Moderate intensity aerobic exercise training reduces total and regional diaphragm hyperemia during submaximal exercise in pulmonary hypertensive rats

by

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Abstract

Introduction: Pulmonary hypertension (PH) is a cardiopulmonary disease characterized by vascular remodeling of the pulmonary arteries, increased respiratory muscle work, dyspnea, and reduced maximal oxygen uptake ($\dot{V}_{O_2 max}$). Previous findings from our lab revealed that, specifically, diaphragm blood flow ($\dot{Q}$) is increased at the expense of hindlimb muscle $\dot{Q}$ during submaximal treadmill exercise in PH, like that seen in chronic heart failure. Exercise training has been demonstrated to improve $\dot{V}_{O_2 max}$, augment muscle $\dot{Q}$, and reduce the "respiratory muscle blood flow steal" phenomenon in heart failure; therefore, in this study, we examined whether moderate-intensity aerobic exercise training would have the same effects in PH. We hypothesized that 4 weeks of exercise training would: 1. Increase $\dot{V}_{O_2 max}$ and exercise tolerance in PH rats towards that of healthy rats. 2. During moderate/heavy exercise, reduce respiratory muscle (diaphragm, intercostal) blood flow "steal" and increase hindlimb muscle $\dot{Q}$ and reduce blood [lactate]. **Methods:** Female Sprague-Dawley rats were randomized into 3 groups: healthy control (HC; *n*=12), sedentary PH (PH-SED; *n*=14), and exercise-trained PH (PH-ExT; *n*=13). All PH rats received a single dose of monocrotaline (MCT; 50mg/kg, i.p.), whilst HC rats received saline vehicle. Echocardiography was used to monitor disease progression (i.e., peak pulmonary arterial acceleration time/ejection time [PA AT/ET]). All rats were acclimated to treadmill running for 5 days before PH-ExT rats performed 4 weeks of moderate-intensity treadmill running (25m/min, 15 min day one, increased by 5 min/day to 60 min). Upon confirmation of PH (AT/ET <0.30), $\dot{V}_{O_2 max}$ testing was performed to assess exercise capacity and tolerance. ~24 hours later, respiratory and hindlimb muscle blood flows ($\dot{Q}$) were determined by infusing fluorescent microspheres at rest and during moderate/heavy-intensity exercise (25m/min). Rest and exercise arterial blood gases, acid-base, and [lactate] were also measured.

Results: All PH rats displayed morphometric and echocardiographic criteria for PH, including elevated right ventricular (RV) systolic pressure, RV hypertrophy (RV/LV+S), and reduced PA AT/ET vs. HC ($P < 0.05$). Exercise training did not increase $\dot{V}_{O_2 max}$ in PH-ExT to that of HC rats (64.9 ± 6.5 vs. 75.4 ± 8.4 ml/kg/min; $P < 0.001$). However, PH-EXT achieved a greater speed during $\dot{V}_{O_2 max}$ testing than PH-SED (51.4 ± 4.1 vs. 44.6 ± 2.6 m/min; $P < 0.001$), which was not different from HC (49.9 ± 3.7 m/min; $P = 0.59$) and a greater tolerable duration of exercise compared to PH-SED (392 ± 50) vs. 268 ± 54 s.; $P < 0.001$) and HC rats (331 ± 55 s.; $P = 0.017$). At rest, total and regional diaphragm $\dot{Q}$ were not different among groups ($P > 0.121$; all groups). However, during exercise, PH-ExT rats had a lower total diaphragm $\dot{Q}$ compared to PH-SED (290 ± 103 vs. 367 ± 149 ml/min/100g; $P = 0.036$), but this was not different from HC (266 ± 68 ml/min/100g: $P = 0.266$). Also, exercising PH-ExT rats displayed a decreased ventral costal (210 ± 63 vs. 304 ± 211 ml/min/100g; $P = 0.05$) medial costal (340 ± 105 vs. 434 ± 235 ml/min/100g; $P = 0.030$), crural (227 ± 55 vs. 474 ± 404 ml/min/100g; $P = 0.006$), and intercostal (45 ± 18 vs. 72 ± 27 ml/min/100g; $P = 0.011$) $\dot{Q}$ compared to PH-SED. Exercising blood [lactate] was 47% lower in PH-ExT versus PH rats (3.2 ± 2.5 vs. 6.0 ± 2.3 mmol; $P < 0.001$). During exercise, PH-ExT rats had lower white gastrocnemius $\dot{Q}$ versus PH-SED (32 ± 21 vs. 54 ± 42 ml/min/100g; $P = 0.050$) and HC (55 ± 27 ml/min/100g; $P = 0.027$). **Conclusions:** Although this moderate-intensity exercise training program did not improve $\dot{V}_{O_2 max}$ in this preclinical model of PH, it improved exercise tolerance and economy and reduced exercising blood lactate. Decreases in total and regional diaphragm and accessory respiratory muscle $\dot{Q}$ support that training decreased the work of breathing in PH. Thus, exercise training can promote cardiorespiratory and muscular adaptations that may help alleviate the burden of PH and potentially increase quality of life.

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List of Abbreviations

6MWD	Six-minute walking distance test
A_b	Reference blood sample intensity
A_t	Individual sample intensity
ANOVA	Analysis of Variance
°C	Degrees Celsius
CO_2	Carbon dioxide
CoA	Coenzyme A
FEV_1	Forced expiratory volume in 1 second
g	Grams
G	Gauge
h	Hour
HC	Healthy controls
Hct	Hematocrit
HR	Heart rate
Hz	Hertz
IC	Intercostal
kg	Kilogram
LSD	Least significant difference
LV	Left ventricle
M	Meters
MAP	Mean arterial pressure
MCT	Monocrotaline
mg	milligram
MG	Mixed gastrocnemius
Min.	Minute
MIP	Maximal inspiratory pressure
mL	Milliliter
mmHg	millimeters of (Hg) mercury
ms	millisecond
MVV	Maximum voluntary ventilation

n	number of
NO	Nitric oxide
O_2	Oxygen
P	Significance value
PA AT	Pulmonary arterial acceleration time
PA ET	Pulmonary arterial ejection time
PA AT/ET	Pulmonary arterial acceleration time/ejection time ratio
PAH	Pulmonary arterial hypertension
PDE-5	Phosphodiesterase 5
pH	Concentration of hydrogen ions
PH	Pulmonary hypertension
PH-ExT	Pulmonary hypertensive exercise trained
PH-SED	Pulmonary hypertensive sedentary
P_{CO2}	Partial pressure of carbon dioxide
P_{O2}	Partial pressure of oxygen
ppm	People per million
$\dot{Q}$	Blood flow
RG	Red gastrocnemius
RV	Right ventricle
RV/LV+S	Fulton index of right ventricular hypertrophy
RVSP	Right ventricular systolic pressure
s	Withdrawal rate (when refering to blood flow assay)
s	Seconds
S_{aO2}	Oxygen saturation
SD	Standard deviation
sGC	soluble Guanylate Cyclase
SV	Stroke volume
T_{lac}	Lactate threshold
µm	Micrometers
VC	Vascular conductance
$\dot{V}_{O_2}$	Oxygen utilization

$\dot{V}_{O_2 max}$	Maximal oxygen uptake
$\dot{V}_{O_2 peak}$	Peak oxygen uptake
$\Delta\dot{V}_{O_2}$	Delta (maximal – resting) oxygen uptake
w	Tissue sample weight
WG	White gastrocnemius

Acknowledgements

I would like to acknowledge my mentors, Dr. Tim Musch and Dr. David C. Poole, for the opportunity to work in their lab and for fostering a nurturing environment for my growth as a researcher and as an individual. Their time, wisdom, and kindness are forever appreciated. I also want to thank my committee for their dedication to the kinesiology graduate students. They have developed a truly special environment allowing for the sharing of ideas, collaboration between labs, and the kindling of valuable friendships. Thank you to Sue Hageman for her contributions to surgical procedures, blood flow assays, rat exercise training, and serving as a maternal figure in Manhattan. Finally, I would like to thank my lab partners, Kiana Schulze and Ramona Weber, for encouraging my pursuit of research, for your priceless guidance, for your words of encouragement, for your willingness to aid in studies, and for our lasting friendship. All of you are such important figures in my collegiate/graduate life, and I am honored to have worked alongside you.

Dedication

To my loving parents Nicholas and Patricia Kenney, thank you for your hard work and sacrifice, allowing us kids to pursue our dreams. To my grandparents, Dean and Carole Lee Kenney, and John and Henrietta Stenger, for your kindness and wisdom in life and for watching over me in death. To my wonderful siblings, Paige, Colin, and Madison, for your lightheartedness and hijinks. To my rock, Abigail Jean Bishop, for being by my side every step of this journey. None of this would have been possible without your words of encouragement, listening ears, and endless support. I love you all!

Chapter 1 - Literature Review

Pulmonary Hypertension

Pulmonary hypertension (PH) is a progressive and debilitating disease of the pulmonary arteries characterized by increased muscularization of the pulmonary arterial vessel walls (Benza et al., 2012) and defined by a resting mean pulmonary arterial pressure (mPAP) > 25 mmHg (Badesch et al., 2010; Hoeper et al., 2017). Patients experience symptoms such as early-onset fatigue during exercise (exercise intolerance), difficulty breathing (dyspnea), and overall decreased quality of life (Simmoneau et al., 2019). PH is a rare cardiopulmonary disease; its symptoms are similar to common pulmonary diseases such as emphysema, often causing patients to be unaware of their condition, leading to delayed treatment and disease progression. The gold standard for PH diagnosis is invasive right ventricular catheterization, which provides direct measurements of pulmonary arterial pressures. An analysis of the U.S. REVEAL registry reports an average survival time from diagnosis of 7 years (Benza et al., 2012). The prevalence of PH varies worldwide due to many factors (poverty, environmental factors, and the prevalence of genetic disease, etc.). It is widely accepted to affect at least 1% of the world population (Hoeper et al., 2016), though the prevalence of PH in the U.S.A. has been estimated at 30.4 ppm (Kirson et al., 2011; Leber et al., 2020). Historically, PH primarily affected young women (NIH, avg. age at diagnosis = 36 years, 59% female; Rich et al., 1987), but over time, with advances in treatment of the disease and the aging population, the COMPERA registry has revealed that the average age of younger individuals at their diagnoses has increased to 54 years favoring females (2.3:1 ratio) (Hoeper et al., 2013).

The increases in pulmonary arterial pressure, definitive of PH are due to chronic inflammation within the lung vasculature (Bowers et al., 2004). This severe oxidative stress

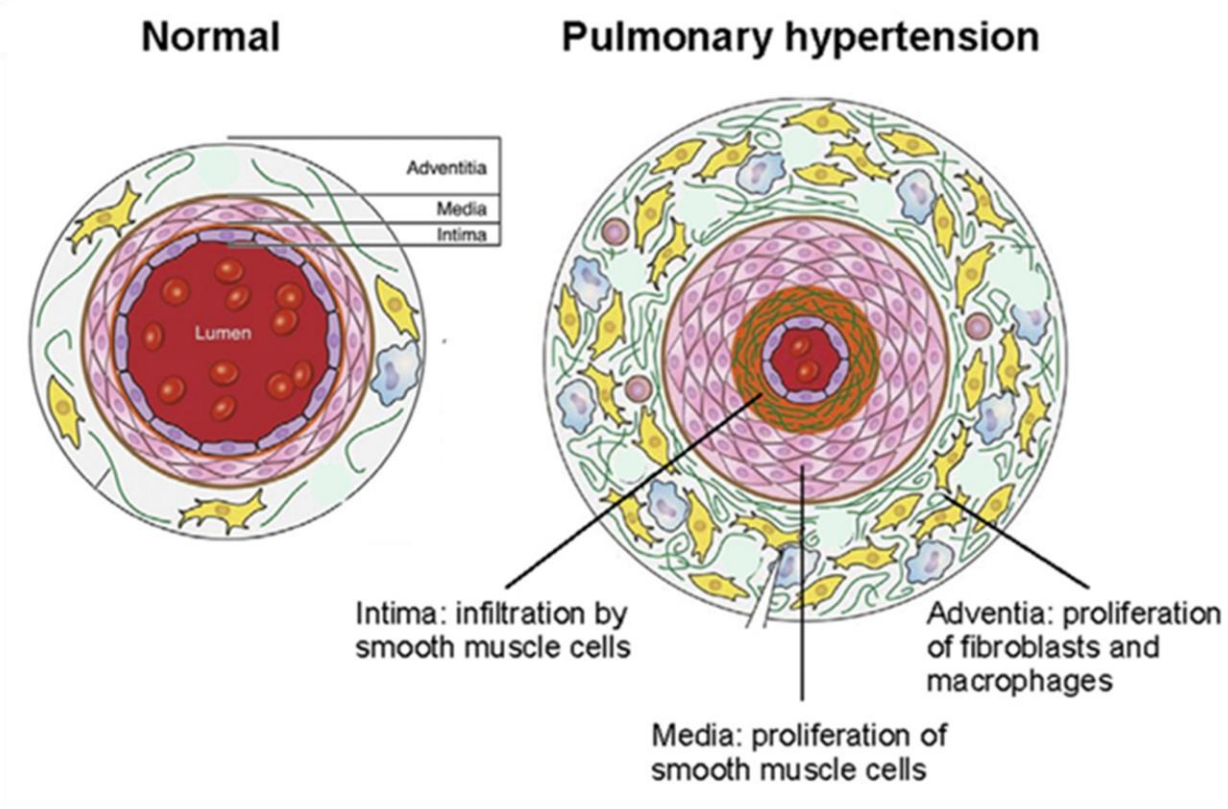

Figure 1.1. Pulmonary vascular remodeling in PH.

This drawing illustrates the vascular dysfunction and remodeling found in PH. The proliferation of the vascular endothelium of the tunica intima and smooth muscle cells of the tunica media causes the narrowing of lumen, resulting in increased pulmonary vascular resistance and pulmonary arterial pressure characteristic of PH. (Gorduek et al., 2016)

damages the vascular endothelium, eventually leading to the proliferation of endothelial and smooth muscle cells within the respective tunica intima and media of the pulmonary arteries (Tuder et al., 2007). This remodeling process results in significant occlusion of the vessel lumen, increasing pulmonary vascular resistance (PVR), and reducing the vasodilatory capacity of the pulmonary arterioles, severely impairing pulmonary blood flow. Such decrements in pulmonary arterial function impair alveolar gas exchange and lead to chronic hypoxia (Sun et al., 2001).

These obstructed vessels markedly elevate right ventricular afterload, thereby contributing to the development of right ventricular dysfunction and, ultimately, failure (Bogaard et al., 2009).

History of Pulmonary Hypertension

Pulmonary hypertension is a relatively "new" disease in terms of awareness of its existence due to the inability to directly study the pulmonary circulation until the late 1920's. Prior to this, investigators and physicians understood how the lungs functioned to allow gas exchange but relied on inaccurate or unreliable methods to evaluate pulmonary hemodynamics and cardiac function, such as the Fick method or CO_2 rebreathing techniques (Newman, 2005). To provide accurate measurements of ventilation and perfusion and venous blood gases, direct measurement techniques such as cardiac catheterization were necessary but deemed too dangerous to perform. The story of the first recorded cardiac catheterization is one of medical malpractice, deception, and the fearless pursuit of knowledge. German physician Werner Forssmann dared to prove that cardiac catheterization was possible via self-experimentation. He convinced a young nurse to aid in this experiment in the guise that she would be the subject. After she prepared the surgical suite for a vena secta, Forssman tied the nurse to the bed and advanced the catheter into his own antecubital vein before walking to the x-ray room to document evidence of the catheter safely located in his heart (Forssman, 1929; Forssman-Falck, 1997). This stunt tarnished Forssmann's career as a physician; however, this promethean event inspired young physiologists, including Dickinson W. Richards, Andre F. Cournand, and colleagues, to use this technique in animal and, eventually, clinical models (Newman, 2005). These experiments established standardized methods to acquire circulatory measurements (Cournand & Ranges, 1941) such as blood gas values (Cournand et al., 1944), pulmonary arterial pressures (Cournand et al., 1945), ventricular and atrial pressures (Cournand et al, 1944;

Cournand & Ranges, 1941), and the effect of disease upon them (Richards, 1957). For their work in revolutionizing physiology via cardiac catheterization, Forssmann, Richards, and Cournand were awarded the 1956 Nobel Prize (West, 2017).

Equipped with the tools to investigate the mysteries of the pulmonary vasculature, the disciples of Cournand and Richards quickly began exploration, with David Dresdale among them. Dresdale is credited as the first to identify cases of increased pulmonary arterial pressures as "primary pulmonary hypertension," describing its association with RV hypertrophy, disease symptoms (Dresdale, 1951), the possibility that the disease might be inherited, and its effects on pulmonary vasculature (lesions, luminal narrowing, medial wall hypertrophy, etc.) (Dresdale et al., 1954). Studies found that the common factor behind PH was hypoxic vasoconstriction, linking the severity of hypoxia with disease progression. By the early 1970s, PH gained worldwide attention after a popular dieting drug, Aminorex, caused a large increase in cases across Europe (Newman, 2005). This event, along with the focus on PH at the 1973 World Health Organization meeting, served as a call to action for developing treatments. Historically, therapies for PH have aimed to reduce hypoxia and chronic vasoconstriction of the pulmonary arteries through vasodilators. Prostacyclin, its analogs, and PDE-5 inhibitors have been commonly prescribed to manage this disease with varying degrees of success, but there was no drug solely dedicated to PH. Following the identification of nitric oxide (NO) by Moncada, Furchgott, Ignarro, and Murad, the understanding of vasodilatory mechanisms expanded. NO induces vasodilation by binding to the heme site of soluble guanylate cyclase (sGC) in vascular smooth muscle, triggering an enzymatic reaction that rapidly increases cGMP production, thereby reducing calcium handling within the vessel and resulting in vasorelaxation. sGC showed promise as a target for PH treatment, and after more than 20 years of research, a tailored

and effective option—sGC stimulator Riociguat—was developed to improve exercise tolerance. Riociguat increases 6MWD and time to exhaustion in patients (Ghofrani et al., 2013), helping to improve quality of life and support other treatments such as exercise training.

The Diaphragm

The diaphragm is a sheet-like muscle that serves as the primary driving force of inspiration, functioning as an essential, continuously active biological pump that maintains ventilatory support from birth until death. Unlike peripheral skeletal muscles, this unique architecture consists of a central tendon anchored to the costal and crural regions, allowing for a piston-like descent that facilitates thoracic expansion and generation of pressure within the thoracic cavity. While the costal segment projects toward the sternum and lower ribs to drive mechanical ventilation, the crural region anchors to the lumbar vertebrae to provide structural stability (Poole et al., 1997).

Given the continuous nature of its activity, the diaphragm exhibits specialized metabolic and contractile properties distinct from other skeletal muscles. This includes a high oxidative capacity, robust vasculature, abundant mitochondrial content, and a greater resistance to fatigue, reflecting its incessant demand for ATP production and sustained contractility. This specialized physiology enables the diaphragm to sustain rhythmic contractions without succumbing to fatigue under normal physiological conditions, a stark contrast to the intermittent activity patterns of other striated muscles (Gransee et al., 2012). However, under conditions of increased respiratory load, such as during intense exercise or in the presence of respiratory disease, the diaphragm's capacity for sustained work can be challenged, leading to the onset of diaphragmatic fatigue. This fatigue can manifest as a reduction in force-generating capacity and altered contractile properties, significantly compromising ventilatory function and overall respiratory

mechanics. Such is seen in PH patients as they present many respiratory muscle abnormalities, including decreased contractile force production of diaphragm muscle fibers (De Man et al., 2009), sarcomeric dysfunction (Manders et al., 2012), and decreased ability to generate inspiratory and expiratory pressures (Meyer et al., 2005; Kabitz et al., 2008) These decrements in respiratory muscle function contribute to dyspnea and exercise intolerance, further exacerbating the clinical presentation of PH.

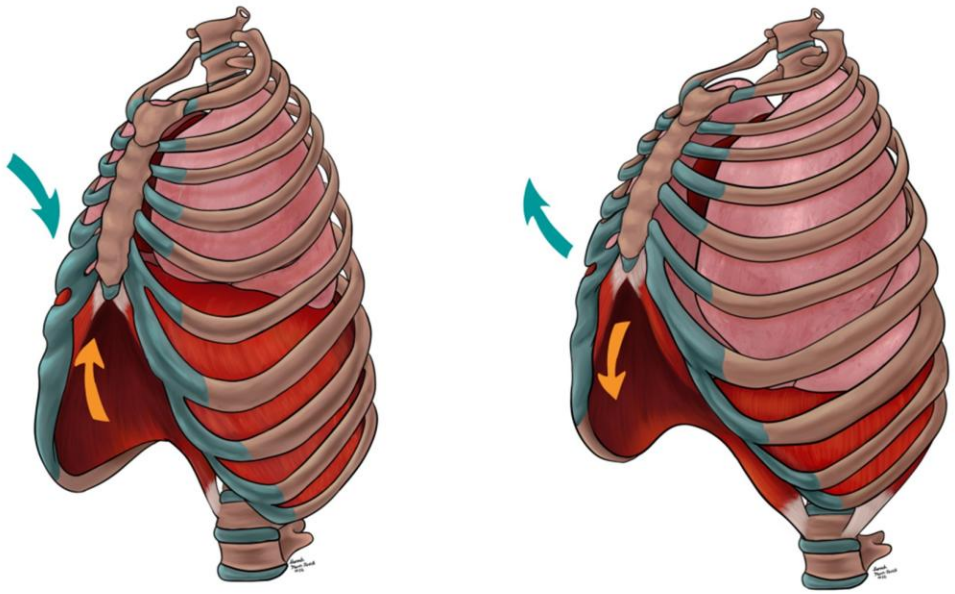

Figure 1.2. Diaphragm movement during inspiration and expiration

This figure shows the orientation of the dome-shaped diaphragm. Additionally displaying the diaphragm's functional movement patterns, increasing intrathoracic volume via contraction of the primary respiratory muscle. These images are original works created by artist Hannah Flores-Ferrell.

During physical exertion, the diaphragm exhibits an exceptional capacity for blood flow and oxidative utilization to sustain elevated ventilatory demands (Poole et al., 2000). Research indicates that the diaphragm possesses a substantial functional vasodilator reserve, allowing for blood flow increases that are commensurate with its high oxidative capacity during maximal

exercise (Poole et al., 2000). This hyperemic response is spatially heterogeneous, as blood flow distribution aligns with regional fiber shortening and ATP consumption rates, specifically favoring the medial costal and crural regions (Brancatisano et al., 1991). However, when the metabolic cost of breathing becomes excessive, the respiratory muscle steal phenomenon occurs, wherein high inspiratory loads divert significant cardiac output away from peripheral locomotor muscles to satisfy the diaphragm's escalating oxygen demands (Harms et al., 1997; Schulze et al., 2024). Consequently, while the diaphragm is uniquely resistant to fatigue due to its distinct fiber-type composition and high capillary density, prolonged exposure to such extreme resistive loading can eventually exhaust its functional reserve (Sieck & Fogarty, 2025). In addition to metabolic challenges, structural remodeling in conditions like emphysema induces chronic hyperinflation, which flattens the diaphragm and significantly reduces the costal surface area available for force generation. This alteration diminishes the muscle's mechanical advantage, causing a compensatory rise in neural drive and increasing diaphragmatic work. Similarly, in pulmonary hypertension, increased pulmonary vascular resistance alters the diaphragm's loading profile, necessitating heightened regional blood flow to meet the accompanying rise in muscular work and oxygen consumption (Schulze et al., 2024).

Exercise Intolerance in PH

The PH patient population is limited in the range of physical activity that they are able to perform due to the oxygen demand of said activities approaching or exceeding that of their own reduced maximal oxygen uptake ($\dot{V}_{O_2\,max}$), resulting in rapid fatigue development and cessation of the activity. There are many factors that contribute to this inability this increased work across different bodily systems. First, due to the nature of the disease and the elevated RV afterload (Bogaard et al., 2009), the interventricular septum is shifted medially, reducing left ventricular

(LV) stroke volume and, consequently, cardiac output, thereby decreasing oxygen delivery. This decreased cardiac output, paired with impaired gas exchange in the lungs, results in a chronic hypoxemia (Sun et al., 2001). Such an environment results in a decreased ability of working skeletal muscle to meet energy demands aerobically, with a reliance on anaerobic glycolysis and the rapid exhaustion of finite fuel sources, accelerating fatigue.

Additionally, there are impairments of the respiratory system, particularly in the primary inspiratory muscle, the diaphragm. Studies have shown that diaphragm function is decreased in PH, as exhibited by decreased forced expiratory volume (FEV_1), maximal voluntary ventilation (MVV) (Sun et al., 2001), and maximal inspiratory pressure generation (MIP), the latter of which is correlated with exercise capacity in PH (Kabitz et al., 2008). Additionally, diaphragm muscle fibers exhibit decreased contractile force in PH (De Man et al., 2009) and in animal models, exhibit impaired vascular function (Schulze et al., 2023), which, in combination, may be a root cause of this diaphragm dysfunction. Further work by Schule (Schulze et al., 2024) found that during submaximal exercise, the diaphragm and its accessory respiratory muscles command greater blood flow at the expense of the hindlimb muscles, accelerating rates of fatigue in what is known as a respiratory muscle “steal” phenomenon (Harms et al., 1997).

These factors, driven by the increased work of breathing and overwhelming dyspnea suffered by PH patients, further limit the ability of this population to perform activities of daily living, decrease quality of life, and further prevent them from performing forms of training that may alleviate these symptoms.

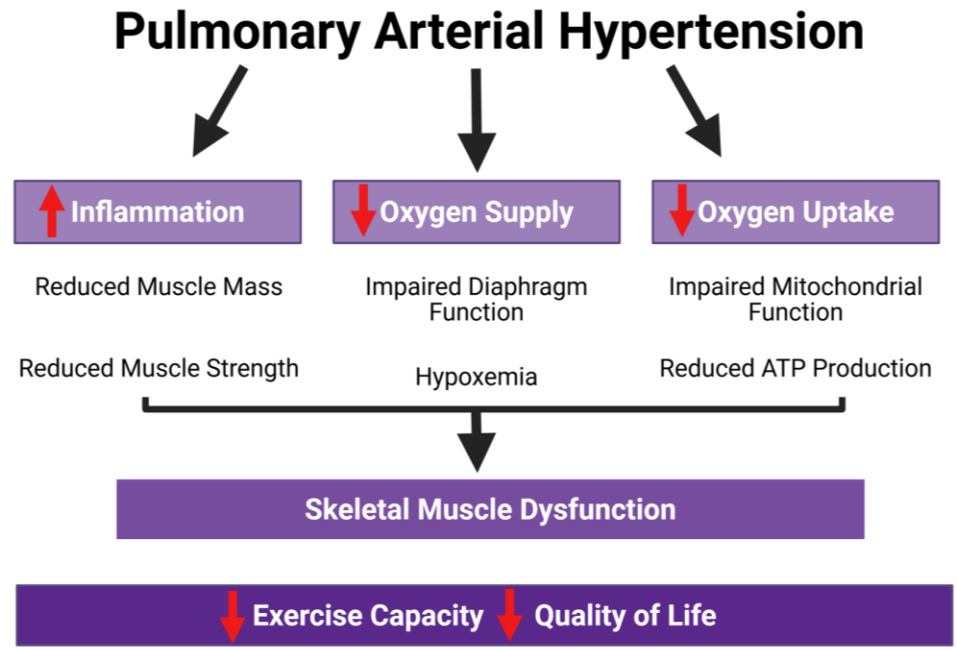

Figure 1.3. Mechanisms of exercise intolerance in PH.

This chart highlights the major impairments in the muscular, respiratory, and immune responses as root causes of exercise intolerance in the PH patient population. Adapted from (Riou et al., 2020).

Summary

Pulmonary hypertension is a silent but deadly disease that greatly decreases the quality of life of its patient population. This disease is defined by the remodeling and elevation of pressures within the pulmonary arteries and characterized by overwhelming dyspnea. PH impairs cardiovascular, muscular, and respiratory function, resulting in a chronic hypoxic environment, increased reliance on anaerobic glycolysis to meet energy demands, accelerated fatigue development, and an inability to sustain most increases in physical activity for extended periods.

Chapter 2 - Introduction

Pulmonary hypertension (PH) is a progressive disease characterized by remodeling of the pulmonary arterial walls and increased resistance within the low-pressure pulmonary circulation. PH impairs gas exchange and prolonged exposure to elevated pressures may progressively induce right ventricular failure (RV) (Bogaard et al., 2009). In addition, elevated RV afterload shifts the interventricular septum leftward, decreasing LV filling and reducing stroke volume and thus cardiac output (Panagiotou et al., 2015; Sun et al., 2001). Such dysfunction results in dyspnea (Sun et al., 2001), tachypnoea, exercise intolerance (Breda et al., 2014; Schulze et al., 2024), and decreased quality of life (Gaine & Rubin, 1998; Halank et al., 2013).

These pernicious adaptations disrupt ventilation-perfusion matching in the lungs, impairing alveolar gas exchange and leading to chronic hypoxemia (Sun et al., 2001). Consequently, there is increased stimulation of peripheral chemoreceptors, which raises ventilatory drive (Farina et al., 2018). These lung impairments, along with the dynamic hyperinflation seen in PH patients, place the diaphragm at a mechanical disadvantage for generating inspiratory flow and increasing lung volume elevating diaphragmatic work (Mitrouska et al., 2022). Evidence supports that the diaphragm is among the first skeletal muscles affected by PH, exhibiting decreased cross-sectional area, reduced number of sarcomeres especially in series (Poole et al. 1994), and diminished contractile force (De Man et al., 2011). The impact of diaphragm dysfunction is further amplified due to increased diaphragm fiber muscle recruitment to meet the energetic demands of the chronic hyperventilation associated with PH.

Accordingly, diaphragm dysfunction and ineffective alveolar ventilation and ventilation/perfusion matching are primary factors contributing to dyspnea in PH. Diaphragmatic weakness further hampers ventilation by reducing the capacity to generate sufficient intrathoracic pressures needed to meet increased ventilatory demands during stress, such as exercise (Sun et al., 2001). Consequently, dyspnea emerges as a significant contributor to exercise intolerance in PH patients, as documented by Kabitz and colleagues (Kabitz et al., 2014) where all subjects reported that their performance in the 6-minute walk distance (6MWD) was limited predominantly by dyspnea rather than muscular fatigue. Additionally, this study found that greater diaphragm strength across patients was positively correlated with 6MWD performance.

Animal studies examining blood flow redistribution in PH models demonstrate increased regional diaphragmatic blood flow during submaximal exercise (Schulze et al., 2024) and during 1-Hz electrically-induced contractions (Schulze et al., 2023) relative to healthy controls. These findings suggest an elevated workload of the diaphragm and a disproportionate increase in demand relative to the limited cardiac output characteristic of PH. This augmented perfusion of respiratory muscles has been shown to be associated with reduced hindlimb blood flow during exercise (Schulze et al., 2024; Long et al., 2022), leading to greater reliance on substrate level metabolism in muscle tissue—specifically, increased glycogen breakdown —to produce ATP, resulting in premature fatigue. This reduction in hindlimb blood flow during exercise has been coined as a respiratory muscle “steal” phenomenon (Harms et al., 1997) and this phenomenon has also been observed in heart failure models in rats (Musch et al., 1993) and in clinical studies of inspiratory loading in individuals (Harms et al., 1997, 2000), providing mechanistic insight

into the maldistribution of cardiac output and inadequate perfusion of peripheral skeletal muscles within PH and the associated exercise intolerance.

Investigations regarding the effect of PH on oxygen delivery during exercise intolerance have recently been conducted in monocrotaline (MCT)-induced PH rats. Studies of rats with severe PH and RV failure performing treadmill running at 50% of their maximal oxygen uptake ($\dot{V}_{O_2 max}$) (Long et al., 2022, 2025) and also moderate PH prior to RV failure running at an absolute submaximal treadmill running intensity revealed similar findings of reduced hindlimb blood flow ($\dot{Q}$) (Schulze et al., 2024; Long et al., 2022, 2025). In addition, PH rats exercising at a fixed absolute submaximal treadmill workload demonstrated increases in respiratory muscle $\dot{Q}$ (Schulze et al., 2024) and blood lactate accumulation during exercise (Schulze et al., 2024; Long et al., 2022, 2025) compared to healthy controls (HC). These data suggest that the exercise intolerance present in PH patients may, at least in part, be related to the maldistribution of cardiac output and also hypoxemia, impacting the locomotory musculature.

Exercise training has been shown in clinical studies of PH patients to increase exercise capacity (Mereles et al., 2006; Mainguy et al., 2010; Grünig et al., 2011, 2012) endurance (De Man et al., 2009), and quality of life (Mereles et al., 2006; Grünig et al., 2011, 2012; Chan et al., 2013). Similarly, exercise training has been demonstrated to produce increases in exercise capacity in animal PH models (Brown et al., 2017). While these studies have provided valuable information on the efficacy of exercise training as an adjunct treatment for the disease, the effect of exercise training on the perfusion of respiratory and peripheral skeletal muscles in PH remains to be determined.

The objective of this investigation was to examine, in PH rats, how exercise training affects bulk and regional diaphragm and hindlimb blood flow ($\dot{Q}$) at rest and during submaximal

exercise, as well as exercise capacity prior to the development of overt RV failure. We hypothesized that MCT-induced PH rats, upon completion of a 4-week moderate-intensity treadmill running program, would show: (1) greater exercise capacity as indicated by an increase in $\dot{V}_{O_2 max}$ and running speed (2) decreased regional and total diaphragm $\dot{Q}$ and vascular conductance (VC) at rest and during submaximal exercise, and (3) higher skeletal muscle $\dot{Q}$ during exercise compared to sedentary PH rats but not different from HC rats. The findings of this study will demonstrate the potential for exercise training adaptations to help correct impaired cardiovascular function, which contributes to respiratory and skeletal muscle dysfunction and exercise intolerance in PH populations.

Chapter 3 - Methods

Animals

This study utilized 39 female Sprague–Dawley rats (aged 4–6 months) sourced from Charles River Laboratories, as most preclinical PH studies fail to account for the clinical sex-specific bias (4:1 female-to-male ratio) (Badesch et al., 2010). The rats were randomized into a healthy control group (HC; $n = 12$), monocrotaline (MCT)-induced pulmonary hypertension group (PH-SED; $n = 14$), and an exercise-trained MCT-induced pulmonary hypertension group (PH-ExT; $n = 13$). After delivery, the rats were kept in a temperature-controlled (23 ± 2°C) environment with a 12:12-h light–dark cycle and were fed and watered ad libitum.

Monocrotaline-Induced Pulmonary Hypertension

The induction of PH involved a solitary intraperitoneal administration of the plant alkaloid monocrotaline (MCT; BOC Sciences, Shirley, NY, USA; 50 mg/kg), a technique known to elicit a moderate-severity phenotype of PH (Gomez-Arroyo et al., 2012; Schulze et al., 2021, 2022, 2023, 2024, 2025). MCT was dissolved in a solution of 50% sterile saline and 50% 200-proof ethanol at room temperature; PH rats were administered a dose of MCT in vehicle, while HC rats received the vehicle alone. While maintained on 1–2% isoflurane, injections were given immediately after baseline echocardiography using a 27 G needle in the lower right abdominal quadrant, with care taken to protect internal organs. Core temperature was maintained at 37°C. Crucially, PH hallmarks—such as increased pulmonary artery pressures, remodeling of small pulmonary vessels, and right ventricular hypertrophy manifest 3–4 weeks following injection, prior to the emergence of congestive heart failure (Gomez-Arroyo et al., 2012). Rats were checked weekly via ultrasound under anesthesia, alongside assessments of body mass and health status.

Echocardiography

Using a commercial system (Logiq S8; GE Health Care, Milwaukee, WI, USA) fitted with a 13 MHz linear transducer (L4-12t), transthoracic echocardiography was conducted before the MCT or saline dosing. Follow-up echocardiography was performed weekly to track the development of the disease. All data were contrasted between the initial measurement and the final assessment performed immediately before the experiment. Animals were initially sedated with a 5% isoflurane-O_2 blend and kept on ≤2.5% isoflurane–O_2. The duration of anesthesia was under 10 minutes for this task. B-mode and M-mode recordings of the left ventricle were taken from the parasternal short axis view and assessed for stroke volume, ejection fraction, and end-systolic/diastolic volumes (Craig et al., 2019). RV images were obtained at the level of the aortic valve near the pulmonary valve. Pulsed Doppler ultrasound was utilized to evaluate pulmonary artery ejection time (ET), acceleration time (AT) , and peak velocity; the AT/ET ratio served as the diagnostic marker for confirming PH (AT/ET < 0.3) (Jones et al., 2002). Upon disease confirmation, the subsequent procedures were executed. In the current study, survival time was measured as the period from the initial injection of MCT to the final echocardiogram confirming PH development.

Treadmill Training

All rats were acclimated to treadmill running 5 days after injection. Rats ran for 5 minutes per day at 25 m/min on a 5% grade for 5 days. This protocol does not produce training adaptations within the animals (Armstrong & Laughlin, 1984; Musch et al., 1992). Following acclimation, PH rats were randomized into exercise training (PH-ExT) and sedentary (PH-SED) groups. Exercise training rats then performed pre-training maximal oxygen uptake ($\dot{V}_{O_2\,max}$) testing. Eleven days after injection, PH-ExT rats began exercise training. On day 1, the rats ran

continuously for 15 minutes at 25 m/min and a 5% grade. The duration of subsequent training sessions increased by 5 minutes each day until reaching a total of 60 minutes per day. Afterward, treadmill grade, speed, and duration remained constant for the rest of the 4-week training period. Untrained rats were restricted to cage activity. During treadmill acclimation and training sessions, manual bursts of compressed air directed at the rat's hindlimb were used to encourage treadmill running. This intensity and duration were chosen to maximize mitochondrial adaptations within the hindlimb (Dudley et al., 1982).

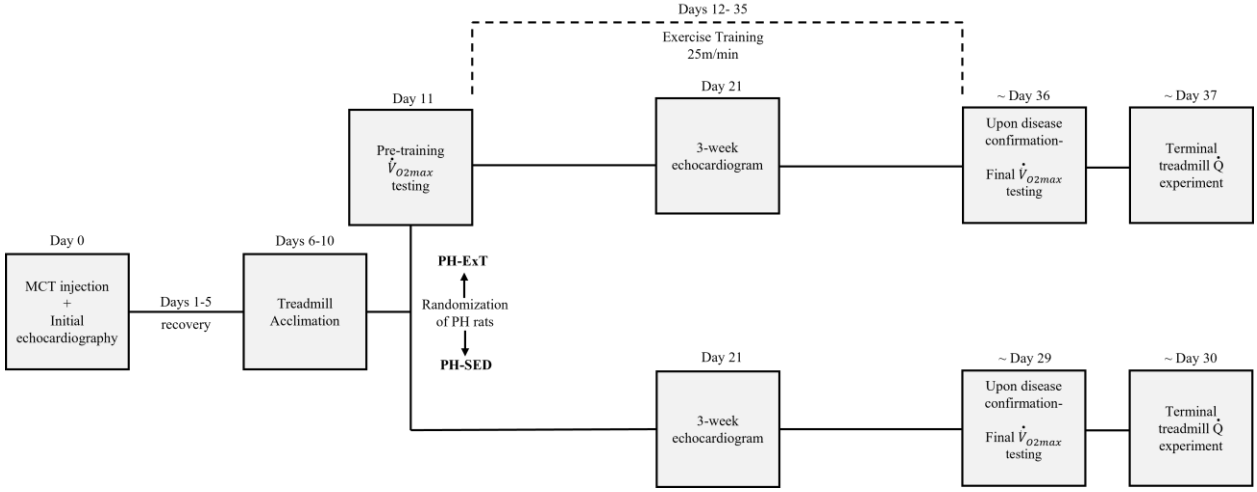

Figure 3.1. Experimental timeline

This schematic displays the timing of key events from MCT injection through initiation of the exercise training protocol to the terminal treadmill Q̇ experiment. "Day(s)" above lines/boxes signify time since MCT injection.

$\dot{V}_{O_2max}$ *Testing*

$\dot{V}_{O_2max}$ assessments were conducted in a Plexiglas metabolic chamber placed on the treadmill (Musch et al., 1988). In all $\dot{V}_{O_2max}$ protocols, rats were prompted to continue via an electrical stimulator at the rear of the chamber. Gas concentrations were recorded in real-time using O_2 and CO_2 analyzers (model S-3A/I and CD-3A; AEI Technologies). The analyzers were standardized before each session using precision gases matching the expected ranges (Musch et al., 1988). Beginning at 25 m/min for 2 min, the speed was increased by 5 m/min each minute

until the rat could no longer keep pace or $\dot{V}_{O_2}$ reached a plateau. Rats were weighed after the test, and these values were used for $\dot{V}_{O_2}$ calculations. High levels of reproducibility for $\dot{V}_{O_{2peak}}$ measurements have been established in our laboratory using these specific methods (Copp et al., 2009). Furthermore, $\dot{V}_{O_{2peak}}$ has been shown to be equivalent to $\dot{V}_{O_{2max}}$ in rats under similar protocols (Bedford et al., 1979).

Hemodynamic Measurements

Surgical Preparation:

Aseptic methods were employed for all surgical interventions. On the day of the terminal protocol, rats were initially anesthetized with a 5% isoflurane–O2 mixture and subsequently maintained on 2.5% isoflurane–O2. Proper depth of anesthesia was confirmed and monitored by the absence of reflexes throughout the ~45-minute preparation. A neck incision was made, and the right jugular vein was isolated and cannulated with a 2-French pressure micromanometer to obtain right ventricular systolic pressure (RVSP) via a PowerLab system. Following measurement, the sensor was removed, the vein ligated, and the right common carotid artery exposed and catheterized for monitoring heart rate, mean arterial pressure, and microsphere delivery. A second catheter was placed in the tail artery for blood sampling. Both lines were tunneled under the skin to the neck and exteriorized. The incisions were closed, anesthesia was stopped, and rats were granted >60 min for recovery (Flaim et al., 1984).

Post-recovery Measurements

After resting for >45 min, a second microsphere injection (with a different color) was performed while the rat remained still to measure resting blood flow. This design reduces the impact of the anticipatory response on HR and blood flow data (Armstrong et al., 1989). The microsphere colors were randomized across the experimental conditions.

Tissue Sampling and Blood Flow Determination

After the final infusion, rats were anesthetized and euthanized with a pentobarbital overdose (>50 mg/kg i.a.) followed by heart removal. Tissues (diaphragm, soleus, plantaris, gastrocnemius, intercostals, and kidneys) were collected, weighed, and stored at −80°C. The soleus and white gastrocnemius were chosen to verify that the intensity was sufficient to increase flow across different fiber types (Delp & Duan, 1996; Laughlin & Armstrong, 1983). Following established templates (Horn et al., 2020, 2022, 2024; Poole et al., 1997; Schulze et al., 2023), the diaphragm was divided into costal and crural segments to assess regional distribution. The microsphere assay followed standard protocols (Deveci & Egginton, 1999). Blood flow (Q̇) was calculated as:

$$(\dot{Q} = [(A_t/A_b) * (s/w)] * 100$$

where Q̇ is blood flow (ml/min/100g), At is the individual sample intensity, Ab is the reference blood sample intensity, s is the withdrawal rate (0.25 mL/min) of the reference blood sample, and w is the tissue weight (g). Vascular conductance was calculated as:

$$VC = \dot{Q}/MAP$$

where VC is vascular conductance (ml/mmHg/min/100 g), Q̇ is blood flow (ml/min/100 g) and MAP is the average mean arterial pressure (mmHg), recorded immediately before and after microsphere infusion. Effective mixing was confirmed by checking for <20% difference between left and right kidneys; failed mixing led to data exclusion.

Postmortem Measurements

After euthanasia, the required muscles, kidneys, RV, LV, and lungs were dissected out and weighed. To quantify RV hypertrophy, the Fulton index was calculated as: RV weight

(mg)/LV + septum (S) weight (mg) (Fulton et al., 1952). Tissue masses w additionally expressed relative to body weight and tibial bone length.

Statistical Analysis

Data analysis and graphing was conducted via GraphPad Prism 11 (GraphPad Software, Boston, MA, USA). One-way ANOVA was used to detect differences in $\dot{V}_{O_2max}$, RVSP, and morphological measurements. Within-animal comparisons were made using paired Student's t-tests. Ultrasound data were analyzed using a two-way ANOVA, while tissue flows were analyzed using a mixed-effects model. In the event that a dataset failed to display equal variance or meet normality, the nonparametric Kruskal-Wallis test was conducted with intergroup comparisons performed via the uncorrected Dunn’s test. The Grubbs' outlier test was applied to identify and remove a singular outlier from each data set (if present). Post hoc testing utilized two-tailed uncorrected Fisher’s LSD test. All results are reported as means (SD), with significance set at $P < 0.05$.

Chapter 4 - Results

Morphometric and Echocardiographic Data

Data were analyzed from 10 PH-ExT (n = 13), 14 PH-SED (n = 14), and 12 HC (n = 12) rats. Three PH-ExT rats were excluded from morphometric measurements due to sudden deterioration of health, resulting in the inability to perform post-training $\dot{V}_{O_2max}$ testing and treadmill blood flow experiments, resulting in a total of n = 37 for morphological and n = 39 for echocardiographic measures across all groups. PH-ExT and PH rats displayed increased lung weight, lung/body mass, lung/tibial bone length, RVSP, and RV/LV+S, compared to HC rats (Table 1). There were no differences in LV and RV echocardiographic parameters or body mass among groups (HC: 304 ± 39, PH-SED: 293 ± 34, PH-EXT: 296 ± 20 g; P > 0.999) at the time of injection. Final measurements revealed that PH rats (ExT and SED) had reduced PA AT/ET (Table 2; P = 0.002), with no differences detected in LV echocardiographic parameters among groups at the time of injection (Table 2; P > 0.345). PH-SED rats had a decreased body mass compared to HC (302 ± 33 vs. 331 ± 35 g; P = 0.021) and PH-EXT (324 ± 15 g; P = 0.045) (Table 1). Additionally, survival time was greater in PH-ExT compared with PH-SED (36.4 ± 7.3 vs. 29.7 ± 6.6 days; P = 0.019), consistent with previous observations (Drummond et al., 2023).

Maximal Oxygen Uptake ($\dot{V}_{O_2max}$)

Four rats (PH-SED; n= 2, PH-ExT; n = 2) were excluded from post-intervention $\dot{V}_{O_2max}$ testing due to injury or rapid disease progression and weight loss, resulting in a total of n = 35 for $\dot{V}_{O_2max}$ measurements across all groups. $\dot{V}_{O_2max}$ was not significantly different in PH-EXT rats from pre- to post-training (68.5 ± 6.7 vs. 64.9 ± 6.5 ml/kg/min; P = 0.249); however, PH-ExT pre-training values were lower than HC (68.5 ± 6.7 vs. 75.41 ± 8.4 ml/min/kg; P = 0.028).

PH rats (ExT and SED) had a lower $\dot{V}_{O_2max}$ compared to HC rats (Figure 4; P = 0.010). Despite this, PH-ExT rats had a greater total running time than both HC (392 ± 50 vs. 331 ± 55 s; P = 0.025) and PH-SED rats (268 ± 5 s; P < 0.001). PH-EXT rats also achieved a greater peak speed compared with PH-SED rats (51.4 ± 4.1 vs. 44.6 ± 2.6 m/min; P = 0.040). The change in $\dot{V}_{O_2}$ for a given speed ($\Delta\dot{V}_{O_2}$/speed) was reduced in PH-ExT compared to PH-SED (0.73 ± 0.17 vs. 0.98 ± 0.15 ml/kg/min/(m/min); P = 0.004) (Figure 5).

Resting and exercising heart rate and mean arterial pressure

At rest, PH-ExT rats exhibited decreased heart rate (HR) compared to PH-SED rats (365 ± 48 vs. 398 ± 50 bpm; P = 0.062), however, mean arterial pressure (MAP) was not significantly different among groups (Table 4). During exercise, HR was lower in PH-ExT compared with both HC (450 ± 53 vs. 486 ± 49 bpm; P = 0.038) and PH-SED (500 ± 33 bpm; P = 0.028), and all groups increased heart rate from rest to exercise. Similarly, exercising MAP was lower in PH-ExT compared to both HC (118 ± 15 vs. 130 ± 17 mmHg; P = 0.026) and PH-SED (vs. 137 ± 12 mmHg; P = 0.003). MAP increased from rest to exercise in HC and PH-SED (P < 0.001) but not PH-ExT (116 ± 14 vs. 118 ± 15 mmHg; P = 0.932).

Resting and exercising arterial blood gases and acid-base parameters

At rest, no differences in arterial blood gases were observed among groups (Table 3). During exercise, arterial pH in PH-ExT rats was not significantly different from HC (7.44 ± 0.04 vs. 7.44 ± 0.06; P = 0.860) but was greater than PH-SED (vs. 7.37 ± 0.12; P = 0.018), where pH decreased from rest to exercise (7.46 ± 0.04 vs. 7.37 ± 0.12; P < 0.001) while PH-ExT did not. Arterial P_{O2} was similarly reduced in PH-ExT and PH-SED compared to HC (95.5 ± 13.3 vs. 119.3 ± 11.8 mmHg; P = 0.005 and 97.2 ± 25.8 mmHg; P = 0.007, respectively), with no

significant difference between PH groups. Blood lactate was lower in PH-ExT compared with PH-SED during exercise (3.2 ± 2.5 vs. 6.0 ± 2.3 mmol; $P < 0.001$), and lactate increased from rest to exercise in all groups ($P < 0.001$). No differences in P_{CO2}, oxygen saturation, or hematocrit were observed among groups during exercise (Table 3). P_{CO2} decreased from rest to exercise in HC and PH-SED ($P < 0.05$) but not PH-ExT, and oxygen saturation and hematocrit did not change from rest to exercise in any group.

Resting and exercising diaphragm blood flow

Four rats (PH-SED, n = 2; PH-ExT, n = 2) were excluded from submaximal microsphere infusion due to injury, trauma during instrumentation, or the rapid onset of disease progression. Exercising flows from 1 HC, and all blood flow data from 3 PH-SED rats were excluded due to procedural error during tissue digestion and during microsphere infusion, resulting in a total of (n = 34) for resting and (n = 30) exercising blood flow measures across all groups. Analysis of increases in absolute $\dot{Q}$ from rest to exercise was performed using data collected from rats that performed both resting and submaximal exercise microsphere infusion. At rest, there were no differences among groups in bulk diaphragm $\dot{Q}$ (Figure 6). Diaphragm VC in PH-ExT rats was not statistically different from either PH-SED (1.10 ± 0.44 vs. 1.51± 0.67; $P = 0.146$) or HC (vs. 0.95 ± 0.32 ml/mmHg/min/100g; $P = 0.519$); however, PH-SED rats had an elevated diaphragm VC compared with HC (Figure 7; $P = 0.035$).

During exercise, total diaphragm $\dot{Q}$ and VC in PH-ExT rats were not significantly different from HC (Figure 6; $P = 0.266$ and $P = 0.270$, respectively). In contrast, PH-SED rats exhibited elevated total diaphragm $\dot{Q}$ compared with both HC (367 ± 149 vs. 266 ± 68 ml/min/100g; $P = 0.007$) and PH-ExT (vs. 290 ± 103 ml/min/100g; $P = 0.032$), and an elevated total diaphragm VC compared with HC (2.63 ± 1.08 vs. 2.05 ± 0.44 ml/mmHg/min/100g; P =

0.024) but not PH-ExT (vs. 2.21 ± 0.70 ml/mmHg/min/100g; P = 0.106) (Figure 7). Total diaphragm $\dot{Q}$ and VC increased from rest to exercise in all groups (Figures 6 & 7).

At rest, no regional differences in diaphragm or intercostal $\dot{Q}$ or VC were observed among groups, with the exception of crural VC, where VC was elevated in PH-SED compared with both HC (1.65 ± 1.60 vs. 1.30 ± 0.60 ml/mmHg/min/100g; P = 0.016) and PH-ExT (vs. 1.37 ± 0.80 ml/mmHg/min/100g; P = 0.047) (Figures 8 & 9).

During exercise, regional diaphragm and intercostal $\dot{Q}$ in PH-ExT rats were not significantly different from HC across all regions (Figure 10). PH-SED rats, however, exhibited elevated $\dot{Q}$ compared with HC in the medial costal (P = 0.007), dorsal costal (P = 0.050), crural (P = 0.008), and intercostal regions (P = 0.008). In addition, PH-SED rats also exhibited elevated $\dot{Q}$ when compared with PH-ExT in the ventral costal ($P \leq 0.05$), medial costal (P = 0.029), crural (P = 0.006) and intercostal muscles (P = 0.011).

PH-ExT rats did not show an increase in ventral costal $\dot{Q}$ from rest to exercise (152 ± 73 vs. 210 ± 63 ml/min/100g; P = 0.121), unlike HC and PH-SED (Figures 8 & 10). $\dot{Q}$ increased from rest to exercise within the medial and dorsal costal regions in all groups. Crural $\dot{Q}$ increased from rest to exercise in PH-ExT and PH-SED but not HC (P = 0.117), and intercostal $\dot{Q}$ increased in all groups.

During exercise, regional VC in PH-ExT rats was not significantly different from HC in any region (Figure 11). In contrast, PH-SED rats exhibited elevated VC compared with HC in the crural region (P = 0.032) and intercostal muscles (P = 0.032), and compared with PH-ExT in the intercostal muscles (P = 0.016).

Ventral costal and intercostal VC increased from rest to exercise in PH-SED and HC but not PH-ExT (ventral costal: 1.37 ± 0.80 vs. 1.74 ± 0.50 ml/mmHg/min/100g, P = 0.266; intercostal: 0.24

± 0.16 vs. 0.37 ± 0.10 ml/mmHg/min/100g, P = 0.089, Figures 9 & 11). Medial and dorsal costal VC increased from rest to exercise in all groups, and crural VC did not increase from rest to exercise in any group.

Overall, absolute increases in regional diaphragmatic $\dot{Q}$ from rest to exercise along with their increases from rest as depicted by the percent increase of $\dot{Q}$ are shown in Figure 12. As demonstrated in this figure, the PH-ExT response to exercise begins to look like the response found in the HC group of rats (and significantly different from those found in the PH-SED group of rats), demonstrating the beneficial effects of exercise training in this preclinical PH model.

Resting and exercising hindlimb blood flow

At rest, no differences in hindlimb $\dot{Q}$ were observed among groups (P > 0.05) (Figure 13). During exercise, PH-ExT rats exhibited a lower white gastrocnemius $\dot{Q}$ compared with both HC (32 ± 21 vs. 55 ± 27 ml/min/100g; P = 0.027) and PH-SED (vs. 54 ± 42 ml/min/100g; P = 0.050) (Figure 14), and did not increase white gastrocnemius $\dot{Q}$ from rest to exercise (P = 0.691), unlike HC and PH-SED. All other hindlimb muscle $\dot{Q}$ were not significantly different among groups during exercise (Figure 14), and increased from rest to exercise in all groups. No differences in kidney $\dot{Q}$ were observed among groups at rest or during exercise (Group: P = 0.377; Condition: P = 0.877; Interaction: P = 0.620). Kidney flows ranged from 740–1000 ml/min/100g, which is slightly greater than previously reported (Schulze et al., 2024, 2025). In PH-Ext rats, soleus $\dot{Q}$ was positively correlated to total diaphragm $\dot{Q}$ during exercise (Figure 15), a relationship not observed in untrained PH rats (Schulze et al., 2024).

Table 1. Morphometric and Millar Measurements				
Animal Characteristic	Healthy Control (n = 12)	PH-SED (n = 14)	PH-ExT (n = 10)	*P*
Body weight (g)	331 ± 35	302 ± 33 *	324 ± 15 †	**≤ 0.05**
Diaphragm (mg)	917 ± 106	891 ± 98	958 ± 84	**0.002**
Diaphragm/body weight (mg/g)	2.89 ± 0.39	2.96 ± 0.27	3.15 ± 0.36	0.182
RV (mg)	174 ± 26	193 ± 46	206 ± 46	0.188
LV+S (mg)	685 ± 45	603 ± 58 *	698 ± 74 †	**<0.001**
RV/LV+S	0.25 ± .03	0.32 ± 0.07 *	0.32 ± 0.09 *	**0.012**
RV/body weight (mg/g)	0.53 ± 0.07	0.64 ± 0.16	0.66 ± 0.16	0.059
Lungs (mg)	1160 ± 158	1795 ± 613 *	1762 ± 640 *	**<0.001**
Lungs/body weight (mg/g)	3.14 ± 0.26	6.07 ± 2.50 *	5.00 ± 1.42 *	**<0.001**
RV/tibial bone length (mg/mm)	3.98 ± 0.61	4.52 ± 1.08	4.81 ± 1.07	0.121
Lung/tibial bone length (mg/mm)	26.5 ± 3.5	42.3 ± 14.8 *	41.1 ± 14.8 *	**<0.001**
RVSP (mmHg)	21.4 ± 2.1	34.9 ± 12.0 *	38.7 ± 17.8 *	**0.001**

Table 4-1. Morphometric and Millar measurements.

Values are means ± SD. RV, right ventricle; LV+S, left ventricle plus septum; RVSP, right ventricular systolic pressure. Analyzed via One-Way ANOVA. * $P \leq 0.05$ vs. healthy, † $P \leq 0.05$ vs. PH-SED.

Table 2. Echocardiographic Measurements									
	Healthy Control		PH-SED		PH-ExT		*P*		
	Pre (n = 12)	Post (n = 12)	Pre (n = 14)	Post (n = 14)	Pre (n = 13)	Post (n = 13)	Group	Time	Interaction
PA ejection time (ms)	88 ± 7	86 ± 7	88 ± 5	85 ± 5	89 ± 4	87 ± 3	0.635	0.062	0.716
PA acceleration time (ms)	32 ± 5	32 ± 6	30 ± 3	23 ± 3 *#	32 ± 3	24 ± 1 *#	**<0.001**	**<0.001**	**<0.001**
PA AT/ET	0.37 ± 0.06	0.38 ± 0.07	0.34 ± 0.04	0.27 ± 0.03 *#	0.36 ± 0.04	0.26 ± 0.03 *#	**0.002**	**<0.001**	**<0.001**
LV ejection fraction (%)	84 ± 7	85 ± 10	86 ± 7	88 ± 6	84 ± 5	85 ± 7	0.351	0.448	0.977
LV stroke volume (ml)	0.55 ± 0.12	0.62 ± 0.19	0.56 ± 0.07	0.53 ± 0.10	0.64 ± 0.13	0.54 ± 0.16	0.345	0.519	0.101
LV fractional shortening (%)	48 ± 7	51 ± 13	52 ± 10	53 ± 8	48 ± 6	50 ± 9	0.345	0.260	0.922

Table 4-2. Echocardiographic measurements.

.Values are means ± SD. PA, pulmonary artery; AT/ET, acceleration time/ ejection time. * $P \leq 0.05$ vs. HC within condition, # $P \leq 0.05$ vs. Pre-injection within group.

Table 3. Arterial Blood Gas Measurements						
	Healthy Control	PH-SED	PH-ExT	*P*		
	(*n* = 12)	(*n* = 12)	(*n* = 10)	Group	Condition	Interaction
Rest						
pH	7.46 ± 0.05	7.46 ± 0.04	7.47 ± 0.04			
P_{CO2} *(mmHg)*	26.5 ± 5.2	24.8 ± 3.4	24.3 ± 4.4			
P_{O2} *(mmHg)*	107.8 ± 13.2	99.3 ± 24.8	92.5 ± 23.1			
S_{aO2} *(%)*	99.7 ± 0.5	98.6 ± 1.9	99.0 ± 1.6			
Hct (%)	32.4 ± 2.0	32.6 ± 3.2	30.9 ± 3.6			
Lactate (mmol)	0.53 ± 0.17	0.86 ± 0.35	0.64 ± 0.23			
Exercise						
pH	7.44 ± 0.06	7.37 ± 0.12 *#	7.44 ± 0.06 #†	0.146	**0.002**	0.070
P_{CO2} *(mmHg)*	22.4 ± 3.2 #	20.8 ± 3.1 #	22.0 ± 4.7	0.445	**0.005**	0.712
P_{O2} *(mmHg)*	119.3 ± 11.8 #	97.2 ± 25.8 *	95.5 ± 13.3 *	**0.021**	0.270	0.215
S_{aO2} *(%)*	99.7 ± 0.5	96.0 ± 6.1	95.4 ± 9.0	0.123	0.064	0.366
Hct (%)	33.7 ± 3.3	33.1 ± 4.0	30.8 ± 4.8	0.110	0.510	0.793
Lactate (mmol)	3.5 ± 1.4 #	6.0 ± 2.3 *#	3.2 ± 2.5 #†	**<0.001**	**0.003**	**0.011**

Table 4-3. Arterial blood gas measurements

Values are means ± SD. S_{aO2}, oxygen saturation; Hct, hematocrit. Analyzed via mixed effects model. * $P \leq 0.05$ vs. healthy within condition, # $P \leq 0.05$ vs. rest within group, † $P \leq 0.05$ vs. PH-SED within condition.

Table 4. Cardiovascular Responses to Exercise						
	Healthy Control	PH-SED	PH-ExT	*P*		
	(*n* = 12)	(*n* = 12)	(*n* = 10)	Group	Condition	Interaction
Pre-Exercise						
HR (bpm)	387 ± 60	398 ± 50	365 ± 48 †			
MAP (mmHg)	120 ± 16	118 ± 13	116 ± 14			
Submax. Exercise						
HR (bpm)	486 ± 49 #	500 ± 33 #	450 ± 53 #*†	**0.019**	**< 0.001**	0.734
MAP (mmHg)	130 ± 17 #	137 ± 12 #*	118 ± 15 *†	0.130	**< 0.001**	**0.020**

Table 4-4. Cardiovascular responses to exercise.

Values are means ± SD. Analyzed via mixed effects model. * $P \leq 0.05$ vs. healthy within condition, # $P \leq 0.05$ vs. rest within group, † $P \leq 0.05$ vs. PH-SED within condition.

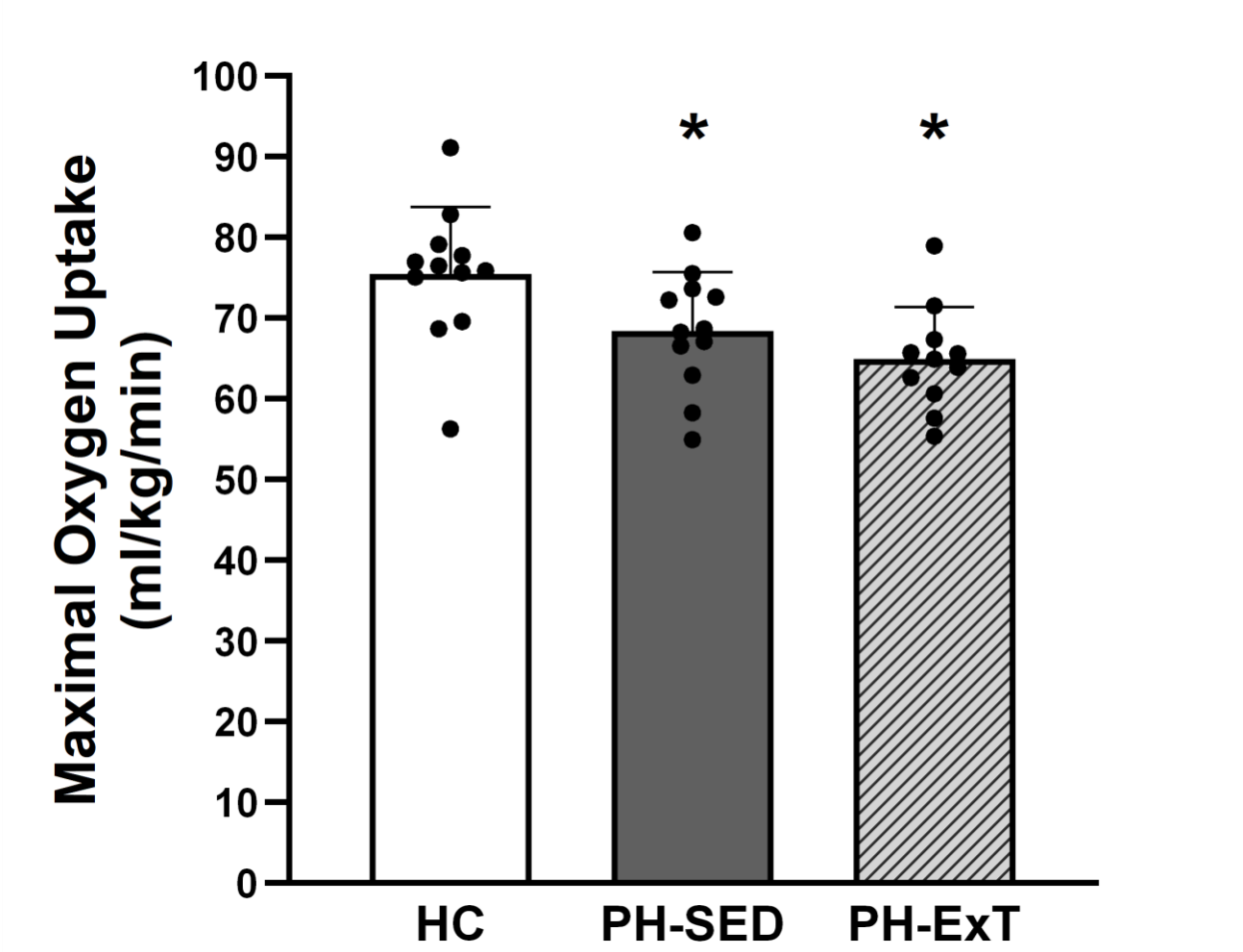

Figure 4.1. Maximal oxygen uptake ($\dot{V}O_{2max}$).

Analyzed via one-way ANOVA. * P < 0.022 vs. healthy.

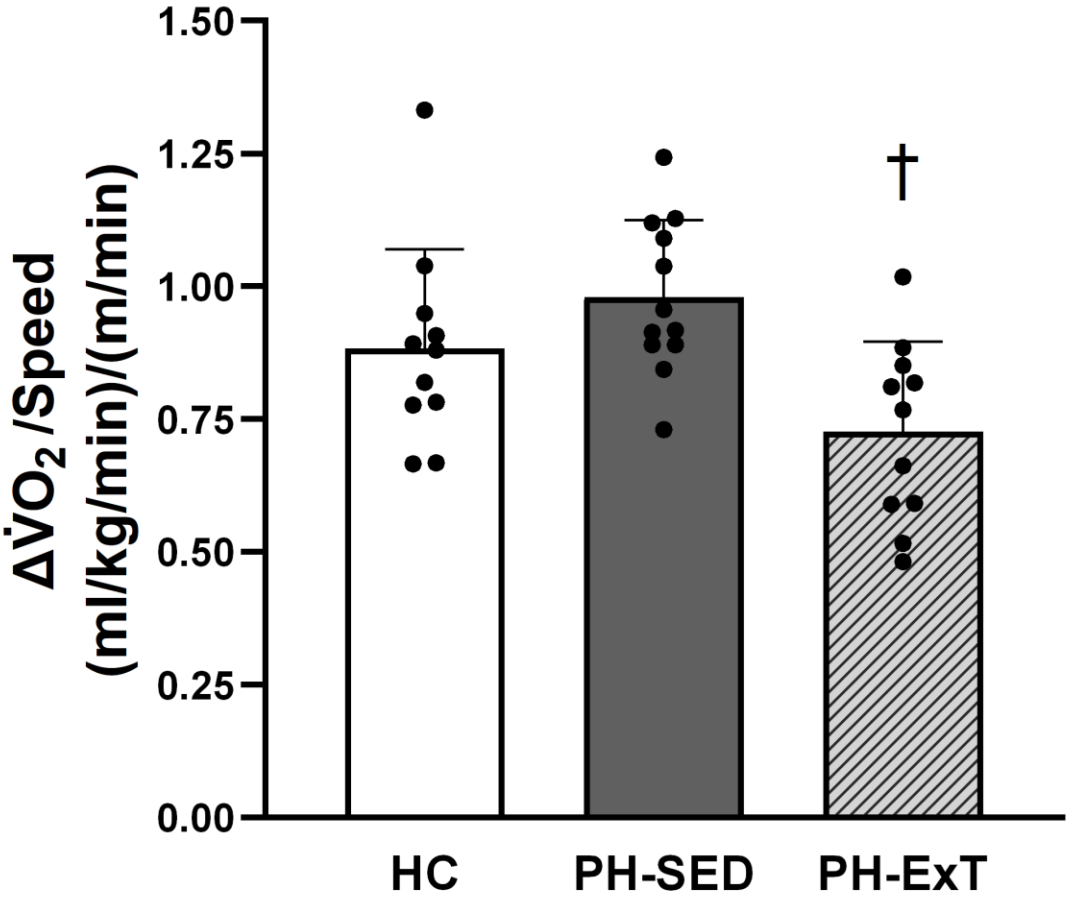

Figure 4.2. Delta $\dot{V}O_2$/Speed.

Analyzed via one-way ANOVA. † $P < 0.004$ vs. PH-SED.

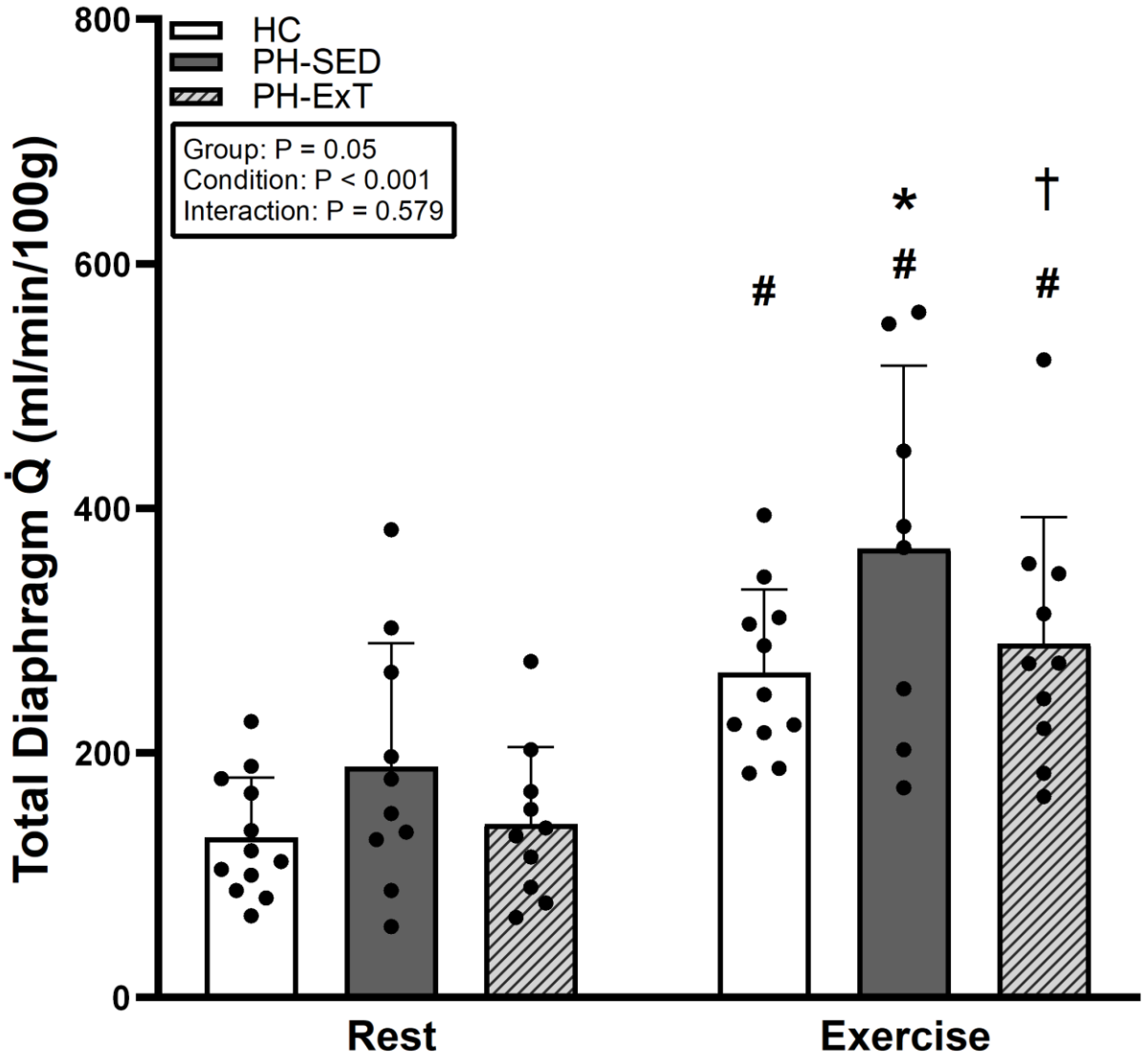

Figure 4.3. Total diaphragm $\dot{Q}$

Values are means ± SD. Analyzed via mixed effects model. * $P < 0.014$ vs. healthy within condition. # $P < 0.001$ vs. rest within group, † $P \leq 0.031$ vs. PH-SED within condition.

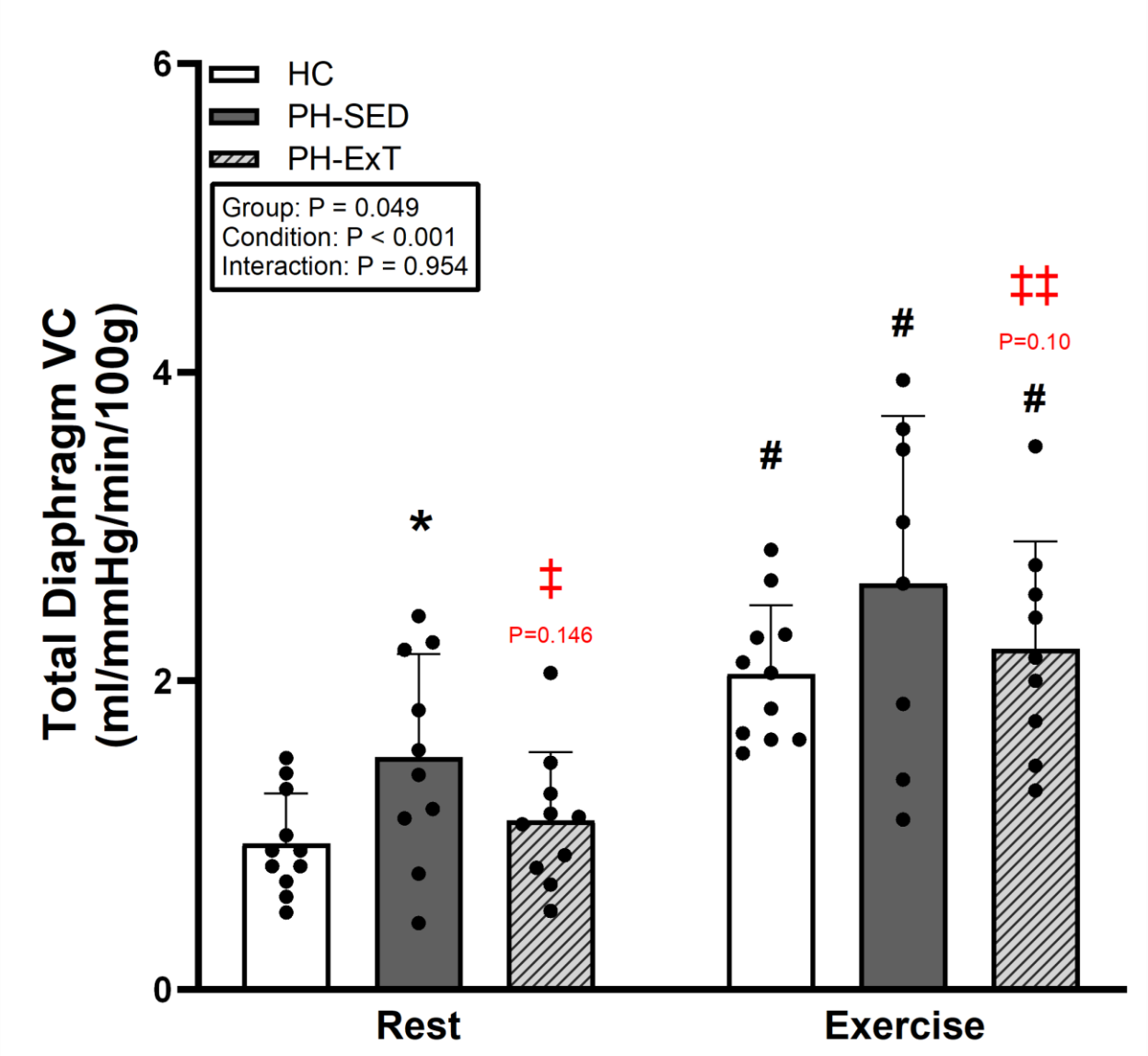

Figure 4.4. Total diaphragm vascular conductance.

Values are means ± SD. Analyzed via mixed effects model. * $P < 0.035$ vs. healthy within condition, # $P < 0.001$ vs. rest within group, ‡ $P = 0.146$ vs. PH-SED within condition, ‡‡ $P = 0.010$ vs. PH-SED within group.

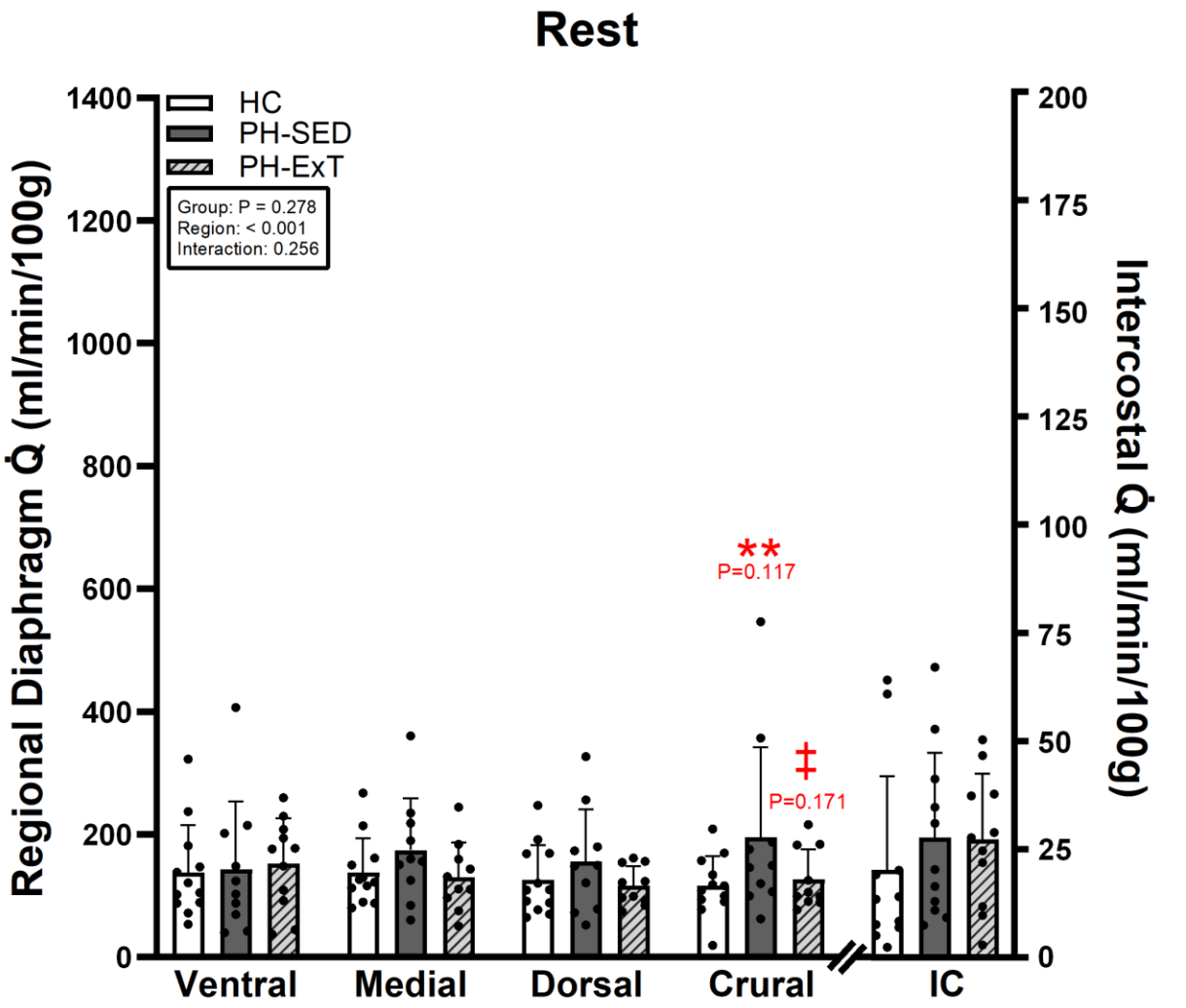

Figure 4.5. Resting regional diaphragm Q̇.

Values are means ± SD. Analyzed via a mixed effects model and multiple one-way ANOVAs**.** ‡ P = 0.171 vs. PH-SED within region. ** P = 0.117 vs. healthy within region.

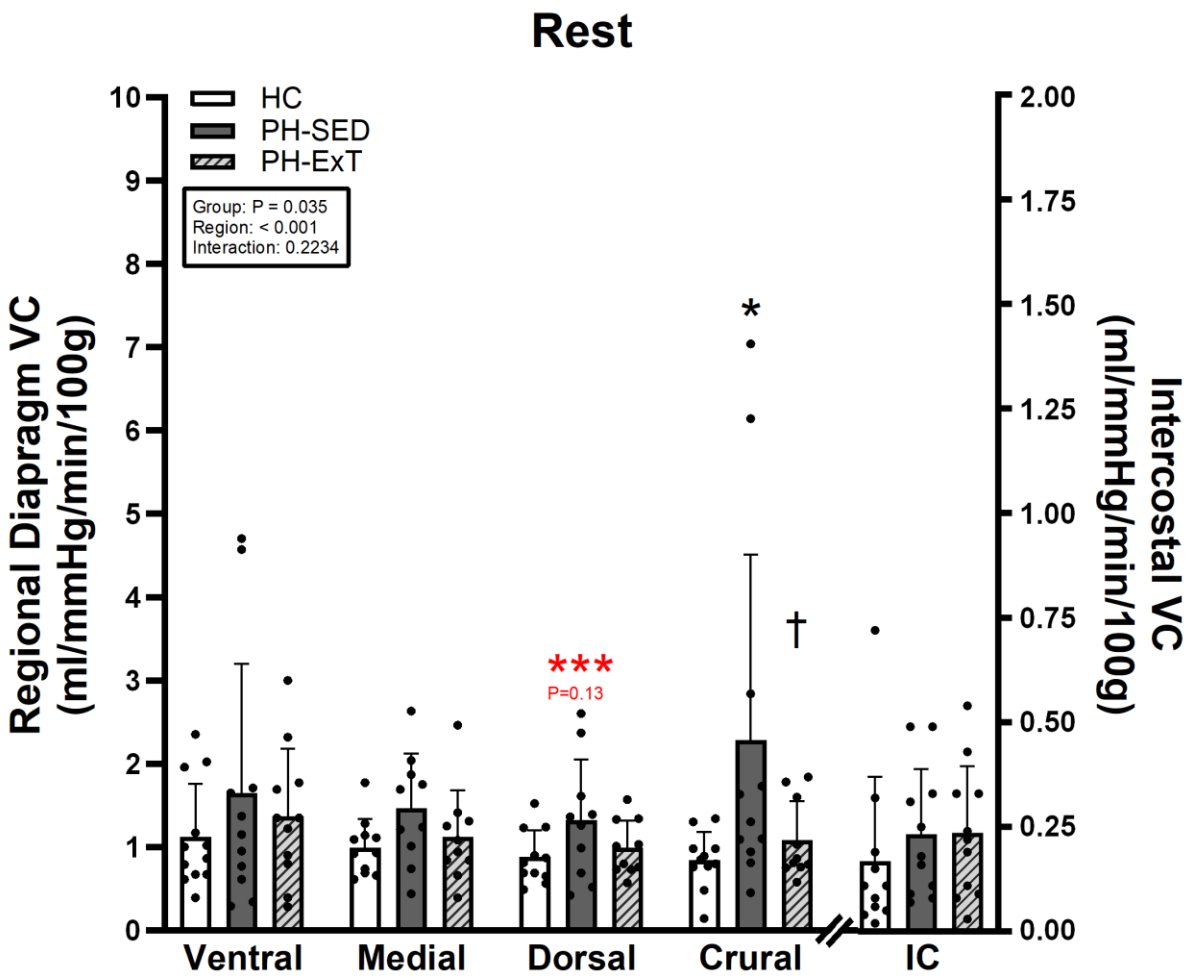

Figure 4.6. Resting regional diaphragm vascular conductance.

Values are means ± SD. Analyzed via a mixed effects model and multiple one-way ANOVAs**. *** P < 0.001 vs. healthy within region, † P < 0.001 vs. PH-SED within region. ** P = 0.13 vs. healthy within region.

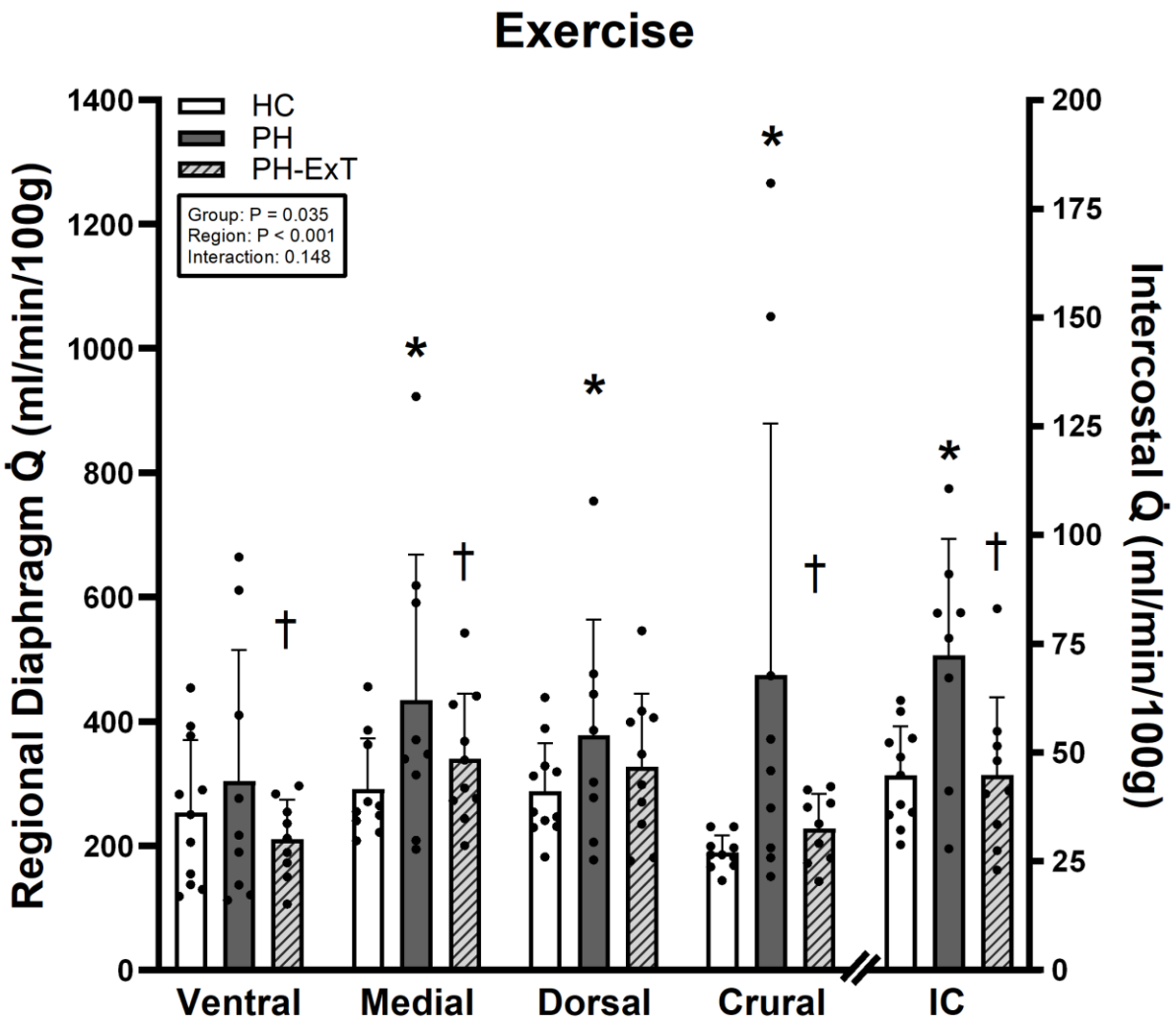

Figure 4.7. Regional diaphragm Q̇ during exercise.

Values are means ± SD. Analyzed via a mixed effects model and multiple one-way ANOVAs**. *** P ≤ 0.05 vs. healthy within region, † P ≤ 0.05 vs. PH-SED within region.

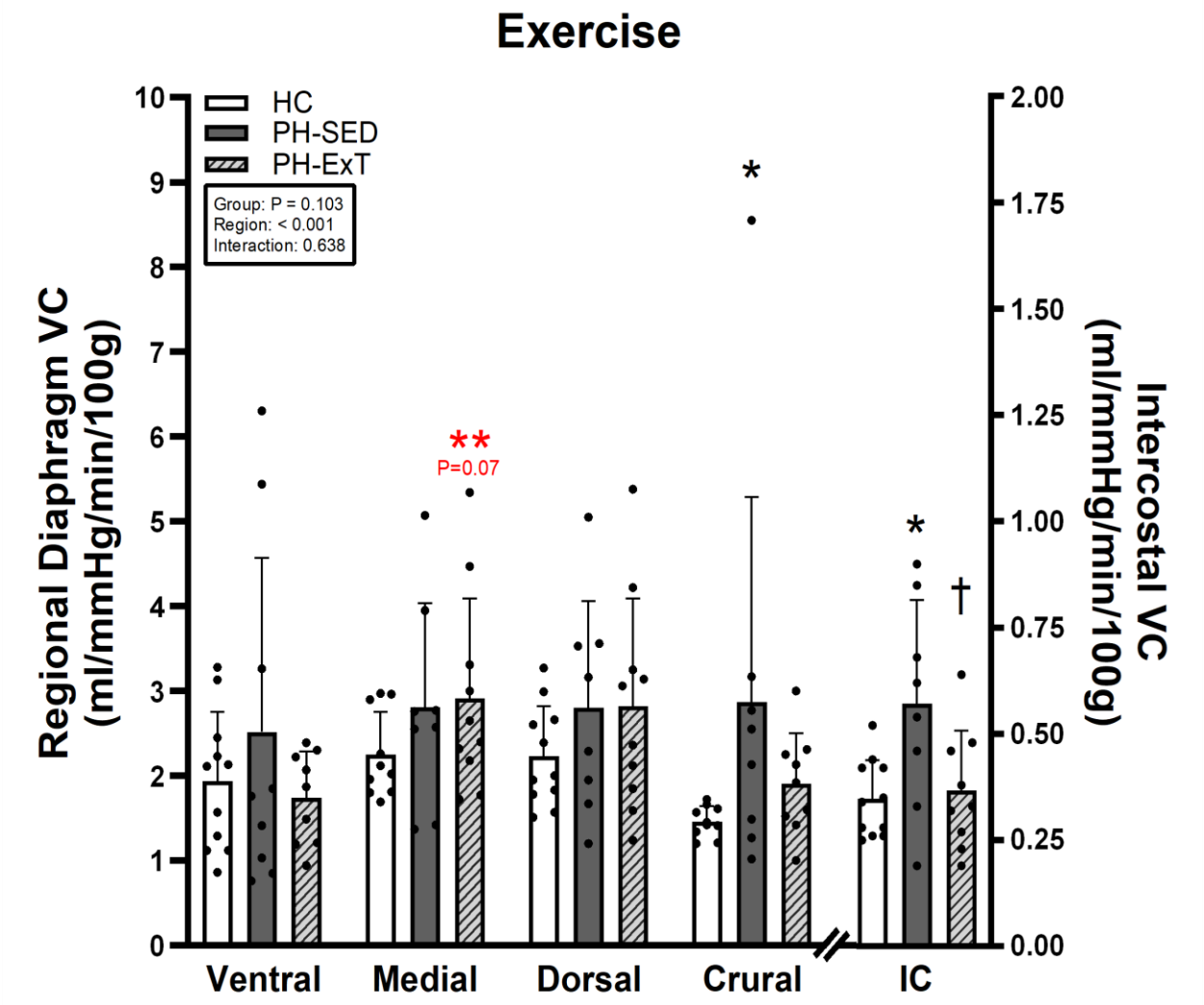

Figure 4.8. Regional diaphragm vascular conductance during exercise.

Values are means ± SD. Analyzed via a mixed effects model and multiple one-way ANOVAs**. *** P < 0.006 vs. healthy within region, † P < 0.015 vs. PH-SED within region. ** P = 0.07 vs. healthy within region.

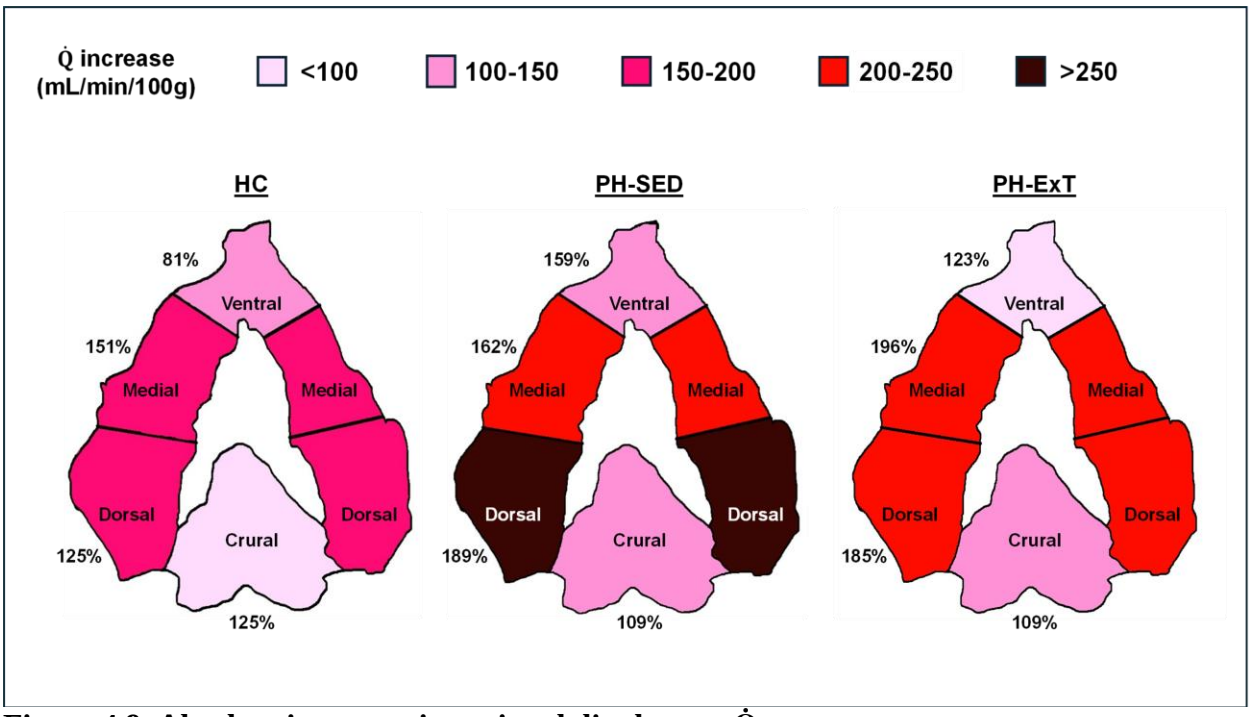

Figure 4.9, Absolute increases in regional diaphragm $\dot{Q}$.

Visual representation of regional exercise-induced hyperemia among groups (absolute $\dot{Q}$ increase from resting value; percentage increase from rest located next to region). Includes paired data from rest to exercise, HC; n = 11, PH-SED; n = 10, PH-ExT; n = 10. Analyzed via mixed-effects model.

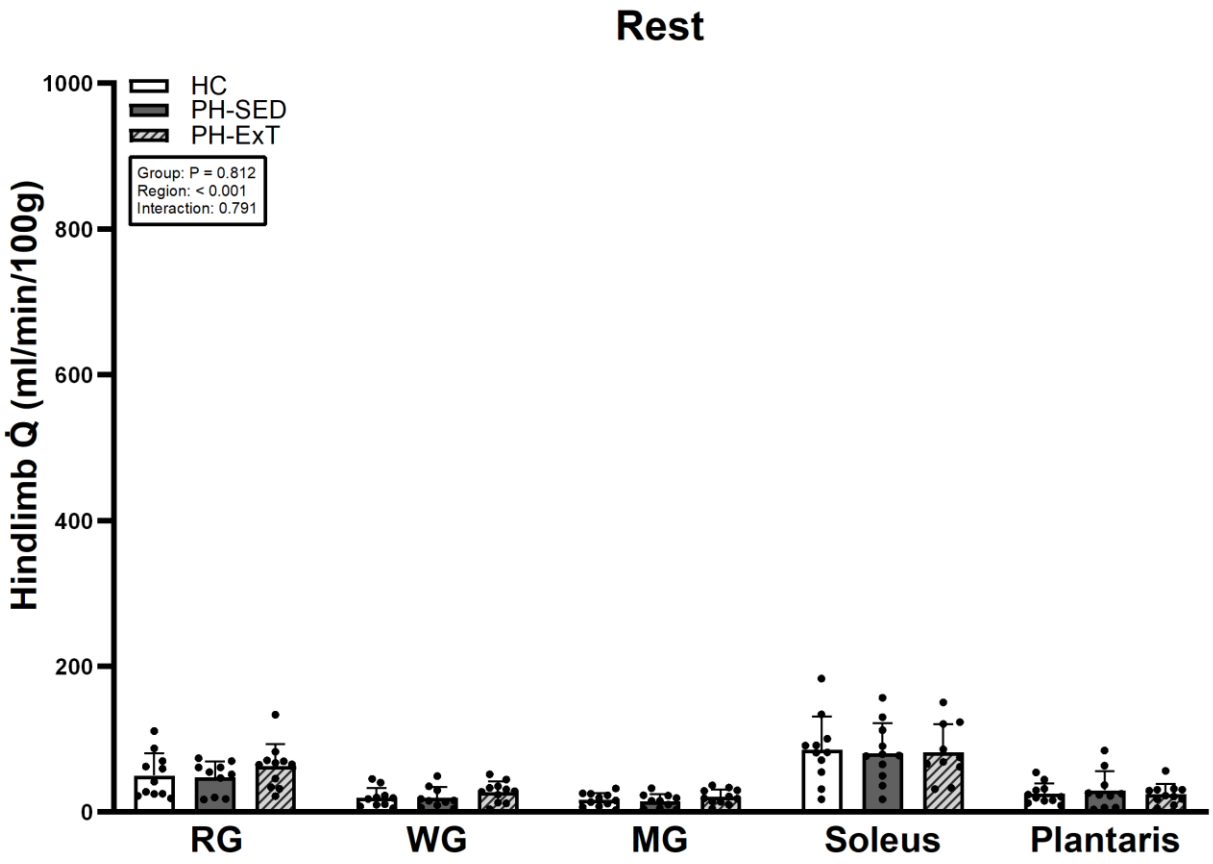

Figure 4.10. Resting hindlimb Q̇.

Values are means ± SD. Analyzed via mixed effects model and individual one-way ANOVAs.

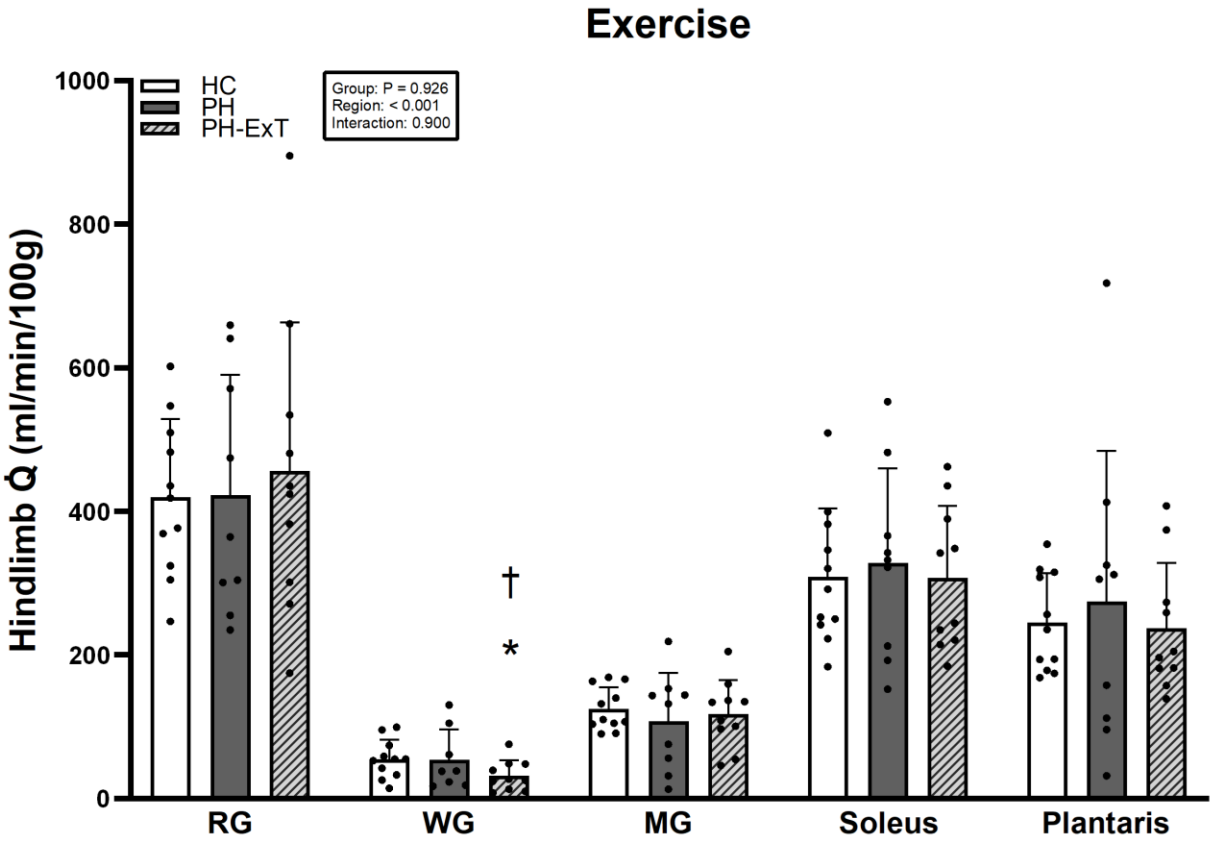

Figure 4.11. Hindlimb $\dot{Q}$ during exercise.

Values are means ± SD. Analyzed via mixed effects model and individual one-way ANOVAs. ***** $P \leq 0.05$ vs. healthy within region, **†** $P \leq 0.05$ vs. PH-SED within region.

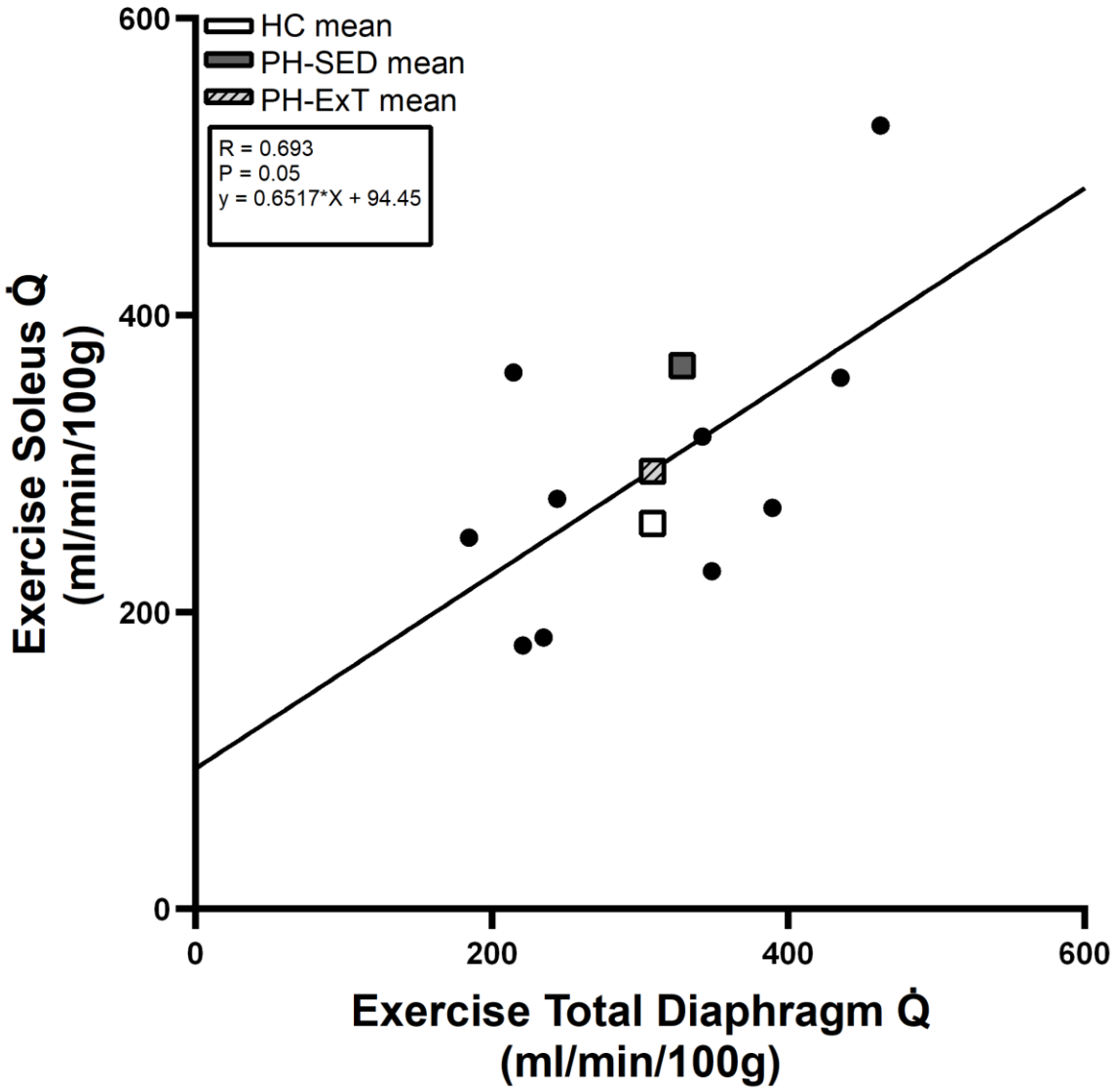

Figure 4.12. Correlation of soleus Q̇ and total diaphragm Q̇ during exercise.

Individual data points represent PH-ExT rats.

Chapter 5 - Discussion

To our knowledge, this is the first investigation into the effects of exercise training on the hyperemic response of locomotor and respiratory skeletal muscles to running in conscious pulmonary hypertensive (PH) rats. Key novel findings include the observations that whereas moderate intensity exercise training may not increase maximal oxygen uptake ($\dot{V}_{O_2\,max}$) in PH, it does increase exercise economy (amount of work per unit $\dot{V}_{O_2}$) and therefore promotes a reduced metabolic cost of exercise, allowing for increased maximal running speeds. Additionally, total diaphragm blood flow ($\dot{Q}$) was reduced during treadmill running at 25m/min (i.e., submaximally) in exercise-trained PH (PH-ExT) rats compared to sedentary PH controls (PH-SED). Whereas PH increases total and regional diaphragm $\dot{Q}$ and intercostal muscle $\dot{Q}$, in PH-ExT rats, these were not different from healthy controls (HC) across all regions at rest and during exercise, suggesting a decreased work of breathing among PH-ExT rats. Contrary to our hypothesis, most hindlimb $\dot{Q}$'s did not differ among groups; however, exercise training reduced white gastrocnemius $\dot{Q}$ in PH during exercise compared to HC and PH-SED. PH-ExT rats showed a positive correlation between total diaphragm $\dot{Q}$ and soleus $\dot{Q}$ during exercise, supporting the notion that exercise training can alleviate the respiratory muscle "steal" phenomenon observed in sedentary PH rats (Schulze et al., 2024), thereby potentially improving quality of life and reducing dyspnea in the patient population.

Monocrotaline (MCT) has been used to produce a reliable and replicable model of PH in animal research within our lab (Schulze et al., 2021, 2022, 2023, 2024, 2025) and others (Brown et al., 2015, 2017; Colombo et al., 2013; Araujo Alves et al., 2026; De Man et al., 2011; Gomez-Arroyo et al., 2012; Long et al., 2022, 2025), inducing cardiopulmonary dysfunction similar to that found in the human PH population. Both PH-SED and PH-ExT rats displayed reduced

pulmonary arterial acceleration time (PA AT), pulmonary arterial acceleration time to ejection time (PA AT/ET) ratios, increased right ventricular systolic pressure (RVSP), and increased lung weight (Tables 1 & 2), similar to the aforementioned studies. The absence of training-induced improvements in left ventricular (LV) function (i.e., ejection fraction, stroke volume) in PH-ExT vs. PH-SED rats may be due to the collection of these measurements after the establishment of PH in the PH-ExT animals. Significantly, increases in cardiac function may have occurred during training but were subsequently diminished due to disease progression.

$\dot{V}_{O_2 max}$ measurements herein agree closely with previous data from our lab collected with the same testing protocol developed in healthy (Copp et al., 2009) and in PH rats (Schulze et al., 2024). Additionally, regional and total diaphragm $\dot{Q}$ and hindlimb $\dot{Q}$ measurements collected at rest and during exercise matched closely those reported previously in studies of healthy and PH rats (Copp et al., 2010; Schulze et al., 2024). Kidney $\dot{Q}$ in all groups was higher than previously reported but remained within physiological limits, resembling flows in healthy rats (Armstrong & Laughlin, 1983). Note that discrepancies in exercising muscle $\dot{Q}$'s between the current study and those reported by Schulze et al. (2024) may be due to the greater absolute intensity during submaximal exercise (i.e., 25m/min vs. 20m/min) during microsphere infusion.

Exercise capacity

In the present investigation, the same absolute submaximal workload of 25m/min was selected for our blood flow determinations for all groups. The rationale behind this is because the performance of an activity, regardless of health state, requires the same oxygen demand. Subsequently, rats ran at 50% (HC), 56% (PH-SED), and 49% (PH-ExT) ($P < 0.001$) of the speed achieved during $\dot{V}_{O_2 max}$ testing. Contrary to our hypothesis, exercise training did not increase $\dot{V}_{O_2 max}$ within PH-ExT rats, which exhibited a continued reduction in $\dot{V}_{O_2 max}$ compared to HC. In addition, $\dot{V}_{O_2 max}$ for PH-ExT rats were not different from PH-SED rats. These findings may be due to the random assignment of groups, pre-training $\dot{V}_{O_2 max}$ values for PH-ExT were

significantly lower than HC prior to the exercise training intervention. Also, it must be acknowledged that $\dot{V}_{O_2 max}$ testing was not performed in PH-ExT rats until significant reductions in PA AT/ET were determined, indicative of significant PH development. The possibility remains that $\dot{V}_{O_2 max}$ may have been elevated during training but was reduced back to the levels of PH-SED rats at the onset of PH. Persistent reductions in $\dot{V}_{O_2 max}$ following continuous exercise training have been reported in MCT-induced PH rats (Brown et al., 2017). Due to a lack of pre-intervention $\dot{V}_{O_2 max}$ testing in HC and PH-SED groups, further comparisons of the changes in $\dot{V}_{O_2 max}$ throughout disease progression could not be made.

While $\dot{V}_{O_2 max}$ was not increased by exercise training, contrary to studies in our lab and others in healthy rats (e.g., Gleeson et al., 1983; Hirai et al., 2015; Musch et al., 1986, 1991; Roca et al., 1992), PH-ExT rats displayed multiple markers indicative of training adaptations during exercise. Specifically, PH-ExT rats were able to achieve the highest work rates (treadmill speed) and time to max/exhaustion during $\dot{V}_{O_2 max}$ testing among all groups, especially when compared to their PH-SED counterparts. Additionally, the lactate threshold ($T_{lac)}$, although not directly measured in this study, is presumed to have shifted to the right in PH-ExT rats, as evidenced by the substantially lower blood lactate accumulation during submaximal exercise compared to PH-SED rats; emblematic of greater muscle oxidative capacity and therefore a better balance of lactate production and clearance. Such adaptations have been observed within 3 weeks of exercise training in healthy populations (Poole & Gaesser, 1985; Gaesser & Poole, 1986), validating the adaptations accrued by PH-ExT rats within their 4-week training window.

Heart rate (HR) at rest and during submaximal exercise was lower in PH-ExT rats than in both HC and PH-SED rats. This training-induced bradycardia may be due to reduced sympathetic activation in PH-ExT rats, as seen in rats (Gleeson et al., 1983) and human models

of aerobic exercise training (Gaesser & Poole, 1986). The reductions in both resting and exercising HR found in the present investigation may have slowed disease progression, as the right ventricle (RV) must overcome increased afterload within the pulmonary arterioles less often. In addition to the reductions in HR found at rest and during exercise, the PH-ExT rats also demonstrated a significant reduction in mean arterial pressure (MAP) during exercise when compared to both HC and PH-SED groups. This reduction in MAP during exercise is again consistent with a possible reduction in the sympathetic response to exercise produced by exercise training (Zanesco & Antunes, 2007)

We did not measure $\dot{V}_{O_2}$ during the submaximal treadmill running when microspheres were infused, but there is the expectation of a decreased $\dot{V}_{O_2}$ slow component after exercise training, decreasing the overall metabolic cost. This was evidenced by the decreased changes in HR, MAP, blood lactate concentrations, and arterial pH measurements in PH-ExT rats (Tables 3 and 4) (Womack et al.,1995). Such decreases in the $\dot{V}_{O_2}$slow component would be presumed to occur primarily due to alterations/adaptations within the respiratory and peripheral skeletal muscle (Poole, 1994) as the reduced intra- and extracellular perturbations, as well as faster $\dot{V}_{O_2}$kinetics will reduce the overall myocyte metabolic stress. This situation will reduce glycogenolysis, better preserving finite glycogen stores in respiratory and peripheral skeletal muscle, thereby increasing exercise tolerance and resilience to fatigue.

A profound finding regarding exercise capacity/tolerance within this study was evidently linked to the reduced $\Delta\dot{V}_{O_2}$/speed slope evinced by PH-ExT rats. This training-induced reduction in the oxidative cost of exercise is a stark contrast to that observed in PH-SED rats. In a clinical population with reduced exercise capacity, maximizing limited resources in this fashion may be crucial for increasing the amount of activity performed and the quality of life in PH.

Blood gas data do not suggest that the lung's ability to arterialize the blood was impacted by training, as PH-ExT rats exhibited similar reductions in P_aO_2 to PH-SED rats at rest and during exercise as compared to HC rats. These decreased driving pressures of O_2 seen in both PH groups (Table 3) are a limiting factor in the peripheral diffusive component of oxygen transport, thus contributing to exercise intolerance in this patient population.

Diaphragm $\dot{Q}$ and vascular conductance

Previous work in our lab by Schulze et al (2024) explored the patterns of $\dot{Q}$ distribution in PH rats during submaximal exercise. That study revealed that respiratory muscle (diaphragm and intercostals) $\dot{Q}$ is increased in sedentary PH rats, with a redistribution in regional diaphragm $\dot{Q}$ and reductions to hindlimb $\dot{Q}$; demonstrating the respiratory muscle "steal" phenomenon as a potential contributory mechanism for exercise intolerance in PH. The current study expands upon that work, investigating the impact of exercise training, a common and effective treatment in clinical models of PH (Grünig et al., 2021; for a review, see Richter et al., 2017) and CHF (Mancini et al., 1995; Tasoulis et al., 2010), on the distribution of $\dot{Q}$ in PH. We found that exercise training decreased total diaphragm $\dot{Q}$ during submaximal exercise when compared to PH-SED rats, suggesting that 4-wks of training potentially decreased the work of breathing within PH. Interestingly, in PH-ExT rats, respiratory muscles commanded less $\dot{Q}$ despite exhibiting the same decreased P_{O2} during exercise found in PH-SED rats in the present study and those of Schulze (2024). Decreased diaphragm recruitment during exercise in PH-ExT rats may, in part, be directly related to decreased lactate production within the musculature during moderate-intensity exercise and the better maintenance of blood pH. These conditions contrast with those seen in PH-SED rats, presumably resulting in decreased stimulation of peripheral chemoreceptors and a lower overall respiratory drive during the exercise bout. Additionally,

respiratory muscle $\dot{Q}$ did not differ between HC and PH-ExT rats, despite increased lung mass, incipient hypoxemia, and increased resistance to flow within the pulmonary arterioles as evidenced by the elevated RVSP characteristic of PH. Both the HC and PH-ExT groups exhibited similar metabolic responses to exercise, such as blood lactate and pH levels, indicating what may be an equivalent stimulation of the peripheral chemoreceptors that influence respiratory drive. These data may signify a training-induced decrease in dyspnea and respiratory work during exercise in PH. This result would signify the amelioration of a primary limitation to exercise tolerance in the PH patient population (Kabitz et al., 2007), lowering the barrier to perform and sustain physical activity in the disease.

Inspection of regional diaphragm $\dot{Q}$ permits investigation of potential mechanisms of diaphragm function in PH, as perturbation of heterogeneous regional diaphragm $\dot{Q}$ may be indicative of suboptimal – or at least changed - breathing mechanics. At rest, PH-ExT rats exhibited crural VC not different from HC but lower than PH-SED (Figure 9). This decreased resistance to flow within a region of the diaphragm that is lightly recruited at rest in health (Sexton & Poole, 1995; Poole et al., 1997) may be due to PH-induced vascular dysfunction within the diaphragm (Schulze et al., 2023), which has been seen in previous studies (Schulze et al., 2025). There were no differences in regional diaphragm $\dot{Q}$ at rest across all groups (Figure 8). However, during submaximal exercise, a stark difference in the hyperemic response across the diaphragm among the PH groups became evident. PH-ExT rats showed reduced ventral costal, medial costal ($P = 0.059$), crural, and intercostal $\dot{Q}$ (Figure 10) and decreased intercostal VC (Figure 11) during treadmill running compared to PH-SED rats. Furthermore, PH-SED rats displayed elevated medial costal, dorsal costal, crural, and intercostal $\dot{Q}$ compared to HC rats during exercise, unlike PH-ExT rats, which displayed similar $\dot{Q}$ and VC to HC rats throughout

the entirety of the diaphragm. Discrepancies in the hyperemic responses between the PH groups are expected due to decreased bulk diaphragm $\dot{Q}$ after training, but they also may reflect differences in respiratory mechanics, with preferentially increased oxygen demand in certain regions which was subsequently altered consequent to exercise training.

In a study of blood flow distribution in healthy hamsters during submaximal exercise, the medial costal diaphragm receives the greatest $\dot{Q}$, supporting that this region performs the most work in producing inspiratory volumes/muscular displacement, followed by the dorsal costal, crural, and then ventral costal regions (Poole et al, 1997). Other regions, such as the ventral costal, play a lesser role in muscular displacement due to their relatively smaller mass and location (Poole et al., 1997). In the present study, the patterns of regional diaphragm hyperemia seen in PH-ExT rats were similar to those displayed in the 1997 study by Poole and colleagues, while HC $\dot{Q}$'s were not different from previous studies in our lab (Schulze et al., 2024) (Figure 10). PH-SED rats displayed an altered hyperemic response and possible impairment of respiratory mechanics as the crural region received the greatest amount of $\dot{Q}$, followed by the medial, dorsal, and then ventral costal regions. This difference in diaphragmatic $\dot{Q}$ distribution seen in PH-SED is similar to patterns seen in emphysematous hamsters during exercise (Sexton & Poole, 1998), suggesting that these rats may display characteristics of lung hyperinflation (Laveneziana et al., 2013) and subsequent flattening of the diaphragm, causing dyspnea, and decreased diaphragm contractile function due to a disadvantaged position on the sarcomere length-tension relationship (Poole et al., 1997) as well as decreased force production of diaphragm muscle fibers (Manders et al., 2012).

Upon the initiation of exercise, oxygen demand and therefore $\dot{Q}$ increases in the respiratory muscle to allow for increased ventilatory muscle work. This absolute increase in

respiratory muscle $\dot{Q}$ also shows the recruitment patterns of the diaphragm regions, allowing for the comparison of the regional hyperemic response among groups (Figure 12). Thus, PH-ExT and HC rats exhibited similar hyperemic profiles, albeit with slightly greater absolute $\dot{Q}$ in the medial and dorsal costal diaphragm within PH-ExT. This greater hyperemia seen in PH was specific to the medial and dorsal costal regions, indicating greater respiratory work performed within the diaphragm regions with the greatest mechanical advantage to generate volumetric change presumably improving overall respiratory mechanics. In comparison, PH-SED rats showed greater increases in each diaphragm region from rest to exercise than PH-ExT rats, while performing the same absolute external workload (i.e., treadmill running at 25m/min). This is reflected by the greater bulk diaphragm $\dot{Q}$ and ventral, medial, and crural $\dot{Q}$ in comparison to PH-ExT rats during exercise. More importantly, the higher absolute $\dot{Q}$ in the ventral costal, combined with the largest increase in $\dot{Q}$ favoring the dorsal costal instead of the medial costal suggests that diaphragm work became more homogeneously distributed in disease. Nonspecific increases in regional diaphragm work suggests elevated metabolic cost of breathing and impaired respiratory mechanics possibly in concert with impaired diaphragm strength (Johnson et al., 2002; Manders et al., 2012; Schulze et al., 2024, 2025).

There were no differences in hindlimb $\dot{Q}$ at rest among groups. Contrary to our hypothesis, during exercise, only the white gastrocnemius $\dot{Q}$ in PH-ExT rats was different from that of HC and PH-SED rats. This decrease in white gastrocnemius $\dot{Q}$ may be due to a preferential use of oxidative fiber types in exercise-trained animals (Armstrong & Laughlin, 1984), but this hypothesis cannot be resolved within this dataset, as other hindlimb muscle flows did not differ among groups. Additionally, such decreases in the white gastrocnemius may be due to the prior exposure and acclimatization of exercise-trained rats to this absolute workload

during microsphere infusion, as they trained at the same speed (Musch et al., 1993). Surprisingly, in this study, hindlimb muscle $\dot{Q}$ in PH-SED rats was not affected by disease progression, contrary to previous investigations (Schulze et al., 2024; Long et al., 2022, 2025). The absence of differences in exercising hindlimb $\dot{Q}$ among PH-SED and PH-ExT rats may be associated with the training-induced reduction of sympathetic activation at the onset of exercise. Lower HR during exercise may have been the product of training-induced increases in stroke volume (SV) to maintain cardiac output during submaximal exercise in PH-ExT rats (Musch, 1992) and, therefore, may have allowed similar hindlimb muscle $\dot{Q}$ between the PH-SED and PH-ExT groups.

PH patients are plagued by dyspnea and increased respiratory work with increases in physical activity (Sun et al., 2001, 2003). This claim has been reinforced by Schulze and colleagues (2024), who demonstrated that cardiac output is preferentially directed to respiratory muscles at the expense of peripheral skeletal muscle, leading to rapid substrate utilization (i.e., glycogen) via anaerobic glycolysis (Brown et al., 2017) and early-onset fatigue of locomotory muscles. Furthermore, that study showed a negative correlation between soleus and total diaphragm $\dot{Q}$ during exercise as evidence of respiratory muscle 'steal' in PH. The present investigation found that exercise training counters this tradeoff as respiratory muscle $\dot{Q}$ is decreased during exercise, resulting in a positive correlation between submaximal exercise soleus and total diaphragm $\dot{Q}$ ($R = 0.693$, $P = 0.05$) (Figure 15). This relationship between respiratory and hindlimb muscle perfusion in PH-ExT rats during exercise is closely similar to that of healthy rats within this study ($R = 0.398$, $P= 0.225$). Importantly, this positive correlation of soleus and total diaphragm $\dot{Q}$ provides evidence for aerobic exercise training as a therapeutic countermeasure to the respiratory muscle steal in PH. Herein, we demonstrate that moderate

intensity exercise training is a beneficial intervention in PH, decreasing drivers for, and indices of, respiratory work during exercise and increasing exercise tolerance. Findings from the present investigation identify the benefits of exercise training and help resolve its novel mechanistic underpinnings, which may, in part, be akin to those seen in clinical inspiratory training studies. Those inspiratory training paradigms decrease dyspnea (da Fontoura et al., 2025; Kahraman et al., 2023), as does aerobic exercise training (Chan et al., 2013; Kabitz et al., 2014), potentially by reducing energetic demands within the respiratory and possibly locomotory skeletal muscles. Therefore, exercise training may be a valuable treatment option for increasing quality of life and exercise tolerance in PH.

Experimental considerations

This investigation used a moderate-severity (50ml/kg) MCT-induced PH model to better understand PH pathophysiology, particularly with respect to exercise hemodynamics, independent of RV failure. MCT is metabolized in the liver and causes endothelial damage and remodeling of the pulmonary vasculature. The dose of MCT used in this study does not cause peripheral vascular damage to the abdominal visceral organs (Lachant et al., 2018), but the potential effects of MCT on the diaphragm or peripheral skeletal muscle have not been studied and should be considered as possible confounding factors in data interpretation. Translation of these findings to human pathology should be employed with caution.

The measurement of citrate synthase activity in soleus muscle was planned for, and assays were performed. Results were excluded from this study because fluorescent microspheres in the sample interfered with limonyl-CoA absorption, leading to non-physiological results.

Future studies might compare how exercise training affects various animal models of PH, such as Sugen hypoxia, chronic hypoxia, and MCT, to better understand the separate impacts of

PH and MCT toxicity on systemic vascular function and $\dot{Q}$ in vivo. Additionally, future studies may collect venous blood samples from the RV (Musch & Larach, 1988) during submaximal exercise to determine O_2 extraction and approximate stroke volumes using the Fick equation.

Due to nature of the MCT model, eliciting PH within 21-28 days post-injection, and our desire to study the effects of PH independent of RV failure, the need to begin 4-week exercise training program prior to the confirmation of PH was necessary. PH-ExT rats performed daily exercise training prior to disease development, possibly attenuating disease progression as seen by the delay in disease confirmation by 7 days compared to PH-SED rats. Future studies may examine whether exercise training could reduce the decrements found in PH by initiating the training protocol after the disease has been established, thereby providing clarification of the mechanisms (preventative or remedial) in which exercise training alters respiratory muscle $\dot{Q}$ as seen in the current study.

Conclusions

Our findings support the concept that moderate-intensity aerobic exercise training decreases the PH-induced higher-than-normal diaphragm and intercostal $\dot{Q}$ during submaximal exercise, whilst not altering skeletal muscle $\dot{Q}$. Whereas $\dot{V}_{O_{2max}}$ was not affected by exercise training, but training adaptations exhibited by PH-ExT rats, such as improved $\dot{V}_{O_2}$ kinetics, decreased metabolic cost of exercise, and increased exercise economy suggest greater exercise capacity in trained PH rats. Also, during exercise and the subsequent elevated ventilatory demand, exercise-trained PH rats showed a preferential redistribution of $\dot{Q}$ to mechanically efficacious regions (medial and dorsal costal) of the diaphragm, unlike sedentary PH rats. In addition to these findings, exercise-trained PH rats showed a positive correlation between soleus and diaphragm $\dot{Q}$ during exercise, supporting the notion that the “respiratory muscle steal”

phenomenon observed in PH may be ameliorated or reversed by exercise training. These data reveal that exercise training reduces ventilatory demands within an animal model of PH prior to RV failure, suggesting that this intervention may reduce the work of breathing, improve diaphragm function, remediate respiratory mechanics, and decrease dyspnea in PH. These findings endorse exercise training as a useful intervention for addressing exercise intolerance in moderate PH, potentially improving both quality of life and exercise capacity for patients.

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