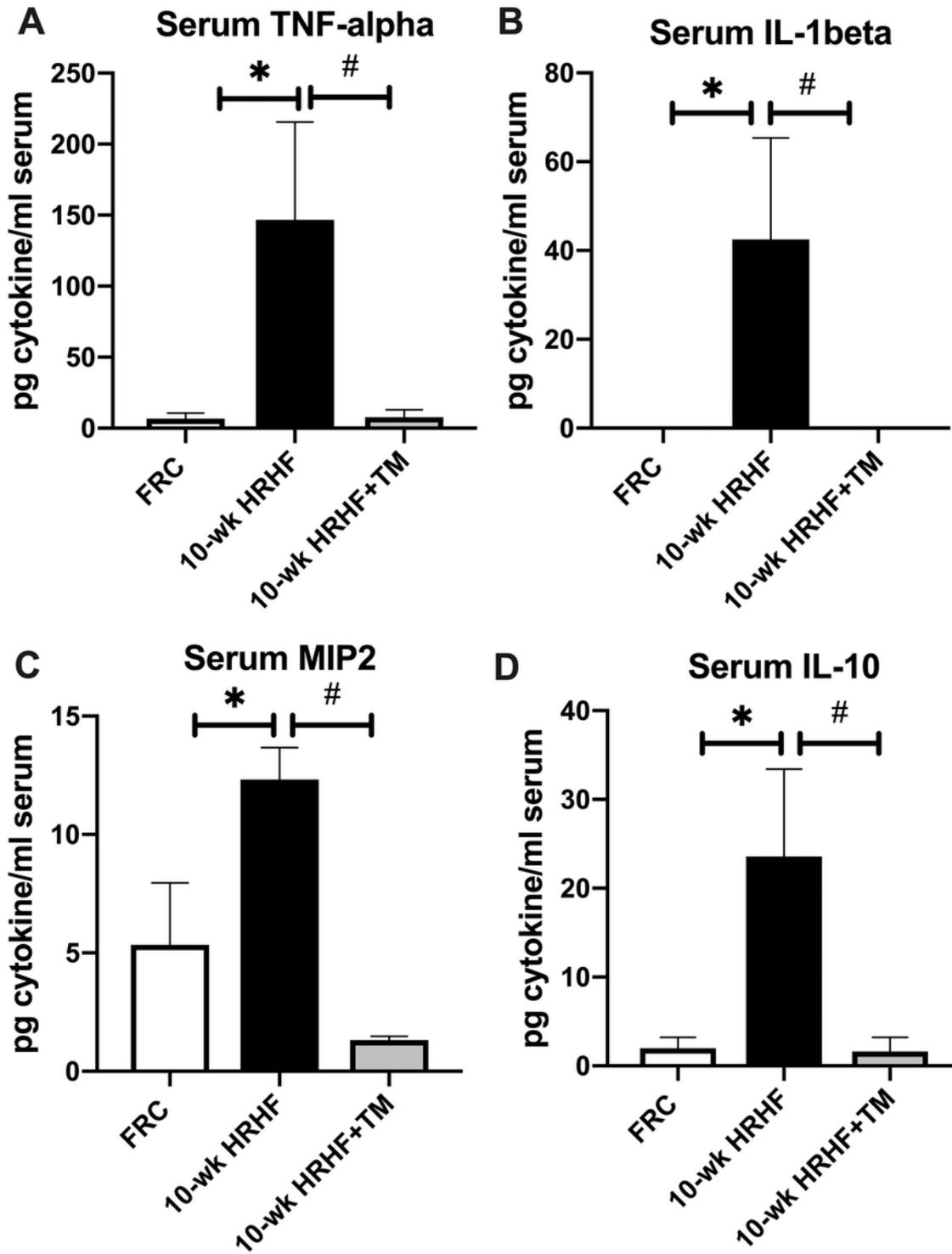


Figure 1

Serum levels of inflammatory cytokines and chemokine, assayed using multi-plex ELISA, presented as pg of cytokine per ml serum. A) TNF-alpha. B) IL-1beta. C) CXCL2 (also known as MIP2). D) IL-10. *: $p < 0.05$, compared to FRC; #: $p < 0.05$, compared to 10-week HRHF rats. Mean + SEM shown for FRC rats (n=8-10/analyte), 10-week HRHF rats (n=8) and 10-week HRHF+TM rats (n=5).

Reach Parameters in HRHF Task Week 10

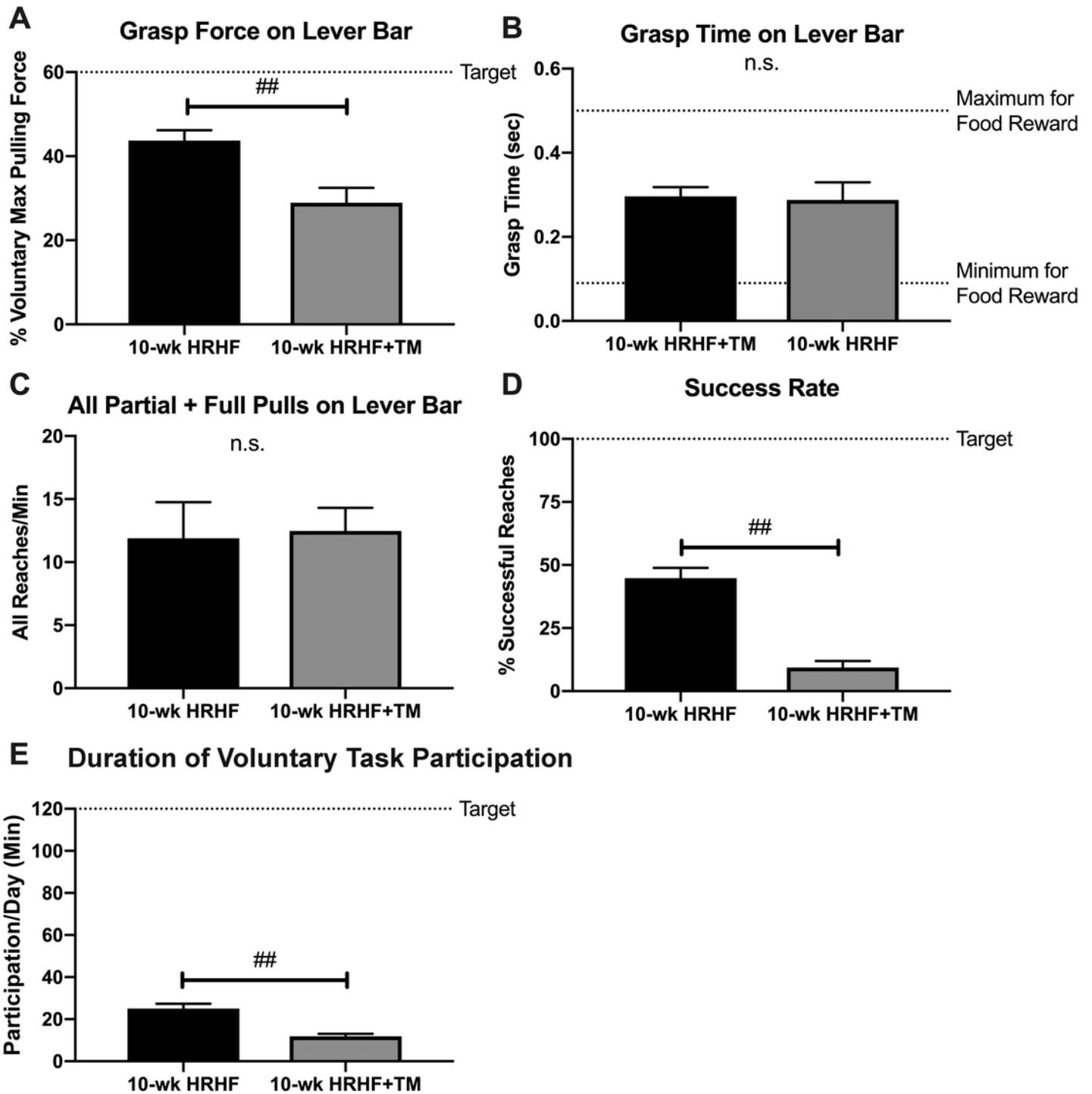


Figure 2

Voluntary Task Performance. A) Grasp force: force exerted by pulling on lever bar (percentage of maximum pulling force [MPF]). B) Grasp time: time spent grasping and exerting force on the lever bar. C) Reach rate: number of partial and full pulls on lever bar per minute. D) Success rate: percentage of successful reaches of all reaches per day. E) Duration of voluntary task participation: Time spent participating per day, in minutes, with 120 minutes per day the target. ##: $p < 0.01$, compared to 10-week

HRHF rats; n.s. = not significant. Mean + SEM shown for 10-week HRHF rats (n=8) and 10-week HRHF+TM rats (n=5).

A Forepaw Mechanical Sensitivity B Macrophages in Median Nerve

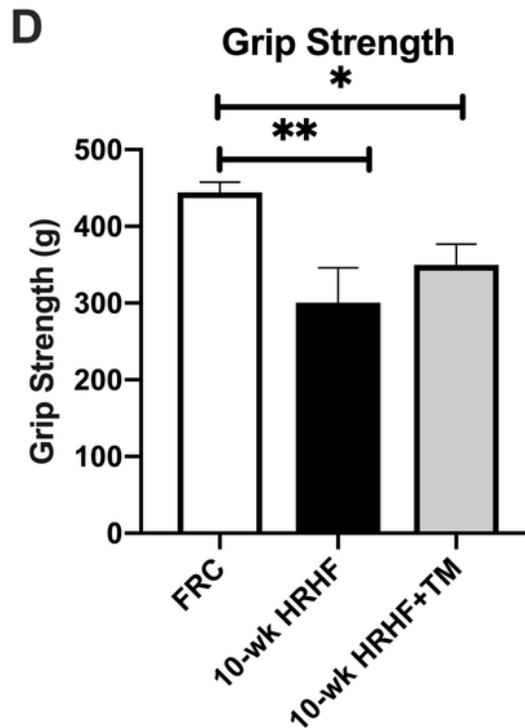
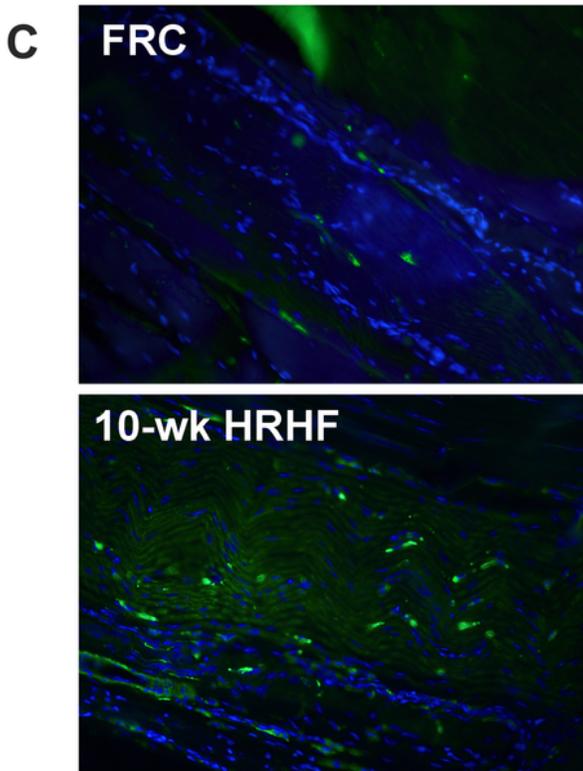
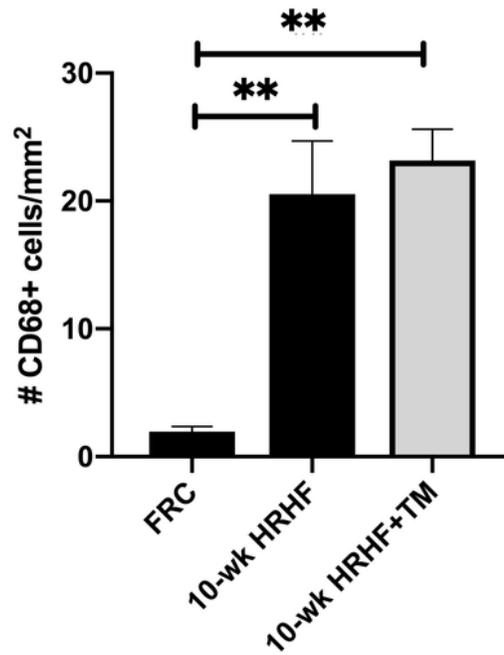
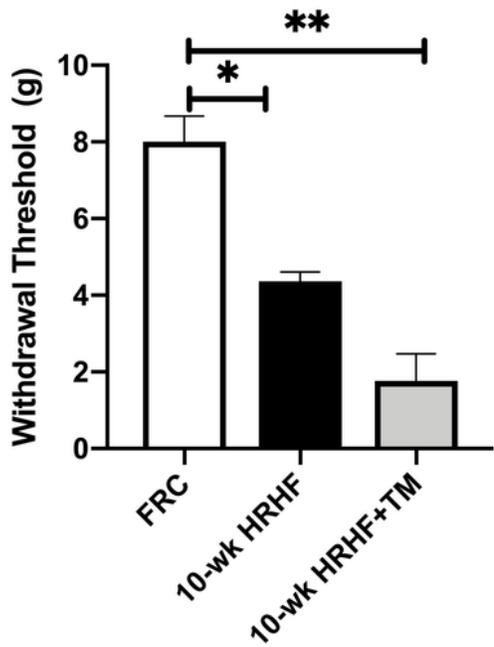


Figure 3

Sensorimotor declines and median nerve inflammation. A) Forepaw Mechanical Sensitivity. B) Number of Activated Macrophages (CD68-immunopositive, green) in the median nerve at wrist level. C) Representative images of CD68+ macrophages in median nerve branches at the level of the wrist in FRC

and 10-week HRHF rats. Images taken with a 20X objective; DAPI was used a nuclear counterstain. D) Reflexive Grip Strength. * and **: $p < 0.05$ and $p < 0.01$, compared to FRC. Mean + SEM shown for FRC rats (n=10), and reach limbs of 10-week HRHF rats (n=8) and 10-week HRHF+TM rats (n=5).



Figure 4

Increased extraneural fibrosis around median nerve of task rats. Representative images of median nerve branches (N) at the level of the wrist in panels A-E, and in the forepaw in panel D in a FRC rat (A), 10-week HRHF rat (B), and from several different 10-week HRHF+TM rats at wrist level (panels C and D) and in the mid-forepaw (panel E). Double-headed arrows in Panel B points out spread of the extraneural connective tissue into surrounding loose areolar connective tissue. Single-headed arrows in panels C and D indicate areas in which the expanded epineurium appears tethered to other structures. Ct = connective tissue; M = lumbrical muscle; N = nerve; T = tendon. Images taken with a 20X objective after H&E staining. (E) Quantification of extraneural fibrosis at the level of the wrist, within a 50 micrometer distance from the edge of the nerve/epineurium juncture. **: $p < 0.01$, compared to FRC; ##: $p < 0.01$, compared to 10-week HRHF rats. Mean + SEM shown for FRC rats (n=10), and reach limbs of 10-week HRHF rats (n=8) and 10-week HRHF+TM rats (n=5).



Figure 5

Epitendon cellularity in flexor digitorum tendons scored using a Bonar scoring system. A) Distal epitendon. B) Intramuscular epitendon. Representative examples of flexor digitorum tendons (T) in a FRC rat (C), 10-week HRHF rat (D) and 10-week HRHF+TM rat (E). *: $p < 0.05$, compared to FRC rats; n.s. = not significant. T = tendon. Images taken with a 20X objective after H&E staining. Mean + SEM shown for FRC rats (n=10), and reach limbs of 10-week HRHF rats (n=8) and 10-week HRHF+TM rats (n=5).