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Title:

High Intensity Interval Training but not Continuous Training Reverses Right Ventricular Hypertrophy and Dysfunction in a Rat Model of Pulmonary Hypertension

Short title: HIIT reverses pulmonary hypertension in rats

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Abstract

Background: Exercise is beneficial in pulmonary arterial hypertension (PAH), although studies to date indicate little effect on the elevated pulmonary pressures or maladaptive RV hypertrophy associated with the disease. For chronic LV failure, high intensity interval training (HIIT) promotes greater endothelial stimulation and superior benefit than customary continuous exercise training (CExT); however, HIIT has not been tested for PAH. Therefore, here we investigated acute and chronic responses to HIIT vs. CExT in a rat model of monocrotaline- (MCT) induced mild PAH. Methods: Six weeks of treadmill training (5x/week) was performed, as either 30 min HIIT or 60 min low-intensity CExT. To characterize acute hemodynamic responses to the two approaches, novel recordings of simultaneous pulmonary and systemic pressures *during* running were obtained at pre- and 2, 4, 6, and 8 weeks post-MCT utilizing long-term implantable telemetry. Results: MCT-induced decrement in VO_2max was ameliorated by both HIIT and CExT, with less pronounced pulmonary vascular remodeling and no increase in RV inflammation or apoptosis observed. Most importantly, only HIIT lowered RV systolic pressure, RV hypertrophy, and total pulmonary resistance, and prompted higher cardiac index; complemented by a RV increase in the positive inotrope apelin and reduced fibrosis. HIIT prompted a markedly pulsatile pulmonary pressure during running and was associated with greater lung endothelial nitric oxide synthase after 6 weeks. Conclusion: HIIT may be superior to CExT for improving hemodynamics and maladaptive RV hypertrophy in PAH. HIIT's superior outcomes may be explained by more favorable pulmonary vascular endothelial adaptation to the pulsatile HIIT stimulus.

Introduction

Pulmonary arterial hypertension (PAH) causes progressive remodeling of small to midsize pulmonary arteries that is dominated by medial hypertrophy and vasoconstriction, and that leads to right ventricular (RV) failure and death (3). Despite the development of several new drug therapies for PAH which have improved overall survival rate (31), most patients continue to exhibit significantly reduced exercise

tolerance(4, 53) and quality of life. Until recently, practitioners generally discouraged aerobic exercise for patients with PAH. Over the past few years, this opinion has shifted toward a more liberal recommendation in favor of exercise, including a grade 1A recommendation at the 5th WHO World Symposium on PH (19). However, this recommendation has been made in the absence of clinical or preclinical evidence about the duration or intensity of exercise. Moreover, there is lack of evidence of salutary effects of exercise training on cardiopulmonary hemodynamics or RV function (13, 17, 23, 40, 43, 48, 62) that would suggest that there are adaptations to the afterload increase in PAH.

Individualized exercise prescription is essential, where exercise parameters are set based on objective evaluation of patient exercise response (42), but optimal mode of delivery for a prescribed exercise workload in PAH has not been investigated. The exercise prescription with the highest therapeutic index for PAH may require an approach alternative to the customary continuous exercise training (CExT) protocol utilized in most cardiopulmonary rehabilitation settings and patient-directed independent programs. Multiple studies indicate greater favorable cardiovascular adaptations after exercise performed at higher intensity than at low or moderate levels for healthy humans(26, 45), and for animal models and humans with coronary artery disease (50), myocardial infarction and left ventricular (LV) dysfunction (14, 25), and chronic obstructive pulmonary disease(15, 47). High intensity interval training (HIIT), alternating intervals of high intensity and low intensity exercise, is superior to CExT in chronic LV failure (2), with evidence even for reversal of LV remodeling in patients following an intervention of HIIT but not of CExT (61). However, the opposite was recently reported in a pre-heart failure hypertension rat model where 4 weeks of HIIT promoted significant pathological LV adaptation not observed for CExT trained and untrained animals (30). Such contradictory findings highlight the importance of studying disease-specific responses to HIIT, or any exercise approach, prior to establishing as clinical practice. Therefore, the purpose of this study was to compare outcomes and training adaptations of the lungs, RV and soleus muscle in a rat model of PAH following a 6 week protocol of HIIT vs. CExT. Moreover, to ascertain dynamic changes in hemodynamic responses during the two types of exercise, a rat was

instrumented with implantable telemetric probes. We hypothesized that since higher exercise pressures prompt greater flow-mediated endothelial shear stress (6), HIIT would provide greater pulmonary vascular endothelial stimulation and more robust training-induced nitric oxide (NO) pathway enhancement for improved hemodynamics, attenuated RV maladaptive remodeling, and better exercise capacity.

Methods

PAH induction and experimental groups. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Indiana University, which is in compliance with National Institutes for Health guidelines. All animals received care in compliance with the Guide for the Care and Use of Laboratory Animals. PAH was induced in male Sprague-Dawley rats (~300g, Charles River and Harlan) by administration of 40 mg/kg monocrotaline (MCT, Sigma Aldrich) subcutaneously (s.q.), which reliably induces stable mild PAH after 2 weeks(54). We employed a mild PAH phenotype to reflect patients in early or controlled disease stages who may be better suited to the intensity of high intervals utilized in traditional HIIT (~90%VO_2R), as we have tested here(25, 60).

Control (CON) animals received s.q. vehicle (saline). Animals were assigned to one of six groups: 1) MCT plus a 6 wk protocol of continuous exercise training (MCT-CExT; n=7), 2) MCT plus a 6 wk protocol of HIIT (MCT-HIIT; n=8), 3) MCT but 'sedentary' with no training program (MCT-SED; n=10), 4) Saline control plus continuous exercise training (CON-CExT; n=5), 5) Saline control plus HIIT (CON-HIIT; n=6), 6) Saline control sedentary (CON-SED; n=6).

VO_2max testing. As running on treadmill is a skilled activity for rats, all rats were familiarized to the treadmill (Columbus Instruments, Columbus, OH) at speeds and inclines that would be required during subsequent testing, as previously performed by our group(9, 38). **Figure 1** presents a schematic of the protocol timeline. Immediately prior to MCT/saline injections, exercise testing was performed for all animals to determine maximal aerobic capacity (VO_2max; expressed relative to body weight) using

indirect open-circuit calorimetry (Oxymax, Columbus Instruments, Columbus, OH) and an incremental treadmill protocol in 3 min stages (8, 59). Exercise testing was repeated for all rats at two and eight weeks post-injection.

Treadmill training. A 6 week treadmill running program was initiated for rats assigned to exercise training, performed in 5 sessions/week. For rats assigned to CExT, uninterrupted steady state running was performed with treadmill speed and incline set to elicit an intensity of 50% of VO_2 Reserve (VO_2R) determined in the second exercise test, calculated for each animal by the method of Karvonen as $[(VO_2max – VO_2resting) \times 0.50] + VO_2resting$. The intensity of 50%$VO_2R$ was chosen as it is within the exercise intensity range recommended by the American College of Sports Medicine for exercise prescription in cardiopulmonary patients(42). Session duration was progressed from 30 min up to 60 min by the end of week 2.

For rats assigned to HIIT, sessions began with a 6 min warm up at 50% VO_2R then proceeded into five 5 min cycles of alternating high and low intensity intervals: 2 min at 85-90% VO_2R and 3 min at 30% VO_2R (totaling 30 min). Training time between HIIT and CExT were intentionally unmatched as it is an important aspect of the HIIT approach that only ~half of the session duration of CExT is required to achieve similar work performed. Rats assigned to remain sedentary (SED) were placed on a stationary treadmill on a matched schedule.

Invasive hemodynamic measurements. Three days following the final exercise test, rats underwent non-survival surgery for invasive hemodynamic measurements. We waited for three days in order to avoid confounding effects of the exercise tests(9, 38). Rats were anesthetized by inhaled inhaled isofluorane, orotracheally intubated, and mechanically ventilated (rate of 68 breaths/min, volume is adjusted as necessary to keep arterial blood gases within normal parameters). Isofluorane delivery was set to 5% for induction and 2% for maintenance, with a gas mixture initially at 100% oxygen then stabilized at room air. The left carotid artery was cannulated with PE-50 tubing and the right internal jugular vein was

cannulated with a 2F Millar catheter (Millar Instruments, Houston, TX) for recordings of pulmonary and systemic pressures as described previously (37), with correct RV catheter position determined by wave form analysis indicating typical RV waveform. RV systolic pressure (RVSP) and mean arterial pressure (MAP) were assessed at room air during normocapnia and normal pH (determined via i-STAT blood gas analyzer [Abbott Point of Care Inc., Princeton, NJ]).

Implantable telemetry. In order to assess dynamic changes in RV and systemic pressure during exercise, a male Sprague-Dawley rat (280g) was instrumented with an implantable telemetry sensor-transmitting probe (model HD-S21 Data Sciences International, DSI, Minneapolis) via laparotomy and thoracotomy as previously described (9). Following surgery, the animal recovered for two weeks prior to exercise testing and MCT administration (40 mg/kg). To assess hemodynamic response to treadmill running loads required by CExT and HIIT protocols, serial exercise testing was performed for the instrumented rat over an 8 week period (pre-MCT, and at 2 week intervals post MCT). The testing was conducted over three consecutive days and consisted of: 1) Determination of VO_2max as described above; 2) 30 min sampling of steady state running at 50%VO_2R, identical to that utilized for training of the CExT rats; and 3) 30 min sampling of alternating high (85-90%VO_2R) and low (30%VO_2R) intensity running, identical to that utilized for training of the HIIT rats. Recordings of systemic blood pressure (abdominal aorta), RV pressures, heart rate (HR), EKG **(**electrodes over the right pectoral muscle and left caudal rib region**)**, and body temperature were obtained at rest and during all treadmill testing, as well as during recovery from testing.

Echocardiography. A recent report of HIIT promoting pathological LV adaptation for pre-heart failure hypertension rats(30) prompted us to include echocardiography to rule out potential worsening of RV function for HIIT-trained MCT rats. Echocardiography was performed before and after the 6 weeks of HIIT and compared to parameters obtained for a subset of untrained (SED) MCT and CON animals. Rats were lightly anesthetized with 1-2% isoflurane via nose cone and images were obtained by a blinded sonographer as previously described by our group(18). Upon determination of all echocardiographic

147 endpoints, rats were recovered from anesthesia. Wall thicknesses, pressures and other derived values were
148 assessed in accordance with published methods(39) including cardiac output (derived from RV outflow
149 tract diameter and velocity time integral), which is expressed relative to body mass as cardiac index.
150 Index of total pulmonary vascular resistance (TPRi) was calculated as reported, which divides the RVSP
151 by the cardiac index.

152 *Tissue harvest.* On the final day of the protocol, immediately following the invasive hemodynamic
153 measurements, rats were sacrificed under anesthesia via exsanguination and lungs, heart, and soleus were
154 harvested as reported previously by our group(37). For consistency, tissues from the telemetry-implanted
155 rat were not included in the morphometry and biochemical assays.

156 *Pulmonary vascular remodeling.* Lungs were flushed with normal saline through a catheter in the
157 pulmonary artery (PA) until clear return was obtained from the left atrium. After excision of the right
158 lung, the left lung was then inflated with formalin/agarose via the trachea with 10% buffered formalin in
159 agarose under constant pressure (15 mmHg), removed from the thoracic cavity, and paraffin-embedded.
160 To characterize the pulmonary vascular phenotype in terms of pulmonary arterial wall hypertrophy,
161 Verhoeff-Van Giesson (VVG) immunohistochemical staining was performed on lung sections of the three
162 MCT groups and the untreated control (CON-SED) animals. Pulmonary vascular wall area was then
163 determined from brightfield microscopy images in a blinded fashion for small and medium-sized PAs
164 (<200 μm diameter, 10 vessels per animal, 20x objective) as previously described by our group(18).

165 *Assessment of lung eNOS.* To investigate training impact on a key regulator of pulmonary vascular tone,
166 endothelial nitric oxide synthase (eNOS) expression and activation status was assessed in lung tissues
167 from the three MCT groups and the untreated control animals. Measurement of lung total eNOS and
168 eNOS phosphorylated at activating site Serine 1177 or at inhibiting site Threonine 495 sites (p-
169 $eNOS^{Ser1177}$ and p-$eNOS^{Thr495}$, respectively) was performed via electrophoresis and immunoblot analysis
170 of lung homogenates as previously described by our group(9). Primary antibodies were a polyclonal

antibody for eNOS (Santa Cruz Biotechnology, 1:200 dilution), p-eNOS^{Ser1177} (Cell Signaling, Danvers, MA, 1:500 dilution), p-eNOS^{Thr495} (Cell Signaling, 1:500 dilution), or vinculin (Sigma Aldrich, 1:1000 dilution) as loading control. The intensity of Western blotting bands was measured by densitometry using ImageJ software (NIH; Baltimore) and expressed normalized to vinculin band intensity and relative to untreated controls.

Right ventricular hypertrophy. RV hypertrophy was assessed by measuring the widely used Fulton index (weight of RV divided by weight of the left ventricle plus septum; RV/[LV+S]) as described previously (37) with a value >~0.30 expected in the PH rat model. Immediately after determination of RV and LV+S weights, sections of the RV were snap-frozen for further biochemical analyses or immersed in 10% buffered formalin for immunohistochemistry studies. An additional assessment of RV hypertrophy was provided by echocardiographic measurement of RV wall thickness and change from baseline in RV wall thickness by blinded sonographer.

RV apelin. Since apelin is recognized as a potent inotropic, anti-apoptotic and anti-inflammatory mediator that has been reported to be down-regulated in the RV of MCT rats (16), RV apelin levels were assessed via electrophoresis and immunoblot analysis of RV homogenates of the three MCT groups and the untreated control animals. Equal amounts of protein (determine by bicinchoninic acid protein assay) were resolved by 7.5% SDS-PAGE, followed by immunoblotting with rabbit polyclonal antibody (1:500, Abcam), followed by secondary anti –rabbit antibody (1:2000, Abcam). The intensity of Western blotting bands was measured by densitometry using ImageJ software (NIH; Baltimore) and expressed normalized to vinculin band intensity and relative to sedentary MCT animals.

RV and skeletal muscle metabolism. To further characterize the MCT phenotype, and to investigate potential training induced adaptations in myocardial or skeletal muscle substrate utilization, indicators of oxidative and non-oxidative (glycolytic) metabolism were assessed in RV and soleus samples of the three MCT groups and the untreated control animals. For western blotting of RV and soleus whole-muscle

195 homogenates, equal amounts of protein (determined by bicinchoninic acid protein assay) were resolved
196 by 7.5% SDS-PAGE, followed by immunoblotting using a total oxidative phosphorylation antibody
197 cocktail (OXPHOS; Abcam, diluted 1:500) and using rat heart mitochondria (Abcam) as a positive
198 control. The intensity of Western blotting bands was measured by densitometry using ImageJ software
199 (NIH; Baltimore) and expressed normalized to vinculin band intensity and relative to sedentary MCT
200 animals. Oxidative capacity of the RV and soleus was also evaluated by assessment of glucose
201 transporter-1 (GLUT-1) abundance as an indicator of cytoplasmic glycolysis. Immunofluorescent staining
202 for Glut-1 (Abcam #ab652; Cambridge, MA, at 1:150 dilution) was performed on cryofixed RV and
203 soleus as previously described by our group(9). Mean pixel intensity of GLUT-1 staining was determined
204 using ImageJ software (NIH; Baltimore) and expressed relative to sedentary MCT animals.

205 *RV inflammation, fibrosis, and apoptosis.* To assess whether the high work rates encountered during
206 intense intervals of a HIIT session may induce detrimental RV responses with chronic use, RV
207 inflammation, apoptosis, and fibrosis were examined for the three MCT groups and the untreated control
208 animals using immunofluorescence and histological assays. Lymphocyte infiltration was assessed as an
209 indicator of RV inflammation via immunofluorescence staining for *CD45* (Santa Cruz Biotechnology,
210 Dallas, TX, 1:20 dilution) in RV cryosections as previously described by our group(9). $CD45^{+}$ counts
211 were expressed as the number of positive stained cells per field, averaging at least six randomly chosen
212 fields per RV. Cardiomyocyte apoptosis was assessed with terminal deoxynucleotidyl transferase dUTP
213 nick end labeling (TUNEL) of RV cryosections according to the manufacturer's instructions (Roche
214 Applied Science, Indianapolis, IN) with DAPI co-staining and expressed relative to nuclei count. RV
215 fibrosis was assessed on formalin-fixed paraffin-embedded RV sections as % positively stained area with
216 Masson's trichrome staining and expressed relative to MCT sedentary animals.

217 *Statistical analyses.* Data are presented in figure graphs as means ± standard error (mean±SEM). An
218 analysis of variance (ANOVA) by group assignment was performed with repeated measures (exercise
219 testing and body mass data at three time points) or without repeated measures (hemodynamic data and

Fulton index) as appropriate; using Tukey's multiple comparison post-test analysis to determine between-group differences. ANOVA was also implemented for data from histological and biochemical assays performed for the three MCT groups and the untreated healthy control group (CON-SED). Echocardiographic data obtained at two time points (pre- and post-training) for HIIT and SED animals (both MCT and CON) were evaluated using repeated measures ANOVA. Pearson product correlations were performed to further explore relationships between dependent variables. All statistical analysis was carried out using SPSS, version 23.0, and differences at α level of 0.05 ($p<0.05$) were considered statistically significant.

Results

CExT and HIIT improve aerobic exercise capacity in mild PH

Aerobic capacity. Two weeks after MCT, rats showed a mild PH phenotype, evidenced by a reduction from baseline in aerobic capacity (by -7±1.5%, **Fig 2A**), and faster time to exhaustion (**Fig 2B**). In contrast, there was no change ($p>0.05$) from baseline for saline-injected animals. Together, these data suggest an expected impairment in physical function in MCT injected rats prior to intervention and this impairment was similar ($p>0.05$) between rats assigned to HIIT vs. CExT groups. Since training workloads were set relative to each rat's individually-determined post-injection maximum, the group means (±SEM) for treadmill speed (m/min) and incline (deg) eliciting a relative training intensity of 50%VO_2R used in CExT was lower as expected for MCT-CExT (9.3±0.6, 2.9±1) compared to CON-CExT (12±1.3, 7±1.2). Likewise, mean (±SEM) treadmill speed utilized for the high intensity intervals of HIIT performed at an incline of 25° to elicit 85-90%VO_2R was lower for MCT-HIIT (14.2±0.3 inc 25) compared to CON-HIIT (16.3±0.2).

Importantly, MCT rats treated with either HIIT ($p<0.01$ vs. MCT-SED) or CExT ($p<0.05$ vs. MCT-SED) exhibited more preserved VO_2max and time to VO_2max, with pre- to post-training change (**Fig 2A,B)** not different ($p>0.05$) from that for CON-SED over the same time period. While the total number of training

minutes for HIIT was half as much as for CExT, calculated cumulative work performed for MCT rats over the 6 weeks was comparable between HIIT and CExT groups as intended (4573±218, 3745±616 joules, p=0.4). Change in body mass over the study period (**Fig 2C**) was not differently affected by group assignment (p>0.05).

HIIT is associated with lower RV pressures

At 8 weeks post-MCT, unexercised (MCT-SED) rats exhibited resting RVSP values (**Fig 3A**) that were ~60% greater compared to CON rats. MCT-HIIT rats, on the other hand, exhibited RVSP values that were similar to CON, and significantly lower than both MCT-CExT and MCT-SED. However, continuous exercise had no salutary effect on RVSP as MCT-CExT exhibited RVSP values that were similar to MCT-SED, and significantly higher than both MCT-HIIT and CON rats. Aortic blood pressures (BP) were similar (p>0.05) among groups, with mean arterial pressure values of 92±4.4, 100±8.7, 99±6.7, 105±7.3, 113±7.0, and 97±5.5 mmHg for MCT-CExT, MCT-HIIT, MCT-SED, CON-CExT, CON-HIIT, and CON-SED, respectively.

Larger post-exercise reduction in RVSP with HIIT

Figures 3B-D show hemodynamics in the chronically instrumented rat, at rest (B), and responses to exercise (C, D). RVSP at rest is increased by MCT injection in this animal by ~60% by week 4 with unchanged systemic pressures (**Fig 3B**). Most importantly, exercise data reveal that, in contrast to the steadier hemodynamics observed during CExT running (right panel, **Fig 3C**), pronounced 'surges' in RVSP occurred during HIIT running which corresponded to the high intensity run intervals (left panel, **Fig 3C**). Further, hemodynamic recordings collected during recovery from these run bouts (striped bars, **Fig 3D**) reveal a more marked post-exercise reduction in RVSP from resting values following a HIIT session compared to a CExT session, suggesting heightened provocation of acute vasodilation by HIIT.

Larger post-exercise reduction in RVSP with HIIT is associated with increased lung eNOS expression

Since the telemetry studies suggested a more pronounced vasodilation effect after HIIT, we measured eNOS expression in lung homogenates of HIIT-, CExT-, and SED- MCT rats and untreated healthy controls (CON-SED). Interestingly, MCT-HIIT exhibited greater fold increase in total eNOS protein than MCT-SED ($p<0.05$) and CON-SED ($p<0.01$) (**Fig 4A**). Phosphorylation of eNOS at activation (serine) and inhibitory (threonine) sites was not different with training (data not shown). Taken together, the telemetric exercise pressure recordings and biochemistry data indicate that HIIT running prompts an acute pulmonary hemodynamic response distinct from CExT running, and is associated with higher lung eNOS abundance and lower resting RVSP in MCT-PH.

Preserved pulmonary vascular structure in exercised MCT rats

Pulmonary arterial wall thickness (by VVG staining, **Fig 4B**) was greater for MCT animals in both small diameter (<100 μm, left panel) vessels, by 30% (MCT-CExT and HIIT) to 45% (MCT-SED), and medium diameter (100 to 200 μm, right panel) vessels, by 26% (MCT-CExT and HIIT) to 50% (MCT-SED). While there was no significant difference in MCT rats between trained vs. sedentary animals, no significant increase in pulmonary arterial wall thickness was observed for the trained animals (both HIIT and CExT), whereas MCT-SED was significantly increased when compared to healthy controls ($p<0.01$for small diameter vessels, $p<0.05$ for medium diameter vessels, vs. CON-SED). This suggests potential beneficial effects of exercise training on pulmonary artery remodeling.

Less RV hypertrophy and better RV function with HIIT

MCT animals treated with HIIT had ratio of RV to LV+S mass (**Fig 5A**) similar to that of healthy (CON-CExT, -HIIT, -SED) animals; this observation was absent in MCT animals treated with CExT. Given the beneficial effects of HIIT on cardiopulmonary hemodynamics and RV hypertrophy, we performed echocardiography to further characterize RV function in these animals. We confirmed the findings suggested by the Fulton index by demonstrating that HIIT-trained MCT animals exhibited an attenuated increase in RV free wall thickness over 6 weeks (**Fig 5B,** upper panel**)** compared to sedentary MCT

(p<0.05), resulting in lower final wall thickness compared to sedentary MCT (p<0.05). Further, echocardiography indicated improvement in RV function in HIIT trained MCT, including better-maintained cardiac output over 6 weeks (cardiac index **Fig 5C,** upper panel), p<0.05), lower final index of total pulmonary vascular resistance (TPRi, **Fig 5D,** upper panel), and tendency for better ratio of pulmonary artery acceleration time over pulmonary artery ejection time (PAAT/PET, p=0.07) vs. MCT-SED. Correlation analysis indicated that final RVSP was related to RV hypertrophy as determined by Fulton index (R= 0.64, p<0.01) as well as by wall thickness in echocardiography (R= 0.65, p<0.01). A differential effect of HIIT on echocardiographic parameters was not observed for CON animals (lower panels, **Fig 5B-D**) except in cardiac output and cardiac index where SED animals exhibited increase over 6 weeks, contrary to that observed in MCT-SED.

Training impact on RV and skeletal muscle metabolism

Untreated MCT rats (MCT-SED) exhibited greater abundance of Glut-1 in immunofluorescence staining of RV (**Fig 6A**) and soleus (**Fig 6B**) compared to untreated healthy controls (CON-SED), suggesting a shift toward glycolytic (non-oxidative) metabolism in both cardiac and skeletal muscle. Interestingly, both HIIT- and CExT- trained MCT rats exhibited less Glut-1 (p<0.01) compared to MCT-SED in the RV (**Fig 6A**). However, only HIIT was associated with increased RV expression of an electron transport chain complex, cytochrome IV (**Fig 6C**). Training effects were also observed for soleus, but in contrast to those observed in the RV, occurred only with a CExT approach. Only MCT-CExT exhibited similar soleus Glut-1 (**Fig 6C**) to CON-SED, with a tendency (p=0.08) for increased soleus expression of an electron transport chain complex, cytochrome III, vs. MCT-HIIT and MCT-SED (**Fig 6D**). Taken together, these data indicate that exercise training- in either a HIIT or CExT regimen- may positively impact MCT-induced glycolytic shift in RV and skeletal muscle.

RV inflammation, fibrosis, and apoptosis

To investigate potential adverse effects of training, biochemical assays were performed to evaluate RV fibrosis, inflammation and apoptosis for all trained and sedentary MCT rats and untreated healthy control rats (CON-SED). Immunoblotting experiments revealed a training effect on RV apelin, an anti-apoptotic and anti-inflammatory mediator and positive inotropic regulator that is inducible by exercise. Much higher RV apelin was observed with HIIT vs. CExT (increased by ~75%, $p<0.01$) (**Fig 7A**). Neither HIIT nor CExT increased RV inflammatory or apoptotic cells as indicated by similar levels ($p>0.05$ vs. MCT-SED) of $CD45^+$ (**Fig 7B**) and $TUNEL^+$ cells (**Fig 7C**). Interestingly, MCT-induced increase in RV fibrosis (trichrome staining, **Fig 7D**) was attenuated by HIIT (by approximately one-third, $p<0.05$ vs. MCT-SED) which supports echo data of improved RV function in these animals.

Discussion

Our most important finding was that while both HIIT and CExT attenuated MCT-induced decrement in aerobic capacity, only HIIT lowered pulmonary pressures and attenuated RV hypertrophy and dysfunction. Previous studies of chronic exercise effects in animals and in patients with PAH revealed mixed impact on hemodynamics, with one study demonstrating a small but statistically significant training-induced lowering of resting pulmonary pressures (23), but all other studies indicating no effect (17, 22, 24, 43). To our knowledge, this is the first exercise intervention associated with robust favorable impact on hemodynamics and the RV in PAH.

Our data show for the first time that chronic exercise-induced upregulation of endogenous pulmonary eNOS expression in PAH, which has previously been demonstrated in healthy animals and models of systemic vascular disease(34, 36, 52, 65). While immunoblotting data may not necessarily reflect nitric oxide (NO) bioavailability, increased eNOS increases NO production(44) and NO-dependent arterial relaxation(21, 33, 36), both typically impaired in PAH (35). We found an increase in total eNOS protein with HIIT, but no change in phosphorylation pattern. This was not surprising since final exercise bout and tissue harvest were separated by three days to mitigate confounding effects of acute exercise on chronic

adaptation. In our previous work, a single bout of moderate intensity exercise (75% VO_2max) induced acute pulmonary eNOS activation (as determined in serine and threonine phosphorylation) in lung tissue collected an hour later and transiently normalized pulmonary pressure in a MCT (50 mg/kg) rat model of moderate PAH (9). In that work, telemetric recordings during running also indicated a running-induced acute pulmonary pressure reduction, as we have seen here (**Fig 3D**, striped bars), and concomitancy with unchanged stroke volume (estimated by O_2 pulse [VO_2 per heart beat]) supported a mechanism of acute pulmonary resistance reduction (e.g. vasodilation) and not an acutely failing RV. We believe this also to be the case for the present telemetric observations of post-running pulmonary pressure reduction (**Fig 3D**, striped bars). However, since assumptions about the relationship between oxygen consumption and heart rate are not upheld during non-steady state running (precluding calculation of O_2 pulse), it is not possible to ascertain relative contribution of acute changes in pulmonary resistance vs. RV contractility underlying the more pronounced acute pulmonary pressure relief evoked by HIIT (black striped bars).

The augmented exercise-induced pulmonary eNOS activation and enhanced acute post-exercise pulmonary vasodilatory response in MCT-PAH rats (9) may be a consequence of higher flow-mediated shear forces. With chronic exercise, only applied as HIIT resulted in greater total pulmonary eNOS protein, and alleviated pulmonary hypertension (**Fig 3 and 4**) while mild intensity continuous exercise stimulus (50% VO_2max, CExT) did not. The induction of eNOS protein and improved pulmonary pressures following HIIT but not CExT may be explained by a difference in the pulmonary vascular stimulation provided by the two training approaches. Telemetric recordings during HIIT and CExT sessions revealed very different exercise hemodynamic profiles generated by the two approaches where HIIT running was accompanied by quick-changing, high magnitude pulmonary pressures (**Fig 3C,** left panel) in contrast to an elevated but relatively unchanging pulmonary pressure in CExT running (**Fig 3C,** right panel). The ability for shear forces at the vessel wall to prompt bursts of NO production acutely and, when applied repeatedly, to enhance NO signaling machinery is enhanced in the presence of quick-changing, high magnitude shear as opposed to statically-applied shear (6). This is in agreement with

multiple studies demonstrating superior improvement in vascular function as assessed via brachial artery flow-mediated dilation with a HIIT approach compared to traditional continuous training (27, 58). Abnormal microvascular shear adaptation has been reported for a rat model of PAH induced by vascular endothelial growth factor receptor blocker Sugen5416 +hypoxia (57), as well as in patients (57), promoting lung endothelial injury and vessel remodeling (57). Therefore, future work should directly investigate if the quick-changing, high magnitude pulmonary pressures we observed during HIIT helps to restore microvascular shear responses in PAH and how this relates to disease progression.

Improved pulmonary hemodynamics in HIIT trained MCT may explain the pronounced reduction in resting RVSP measures (**Fig 3A**) and RV hypertrophy (**Fig 5A,B**), which we interpret as evidence of benefit. Serial echocardiographic measures revealed lower pulmonary resistance and higher cardiac output (TPRi and cardiac index, **Fig. 5C,D**) for HIIT trained animals. Superior outcomes with HIIT may have also been derived from direct effects on the RV. Histological and biochemical assessment of the RV indicated a healthier myocardium for HIIT-trained MCT including greater apelin expression (**Fig 7A**), less fibrosis (**Fig 7D**), and potentially less metabolic dependence on cytoplasmic glycolysis (**Fig 6A,B**). Apelin has been recognized as a potent inotropic substance and strong vasodilator, hence its increased interest as a potential treatment/biomarker of PAH(1), and its response to treatment, including exercise(63). We previously identified apelin to be an important contributor to RV function in our lab, which prompted us to include RV apelin expression as a secondary endpoint. To our knowledge, ours is the first report of training induced changes in RV apelin. The higher RV apelin observed for MCT rats following 6 weeks of HIIT may have contributed to the improved RV function observed in these animals via direct effects on myocardial contractility.

Our observations regarding training impact on indicators of glycolytic vs. oxidative metabolism are particularly relevant in light of the growing evidence that PAH is associated with an inefficient metabolic shift(56) in both cardiac and skeletal muscle mitochondrial substrate utilization from aerobic to anaerobic metabolism that contributes to diminished exercise tolerance(41, 49). Direct measures of myocyte

metabolism were beyond the scope of this work; however, immunostaining and immunoblotting data (**Fig 6A,B**), indicate an interesting possible differential response of skeletal and cardiac muscle to training approach. More robust oxidative metabolic adaptations were observed in the RV myocardium in response to HIIT, while training induced metabolic adaptations in the soleus were only observed for the CExT approach. This is in agreement with other studies that have reported divergent adaptive responses by muscle types, for example, in a Dahl sodium sensitive rat model of hypertension, not only did CExT prompt greater oxidative metabolic adaptations compared to HIIT in skeletal muscle, findings in white vs. red gastrocnemius indicated a fiber type-specificity to this response (29). The absence of a robust skeletal muscle metabolic training adaptation in our HIIT rats may be a consequence of the lower absolute workloads and session duration of our protocol compared to that in other studies(46, 64) utilizing more physically-able disease models, and workloads set relative to calculated critical power(11), and thus may not have provided a sufficient stimulus to achieve these effects.

Exercise may worsen RV inflammation in PAH if coincident with excessive RV wall stress (28), as reported with high left ventricular (LV) wall stress in an LV overload rodent model (55), and systemic hypertension rodent models(12, 51). Despite the high work-rate run intervals, HIIT treated rats had no evidence of heightened RV pro-inflammatory/apoptotic signaling for MCT, nor was this observed for CExT trained MCT (**Fig 7 A-D**), which is in contrast to that reported after 6 weeks of treadmill (CExT-type) training for rats that received 60 mg/kg MCT (28). The reasons we did not observe increased inflammation may result from a less severe model of PAH, and because we adjusted the treadmill workload relative to each animal's post-disease VO_2max, in order to avoid greater relative strain on animals with worse disease, and more closely reflect individualized exercise prescription for patients.

Limitations and future directions. First, the relationship between higher exercise pressures of HIIT and the positive adaptations we observed (enhancement of hemodynamics and exercise capacity, attenuation of RV maladaptive remodeling), is indirect and remains associative until future mechanistic experiments directly interrogate causation of these adaptations by HIIT's higher exercise pressures. Second, we tested

413 the effect of exercise on male rats with a mild PH phenotype. While this may suggest the translation of
414 our findings to clinical exercise interventions may be focused on early disease, it remains to be
415 determined if prescribed training applications as we have tested here will have similar benefit with no
416 detrimental RV effects in more angioproliferative severe PH, as well as in females. Given the known
417 sexual dimorphism in PAH incidence(5), outcomes(31, 32), and acute exercise responses(38), sex
418 differences in the training adaptations we have described here remain to be investigated. Lastly, the
419 exercise sessions of the HIIT and CExT groups were performed with identical session frequency but were
420 not time matched, with CExT sessions lasting 60 min but HIIT sessions only half of that duration. While
421 this more accurately reflects how HIIT is performed in clinical practice, it introduces the possibility that
422 superior outcomes with HIIT are resultant from briefer session durations provoking less cumulative
423 training-induced RV wall stress. Since calculated cumulative work performed over the 6 weeks was
424 comparable with HIIT and CExT, we believe that this alternative explanation is not as likely, and chose to
425 keep HIIT session durations unmatched to facilitate translation of findings to a customary HIIT
426 prescription for patients. In terms of time cost, the shorter training sessions of HIIT may provide
427 additional appeal to patients as time constraints are commonly cited as barriers to exercise adherence (20).
428 Not only may short bouts of higher intensity exercise may be more enjoyable than longer, steady state
429 effort of continuous exercise (7), the anaerobic energy utilization required in HIIT may better mimic the
430 physiological requirements of activities of daily living in those with cardiopulmonary disease (10).

431 *Perspectives and Significance.* Our report identifies for the first time that exercise training using a HIIT
432 approach is superior to customary CExT for improving hemodynamics and RV remodeling and
433 dysfunction in a rat model of PAH, and does not promote RV inflammation or cardiomyocyte apoptosis.
434 These outcomes with HIIT may be explained by pulsatile pulmonary vascular exposure to flow-shear
435 stimulus promoting pulmonary eNOS upregulation, coupled with reduced fibrosis, apelin upregulation,
436 and improved metabolic profile in the RV muscle. The pulmonary pressure- reducing and RV-preserving

effect observed only with HIIT encourages further investigation of this alternative training approach in other models and in patients as a potentially more optimal exercise regimen for PAH.

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Disclosures

None.

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651 **Figure legends**

652 **Figure 1.** Study protocol. Following a period of treadmill familiarization, maximal oxygen uptake
653 (VO_2max) was measured. Rats then received either monocrotaline (MCT, 40 mg/kg, i.p.) to induce PAH
654 (n=25), or saline, for healthy controls (n=17). Two weeks later, VO_2max testing was repeated to establish
655 pre-training values, and echocardiography was performed on a subset of rats. A 6-week treadmill program
656 was then initiated for rats assigned to exercise training, performed in 5 sessions/week. CExT (n=12)
657 performed 60 min of uninterrupted steady state running at 50% of VO_2 Reserve (VO_2R) determined in the
658 second exercise test. HIIT (n=14) performed a brief warm up then five cycles of alternating high and low
659 intensity intervals: 2 min at 85-90% VO_2R and 3 min at 30% VO_2R, totaling 30 min). Rats assigned to
660 remain sedentary (SED, n=16) were placed on a stationary treadmill on a matched schedule. At the
661 conclusion of 6 weeks, final VO_2max testing and echocardiography was performed, and three days later,
662 invasive hemodynamic measures were performed, followed by sacrifice and tissue harvest.

663 **Figure 2.** Aerobic exercise capacity and body mass. A) For rats with monocrotaline (MCT, 40 mg/kg)
664 induced PAH (black lines/symbols), decrement in VO_2max (expressed relative to body mass) was
665 ameliorated by both a high intensity interval training approach (HIIT, dashed line/triangles, n=8), and a
666 continuous exercise training approach (CExT, dotted line/circles, n=7), vs. untrained MCT (SED, solid
667 line/squares, n=10), with change after 6 wks of training (8 wk time point) compared to pre-training (2 wk
668 time point) not different ($p>0.05$) from that in healthy HIIT, CExT, and SED control rats (CON, gray
669 lines/symbols, n=5-6 ea). B) MCT-induced decrement in treadmill run time (time to VO_2max) was also
670 significantly improved by CExT and HIIT. C) Body mass was significantly impacted by time for all
671 groups, but not differently affected by group assignment. *$p<0.05$, **$p<0.01$

672 **Figure 3.** Pulmonary hypertension and exercise hemodynamics. A) For rats with monocrotaline (MCT,
673 40 mg/kg) induced PAH (black bars), MCT-induced elevation in resting RV systolic pressure (RVSP)
674 was attenuated by a high intensity interval training (HIIT, n=8) but not continuous exercise training

675 (CExT, n=7) approach, with RVSP of MCT-HIIT similar to that in healthy HIIT, CExT, and SED control
676 rats (CON, gray bars, n=5-6 ea). *p<0.05; **p<0.01 B) Serial measures of simultaneous RV systolic and
677 mean systemic resting pressures (BP) in an awake, freely-moving rat instrumented for implantable
678 telemetry. Recordings are shown for pre-, and 2, 4, 6, 8, and 10 weeks post-MCT (40 mg/kg) injection.
679 C) Real-time tracings of heart rate (HR, upper), and pulmonary (RVSP, lower) and systemic (mean BP,
680 middle) pressures recorded via implantable telemetry are shown during HIIT running (left panel) and
681 CExT running (right panel) for a rat at 4 wks post-MCT (40 mg/kg). Note the intermittent surges in RVSP
682 that correspond to high (HI) and low (Lo) intensity intervals during HIIT which are absent during CExT.
683 D) Change in RVSP relative to resting values during a session of CExT (solid gray), and during the high
684 intensity ('Hi', solid black) and low intensity ('Lo' solid white) intervals of a HIIT session recorded in the
685 instrumented rat at baseline (pre-MCT), and at 2, 4, 6, and 8 wks post-MCT. Change in RVSP from
686 resting values during recovery from both CExT and HIIT run session are also indicated at each time point,
687 as striped gray and black bars, respectively.

688

689 **Figure 4.** Lung eNOS expression and vascular remodeling. A) For rats with monocrotaline (MCT, 40
690 mg/kg) induced PAH (black bars), only high intensity interval training (HIIT, n=8), and not continuous
691 exercise training (CExT, n=7), significantly increased lung eNOS abundance vs. untrained animals (SED,
692 n=10) as assessed by immunoblotting (expressed as fold difference from mean value for untreated
693 controls (CON-SED, gray bar, n=6). A representative immunoblot showing the band corresponding to
694 eNOS and to vinculin (loading control) is also shown. B) MCT-induced thickening of pulmonary arterial
695 walls (PA wall thickness as fraction of vessel area) was not significantly different following either HIIT
696 or CExT compared to SED, for either small diameter (<100 μm, left panel), or medium diameter (100-200
697 μm, right panel) vessels in MCT; however PA wall thickness in both small and medium vessels was only
698 significantly greater for untrained MCT (MCT-SED) when compared to untreated healthy controls (CON-

SED). Representative images of VVG-stained elastin surrounding arteries (arrows) in lung sections is also provided. *p<0.05; **p<0.01

Figure 5. RV hypertrophy and function. A) For rats with monocrotaline (MCT, 40 mg/kg) induced PAH (black bars), elevation in ratio of RV to LV+S mass (Fulton index, a measure of RV hypertrophy) was attenuated by a high intensity interval training (HIIT, n=8) but not continuous exercise training (CExT, n=7) approach, with values for MCT-HIIT significantly lower (p<0.01) than MCT-CExT and untrained (SED) MCT (n=10), and similar to that for healthy HIIT, CExT, and SED control rats (CON, gray bars, n=5-6 ea). Representative photographs show a top-down view of hearts (great vessels and atria removed) from MCT-CExT, MCT-HIIT, MCT-SED, and CON-SED where increased thickness of RV (far right wall) relative to LV (far left wall) for MCT-CExT and MCT-SED can be appreciated. B through D) Echocardiography performed pre- ('2 wks') and post- ('8 wks') intervention for HIIT (dashed line/triangles, n=8 MCT [black, upper panels], n=4 CON [gray, lower panels]) and SED (solid line/squares, n=8 MCT, n=4 CON) demonstrate that in HIIT trained MCT rats, increase in RV wall thickness (B) was ameliorated, and cardiac output (expressed normalized by body mass as cardiac index, C) was better maintained. Calculated final total pulmonary vascular resistance index (TPRi, panel D) was also lower for MCT-HIIT vs MCT-SED. *p<0.05; **p<0.01

Figure 6. RV and soleus metabolism. Abundance of glucose transporter Glut-1 was greater for RV (A) and soleus (C) in sedentary rats with monocrotaline (MCT, 40 mg/kg) induced PAH (black bars, MCT-SED, n=10) vs. sedentary healthy controls (gray bars, CON-SED, n=6). In the RV (A), trained MCT had less Glut-1 compared to SED MCT whether they followed either a high intensity interval training (HIIT, n=8) or continuous exercise training (CExT, n=7) approach. Immunoblotting for oxidative phosphorylation proteins (OXPHOS) in RV homogenates (B) indicated that training also increased expression of electron transport chain cytochrome IV (expressed as fold difference from MCT-SED for loading control-normalized densitometry value), but only for rats trained with a HIIT approach. For the soleus (C and D), a training effect toward attenuating MCT-induced dependence on glycolytic

metabolism was also observed, but opposite to the RV, only occurred for CExT and not HIIT. CExT-trained MCT tended to express less Glut-1 (C) and more cytochrome III in OXPHOS immunoblots of soleus homogenates (D) compared to MCT-HIIT, with mean values not significantly different than that for CON-SED. Abundance of Glut-1 was measured by mean pixel intensity of red immunofluorescent staining shown in representative images, with green representing wheat-germ agglutin stained myocyte membrane and blue representing nuclei. A representative immunoblot showing the band corresponding to cytochrome III and to vinculin (loading control) is also shown. Results depicted in bar graphs to the left of images is expressed as fold difference from MCT-SED in mean pixel intensity. *$p<0.05$; **$p<0.01$

Figure 7. RV inflammation, apoptosis, fibrosis, and apelin expression. A) Immunoblotting of RV homogenates for the anti-apoptotic/anti-inflammatory mediator and positive inotropic regulator apelin in rats with monocrotaline-induced (40 mg/kg) PAH (MCT, black bars) and untreated healthy control rats (CON, gray bars) revealed a higher protein abundance in MCT that were trained with high-intensity interval training (HIIT, n=8) but not with continuous exercise training (CExT, n=7). Values are expressed as fold difference from untrained (SED) MCT (n=8). In fixed RV sections of MCT rats (MCT, black bars) and untreated healthy control rats (CON, gray bars) infiltration of CD45+ cells (lymphocytes, B) measured by immunofluorescent staining (count per field, mean±SEM) was not different from untrained animals (SED), either after a HIIT or CExT approach. Adjacent panels are representative images with arrows indicating examples of CD45+ (red) cells, with green representing wheat-germ agglutin stained myocyte membrane and blue representing nuclei. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for myocyte apoptosis was also performed (% of TUNEL+ cells, mean±SEM, C). Adjacent panels are representative images, including a positive and negative control slide, with arrows indicating examples of TUNEL+ (bright green) cells, and blue representing non-apoptotic nuclei. RV sections were additionally assessed for fibrosis with Masson's trichrome (blue, in images) staining (D) and MCT-induced increase in RV fibrosis (expressed as fold difference from MCT-SED in % positively-stained field) was less for HIIT-trained MCT. *$p<0.05$; **$p<0.01$

Figure 1

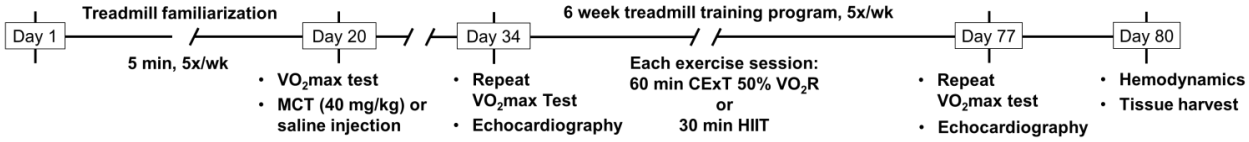

Treadmill familiarization
6 week treadmill training program, 5x/wk
Day 1
Day 20
Day 34
Day 77
Day 80
5 min, 5x/wk
• VO2max test
• MCT (40 mg/kg) or saline injection
• Repeat VO2max Test
• Echocardiography
Each exercise session:
60 min CExT 50% VO2R
or
30 min HIIT
• Repeat VO2max test
• Echocardiography
• Hemodynamics
• Tissue harvest

Figure 2

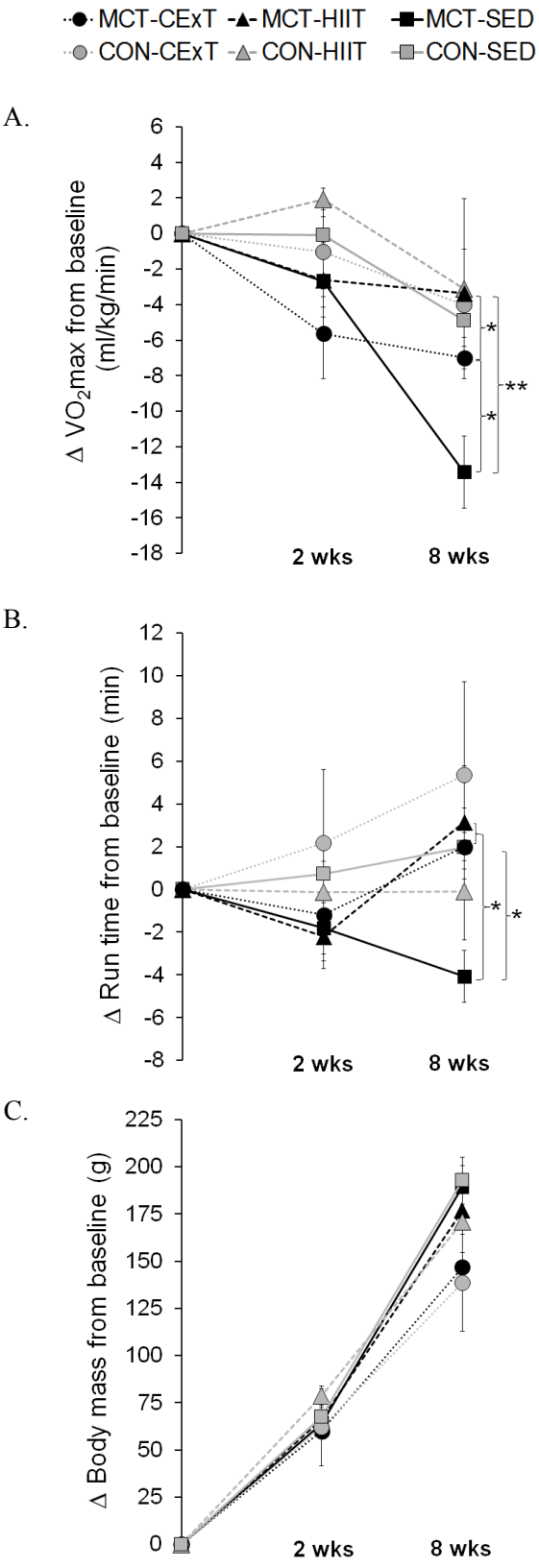

MCT-CExT
MCT-HIIT
MCT-SED
CON-CExT
CON-HIIT
CON-SED
A.
Δ VO2max from baseline (ml/kg/min)
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
2 wks
8 wks
*
*
**
B.
Δ Run time from baseline (min)
12
10
8
6
4
2
0
-2
-4
-6
-8
2 wks
8 wks
*
*
C.
Δ Body mass from baseline (g)
225
200
175
150
125
100
75
50
25
0
2 wks
8 wks

Figure 3

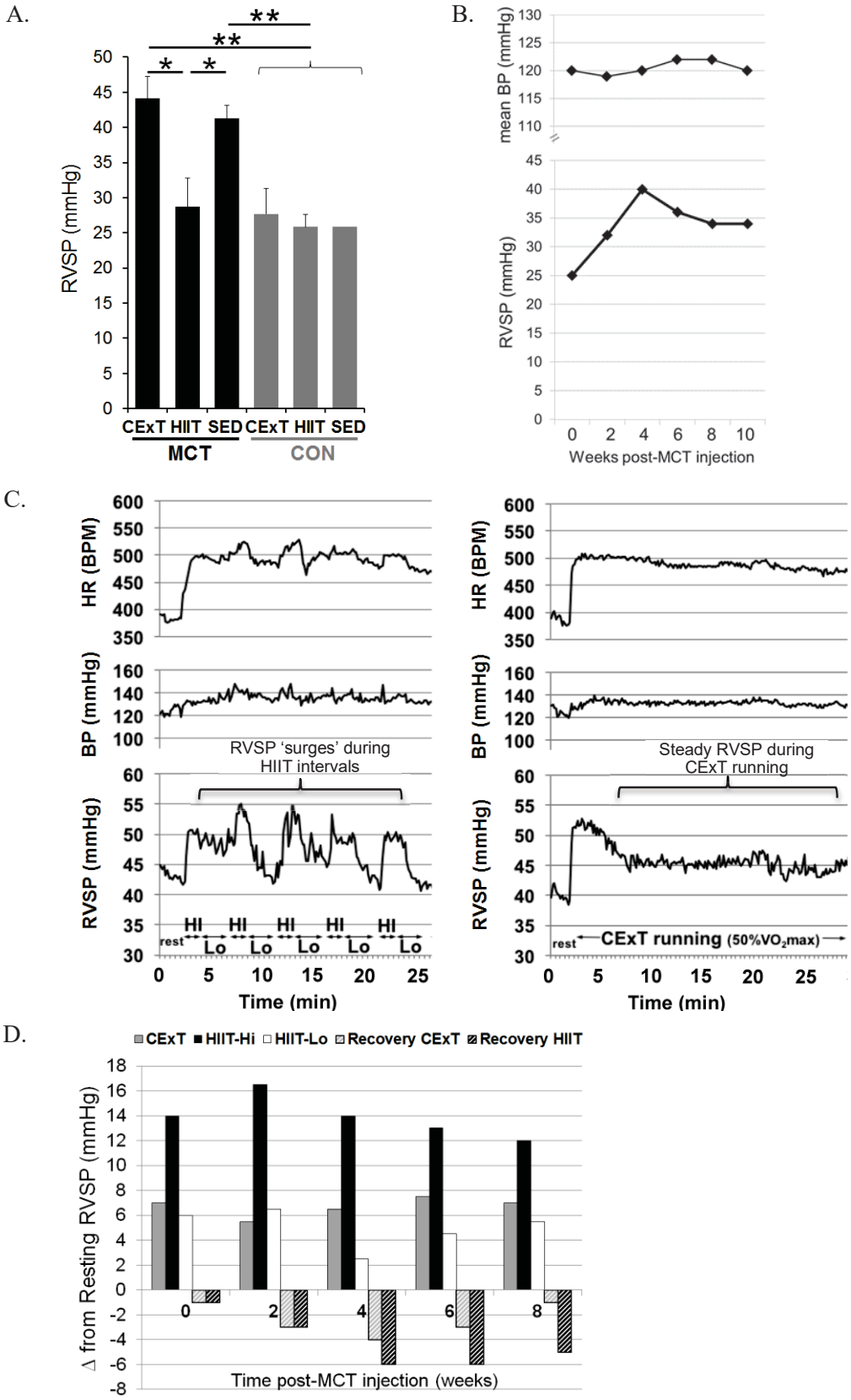

A.
RVSP (mmHg)
CExT HIIT SED CExT HIIT SED
MCT
CON
B.
mean BP (mmHg)
RVSP (mmHg)
Weeks post-MCT injection
C.
HR (BPM)
BP (mmHg)
RVSP (mmHg)
RVSP 'surges' during HIIT intervals
Steady RVSP during CExT running
rest
HI
Lo
CExT running (50%VO2max)
Time (min)
D.
CExT
HIIT-Hi
HIIT-Lo
Recovery CExT
Recovery HIIT
Δ from Resting RVSP (mmHg)
Time post-MCT injection (weeks)

Figure 4

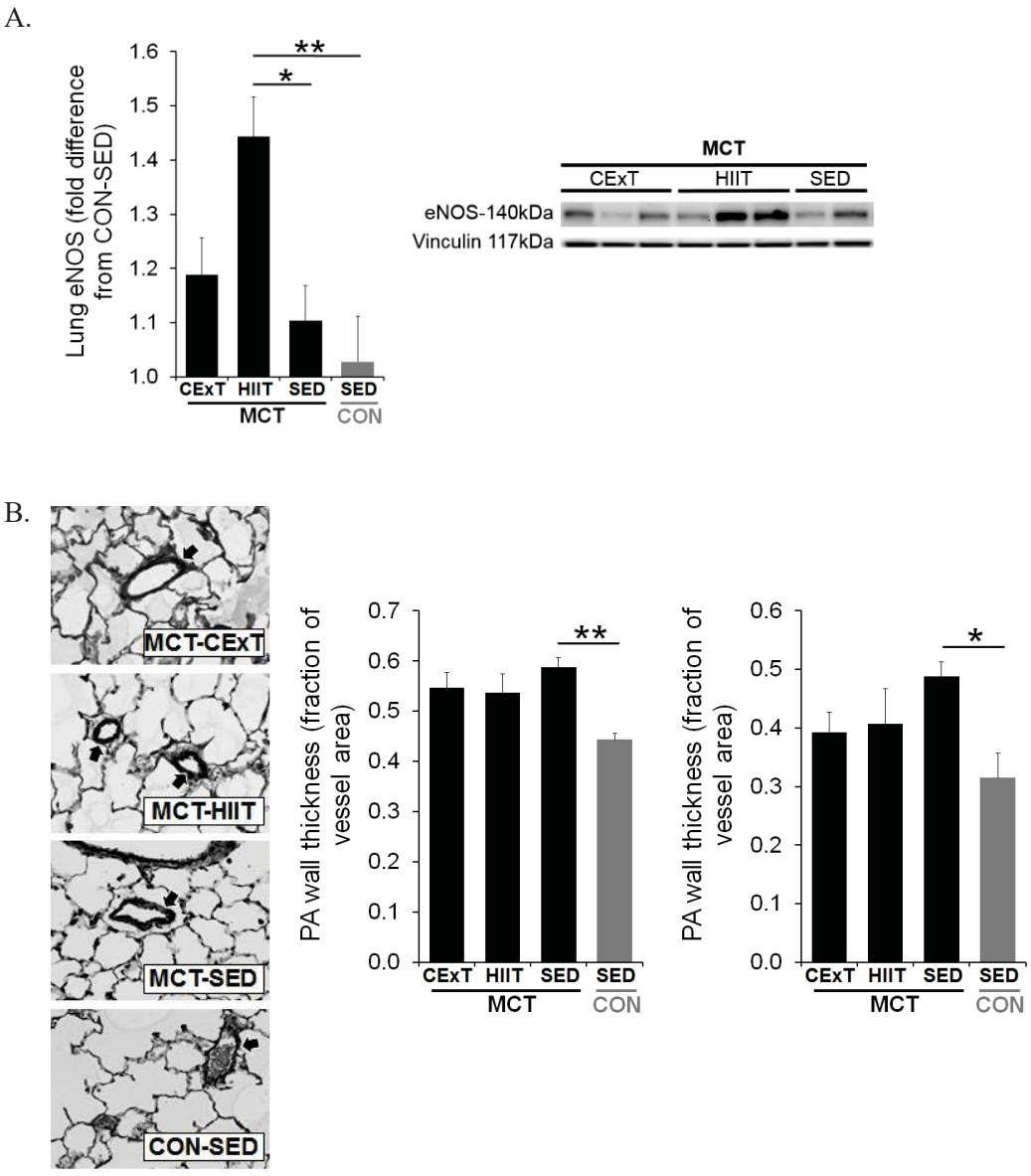

A.
Lung eNOS (fold difference from CON-SED)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
**
*
CExT
HIIT
SED
SED
MCT
CON
MCT
CExT
HIIT
SED
eNOS-140kDa
Vinculin 117kDa
B.
MCT-CExT
MCT-HIIT
MCT-SED
CON-SED
PA wall thickness (fraction of vessel area)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
**
CExT
HIIT
SED
SED
MCT
CON
PA wall thickness (fraction of vessel area)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
CExT
HIIT
SED
SED
MCT
CON

Figure 5

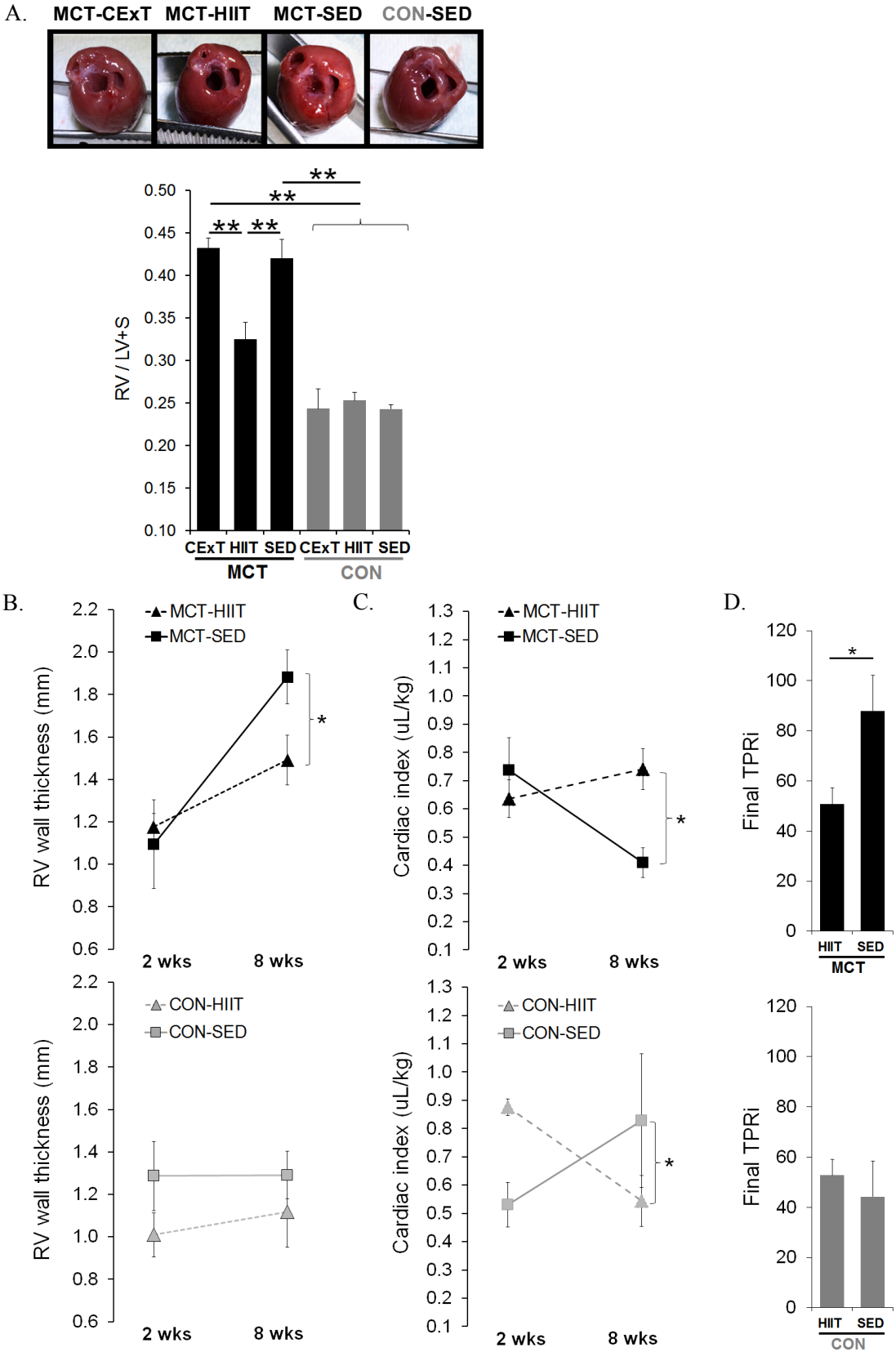

A.
MCT-CExT
MCT-HIIT
MCT-SED
CON-SED
RV / LV+S
CExT HIIT SED CExT HIIT SED
MCT
CON
B.
MCT-HIIT
MCT-SED
RV wall thickness (mm)
2 wks
8 wks
CON-HIIT
CON-SED
C.
Cardiac index (uL/kg)
D.
Final TPRi
HIIT SED
MCT
CON

Fig 6

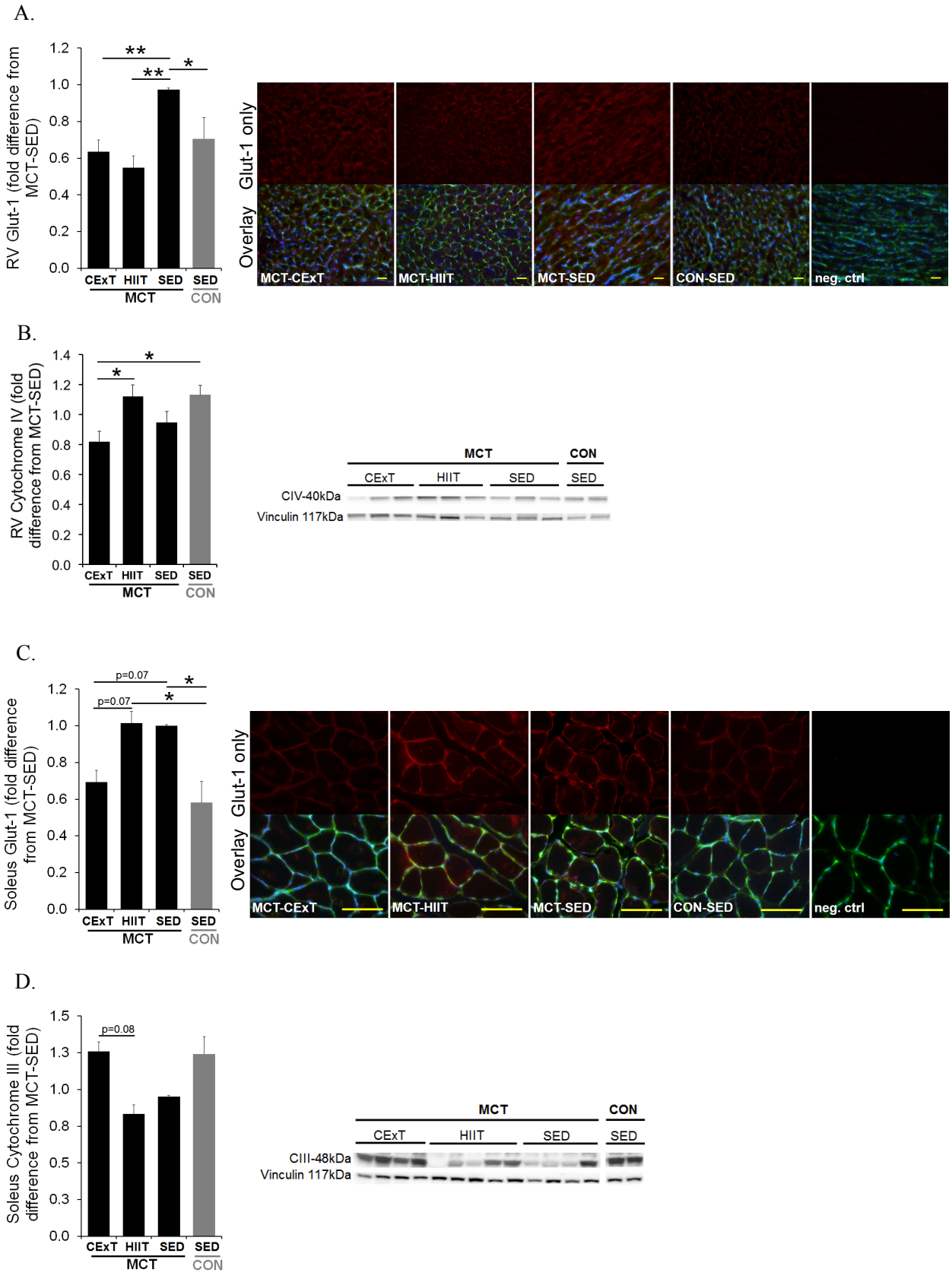
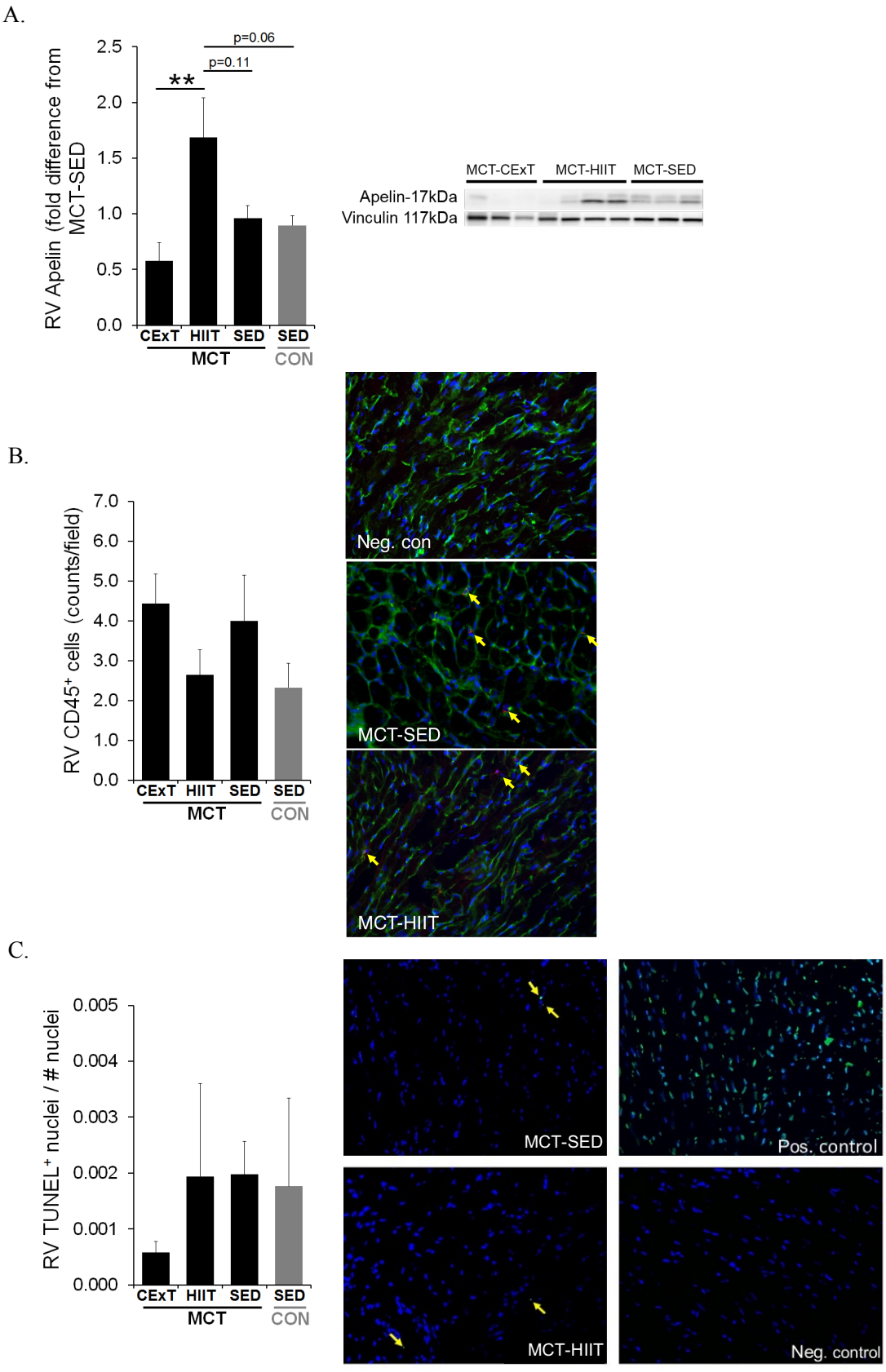
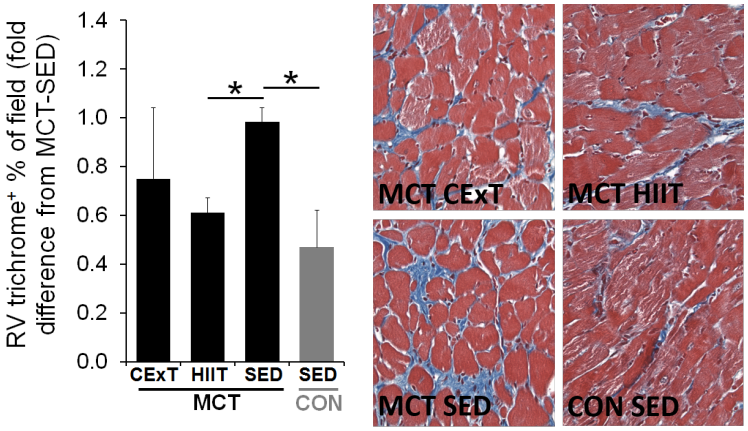

A.
RV Glut-1 (fold difference from MCT-SED)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
*
CExT
HIIT
SED
SED
MCT
CON
Glut-1 only
Overlay
MCT-CExT
MCT-HIIT
MCT-SED
CON-SED
neg. ctrl
B.
RV Cytochrome IV (fold difference from MCT-SED)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
CExT
HIIT
SED
SED
MCT
CON
MCT
CON
CExT
HIIT
SED
SED
CIV-40kDa
Vinculin 117kDa
C.
Soleus Glut-1 (fold difference from MCT-SED)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
p=0.07
p=0.07
*
*
CExT
HIIT
SED
SED
MCT
CON
Glut-1 only
Overlay
MCT-CExT
MCT-HIIT
MCT-SED
CON-SED
neg. ctrl
D.
Soleus Cytochrome III (fold difference from MCT-SED)
1.5
1.3
1.0
0.8
0.5
0.3
0.0
p=0.08
CExT
HIIT
SED
SED
MCT
CON
MCT
CON
CExT
HIIT
SED
SED
CIII-48kDa
Vinculin 117kDa

Figure 7


A.
RV Apelin (fold difference from MCT-SED)
2.5
2.0
1.5
1.0
0.5
0.0
**
p=0.11
p=0.06
CExT
HIIT
SED
SED
MCT
CON
MCT-CExT
MCT-HIIT
MCT-SED
Apelin-17kDa
Vinculin 117kDa
B.
RV CD45+ cells (counts/field)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Neg. con
MCT-SED
MCT-HIIT
C.
RV TUNEL+ nuclei / # nuclei
0.005
0.004
0.003
0.002
0.001
0.000
MCT-SED
Pos. control
MCT-HIIT
Neg. control

D.


RV trichrome+ % of field (fold difference from MCT-SED)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
CExT
HIIT
SED
SED
MCT
CON
MCT CExT
MCT HIIT
MCT SED
CON SED