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Effect of Chronic Heart Failure in Older Rats on Respiratory Muscle and Hindlimb Blood Flow during Submaximal Exercise

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Abstract

Submaximal exercise diaphragm blood flow (BF) is elevated in young chronic heart failure (CHF) rats, while it is unknown if this occurs in older animals. Respiratory and hindlimb muscle BFs (radiolabeled microspheres) were measured at rest and during submaximal exercise (20 m/min, 5% grade) in older healthy (n=7) and CHF (n=6) Fischer 344 × Brown Norway rats (27–29 mo old). Older CHF, compared to healthy, rats had greater (p<0.01) left ventricular end-diastolic pressure and right ventricle and lung weight (normalized to body weight). During submaximal exercise, respiratory and hindlimb muscle BFs increased (p<0.02) in both groups, while diaphragm BF was higher (CHF: 257±32; healthy: 121±9 mL/min/100g, p<0.01) and hindlimb BF lower (CHF: 111±10; healthy: 133±12 mL/min/100g, p=0.04) in older CHF compared to healthy rats. Submaximal exercise hindlimb BF was negatively related (r=-0.93; p=0.03) to diaphragm BF in older CHF rats. During submaximal exercise, diaphragm BF is elevated in older CHF compared to healthy rats in proportion to the compromised hindlimb BF.

Keywords

diaphragm; aging; pulmonary congestion; intercostals; inspiratory muscle metaboreflex

Introduction

Chronic heart failure (CHF) afflicts over 6 million Americans (Benjamin et al, 2017). CHF is characterized by impaired cardiac output and heightened sympathoexcitation constraining locomotor muscle blood flow (BF) and limiting exercise tolerance, the hallmark symptom of CHF (rev. Poole et al, 2012). Furthermore, CHF is associated with pulmonary abnormalities such as increased physiological dead space, mild ventilation/perfusion mismatch, lower lung diffusion capacity, and obstructive-restrictive lung disorders (rev. Poole et al, 2012). During submaximal exercise, the pulmonary abnormalities in concert with the exaggerated

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ventilatory response (Sullivan et al, 1988; Agostoni et al, 2003; Myers et al, 1992) results in a greater work of breathing in CHF patients compared to healthy individuals (Cross et al, 2012).

Animal models of CHF provide essential insights into the cardiopulmonary pathophysiological mechanisms contributing to exercise intolerance in this disease. For example, in CHF rats during submaximal exercise, diaphragm BF is considerably higher (Musch, 1993), while hindlimb BF is constrained (Musch & Terrell, 1992) compared to their healthy counterparts. The redistribution of cardiac output during submaximal exercise from the locomotor to the inspiratory muscles in CHF is mediated partially via the inspiratory muscle metaboreflex during submaximal exercise (Miller et al, 2007; Musch et al, 1993). Thus, unloading the inspiratory muscles during submaximal exercise leads to greater hindlimb BF and vascular conductance (VC) in canines with CHF (Miller et al, 2007). However, a significant limitation of these previous studies (Musch, 1993; Miller et al, 2007; Musch & Terrell, 1992) is that diaphragm and hindlimb BF were not measured simultaneously. Furthermore, these studies (Musch, 1993; Miller et al, 2007; Musch & Terrell, 1992) investigating CHF in animals have exclusively used young animals whereas CHF disproportionately afflicts older adults, with ~80% of the patients being 60 years and older (Benjamin et al, 2017). Consequently, as the population ages, the incidence of CHF in America is spiraling upwards. However, within established CHF animal models, there is almost a complete absence of investigations that have examined CHF against a background of aging. This is important considering aging and CHF independently enhance sympathetic nerve activity (Koch et al, 2003; Ferguson et al, 1990), impair nitric oxide vasodilation (Hirai 1995; Hirai et al, 2011), and redistribute blood flow from highly oxidative to highly glycolytic muscles during exercise (Musch et al, 2004; Musch & Terrell, 1992).

In this context, we sought to determine respiratory muscle (diaphragm, intercostal, and transversus abdominis) and hindlimb BFs and VCs in older CHF rats (induced by myocardial infarction (MI)) as well as in older healthy rats at rest and during submaximal exercise at the same absolute workload. This paradigm was specifically selected because older CHF and healthy individuals perform daily tasks that are equivalent in absolute terms. We hypothesized that respiratory muscle BF and VC would be higher, while hindlimb BF and VC lower during exercise in older CHF compared to older healthy rats. Furthermore, we tested the hypothesis that the elevated diaphragm BF would relate quantitatively to the reduced hindlimb BF; both being affected in proportion to the CHF-induced increases in lung mass.

2. Methods

2.1 Ethical approval

Thirteen older (27-29 mo old) Fischer 344 X Brown Norway (F344/BN) male rats were used in this investigation. These rats were specifically selected for this study because they represent old (senescent) rats according to the lifespan for the F344/BN strain (Larkin et al, 1996). Furthermore, the F344/BN rat has the advantage over the Fischer 344 rat because it does not develop many of the age-related pathologies that proliferate in their highly inbred Fischer 344 cousins (Lipman et al, 1996; Bronson, 1990). Rats were maintained at

accredited animal facilities at Kansas State University on a 12:12 h light-dark cycle with food and water provided *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University.

2.2 Surgical instrumentation

Six rats received an MI as described previously (Musch & Terrell, 1992). Briefly, under aseptic conditions, rats were initially anesthetized with a 5% isoflurane-oxygen mixture, intubated, and connected to a rodent respirator and maintained on a 2% isoflurane-oxygen mixture. A left thoracotomy was performed between the fifth and sixth rib, allowing for access to the heart. The pericardium was then opened, and the left main coronary artery was ligated with 6-0 Ti-cron suture. The lungs were hyperinflated and the ribs were sutured back together using 2-0 gut. The muscles of the thorax along with the skin were sewn together using 4-0 gut and 3-0 silk suture, respectively. Lidocaine (1.5 mg/kg every 2 h for 8 h) and buprenorphine (0.03 mg/kg every 12 h for 24 h) were administered subcutaneously for postoperative pain alleviation, and ampicillin (50 mg/kg every 24 h for 10 days) was injected subcutaneously to minimize the chance for infection. After surgery, anesthesia was removed and animals were extubated. The rats were given 5-6 wks to recover from surgery.

All rats were familiarized with running on a custom-built, motor-driven treadmill for two weeks consisting of exercising 5-10 min/day at 20 m/min, 10% grade. The speed of the treadmill was gradually increased to 28 m/min, while exercise duration was reduced to <5 min/day to ensure a training effect was not incurred (Musch & Terrell, 1992).

2.3 Instrumentation and final experiment protocol

On the day of the experiment, all rats were anesthetized with a 5% isoflurane-oxygen mixture and maintained subsequently on a 2% isoflurane-oxygen mixture. Under aseptic conditions, a shallow incision was made on the midline of the anterior portion of the neck to allow access to the carotid artery. The right carotid artery was exteriorized and cannulated with a 2-Fr-catheter-tipped pressure micromanometer (model TC-510, Millar Instruments). While heart rate (HR) and the arterial pressure waveform was being monitored, the pressure transducer was advanced into the left ventricle (LV) in a retrograde fashion and LV end-diastolic pressure (LVEDP) and the derivative of the pressure wave form (LV dP/dt) were measured and recorded. After LVEDP and LV dP/dt measurements were completed, the micromanometer was retracted, HR and arterial pressure were measured and recorded, while the micromanometer was placed in the aortic arch, and then the micromanometer was removed. One catheter (PE-10 connected to PE-50, IntraMedic polyethylene tubing, Clay Adams, Becton, Dickinson, Sparks, MD) was placed in the ascending aorta via the right carotid artery for the measurement of mean arterial pressure (MAP) and HR (model 200, DigiMed BPA, Louisville, KY) and the infusion of radiolabeled microspheres. A second catheter was placed in the caudal (tail) artery for arterial blood sampling (Musch & Terrell, 1992). Both catheters were tunneled subcutaneously through the dorsal aspect of the cervical region and exteriorized through a puncture in the skin. The incisions were then closed, anesthesia was terminated, and the rats were given >90 min to recover (Flaim et al, 1984).

2.4 Measurement of respiratory muscle and hindlimb BF

Following recovery, each rat was placed on the motor-driven treadmill and the carotid artery catheter was connected to the pressure transducer (model P23ID, Gould Statham, Valley View, OH). After the stabilization period, the caudal artery was connected to a 1-mL syringe chambered in an infusion/withdrawal pump (model 907, Harvard Instruments). Exercise was initiated, and the speed of the treadmill was increased progressively during the next 30 s to a speed of 20 m/min (5% grade). The rat was then required to exercise at the speed and grade for another 3 min. During this time, radiolabeled microspheres (^{46}Sc , ^{85}Sr , ^{141}Ce , or ^{113}Sn in random order; New England Nuclear, Boston, MA) were mixed by a vortex agitator (Fishers Scientific, Waltham, MA). At the 3 min exercise mark, the carotid artery catheter was disconnected from the pressure transducer and $0.5\text{--}0.6 \times 10^6$ microspheres with a 15- μm diameter were injected into the aortic arch to determine hindlimb and respiratory muscle BFs. Simultaneously, the pump connected to the caudal artery catheter was activated and blood withdrawal was initiated at a rate of 0.25 mL/min. Blood withdrawal was terminated 30 s following the microsphere infusion and then exercise was terminated.

After >60 min of recovery, a second microsphere infusion was performed (radiolabeled different from the first microsphere infusion), while the rat sat quietly on the treadmill for determination of resting hindlimb and respiratory muscle BFs, MAP and HR. This experimental protocol minimizes the potential influences of the pre-exercise anticipatory response on resting muscle BFs, MAP and HR measurements (Armstrong et al, 1989).

2.5 Determination of BF and VC

Following the completion of the exercise protocols and resting measurements, rats were euthanized with an overdose of sodium pentobarbital (>50 mg/kg body wt.) via the right carotid artery catheter and placement of each catheter was verified by anatomic dissection. The lungs were excised and weighed. The heart was removed and examined for scar tissue on the LV free wall for documentation that a large MI had been produced in the animal. The right ventricle (RV) was then separated from the LV and septum, and both tissues weighed. The diaphragm, intercostals, transversus abdominis, and hindlimb muscles of each rat were dissected out. The tissues were blotted, weighed, and placed immediately into counting vials. Tissue BFs were determined using the radionuclide-tagged microsphere technique that has previously been used in the exercising rat (Musch & Terrell, 1992). Before each injection, the microspheres were thoroughly mixed and agitated by sonication to prevent clumping. Each group of microspheres ($0.6\text{--}0.7 \times 10^6$ in number) was injected into the ascending aorta of the rat in a 0.15–0.20 mL volume. The radioactivity of each tissue was determined with a gamma scintillation counter (model 5230, Auto Gamma Spectrometer, Packard, Downers Grove, IL). The radioactivity of the tissues was then analyzed by computer, taking into account the cross-talk fraction between the different isotopes. Absolute muscle BF was then calculated by the reference sample method (Ishise et al, 1980) and expressed in milliliters per min per 100g of tissue. VC was then calculated by normalizing BF to MAP measured at the time of the microsphere infusion and expressed as mL/min/mmHg/100g.

2.6 Statistical analyses

Values are reported as mean $\pm$ standard error (SE). All statistical analyses were performed by using SigmaStat 2.0 (Jandel Scientific, San Rafael, CA). Unpaired *t*-tests were used to compare body weight and heart morphometrics. MAP, HR as well as respiratory muscle (diaphragm, intercostal, and transversus abdominis) and hindlimb BFs and VCs were compared within (rest vs 20 m/min, 5% grade) and among (healthy vs CHF) groups using mixed factorial analysis of variance and Student-Newman-Keuls post-hoc tests when appropriate. An unpaired *t*-test was used to compare changes in diaphragm BF (from rest to exercise) between older healthy and CHF rats. Linear regressions were used to determine if diaphragm and hindlimb muscle BFs were correlated with normalized lung weight as well as if diaphragm and hindlimb BFs were correlated. An influential outlier was detected in Fig. 5B via Cook's distance and DFFITS, thus the data point was shown for transparency, but not included in the correlation. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Body weight and heart morphometrics

Body weight was not different ($p = 0.73$) between older CHF and healthy rats (Table 1). Older CHF rats had greater ($p < 0.01$) LVEDP and normalized RV and lung weight as well as lower LV dp/dt ($p = 0.04$) compared to older healthy rats.

3.2 Cardiovascular responses

MAP was lower ($p < 0.01$) in older CHF compared to the healthy rats at rest and during submaximal exercise. From rest to submaximal exercise, MAP increased ($p < 0.01$) in older CHF, but not older healthy rats. At rest and during submaximal exercise, HR was lower ($p < 0.01$) in older CHF compared to older healthy rats. HR increased from rest to exercise ($p < 0.01$) in both older CHF and healthy rats.

3.3 Respiratory muscle BFs

At rest, diaphragm (Fig. 1A), intercostal (Fig. 1B), and transversus abdominis (Fig. 1C) BFs were not different ($p > 0.34$) between groups. From rest to submaximal exercise, diaphragm, intercostal and transversus abdominis BFs significantly increased ($p < 0.02$) in both groups. However, older CHF rats had higher diaphragm BF ($p < 0.01$) during submaximal exercise compared to older healthy rats. Older CHF rats also had a greater increase in diaphragm BF ($p < 0.01$) from rest to submaximal exercise compared to older healthy rats (Fig. 2). No differences ($p = 0.69$) were found in intercostal and transversus abdominis BFs between groups during submaximal exercise.

At rest, diaphragm (Fig. 3A), intercostal (Fig. 3B), and transversus abdominis (Fig. 3C) VCs were not different ($p > 0.22$) between groups. From rest to submaximal exercise, diaphragm and intercostal VCs increased ($p < 0.04$) in both groups, while transversus abdominis VC increased ($p = 0.02$) only in older CHF rats. During submaximal exercise, older CHF rats had higher diaphragm VC ($p < 0.01$) compared to older healthy rats. No other differences ($p = 0.11$) were present in VC between older healthy and CHF rats.

3.4 Hindlimb BF

At rest, hindlimb BF (Fig. 4A) and VC (Fig. 4B) were not different ($p=0.84$) between groups. From rest to submaximal exercise, hindlimb BF and VC increased ($p<0.01$) in both groups. During submaximal exercise, older CHF rats had lower ($p=0.04$) hindlimb BF compared to older healthy rats, while hindlimb VC was not different ($p=0.79$).

3.5 Relationships

In older CHF rats, submaximal exercise diaphragm BF correlated positively ($r=0.94$; $p<0.01$) with normalized lung weight (Fig. 5A). Submaximal exercise hindlimb BF was negatively correlated ($r=-0.91$; $p=0.03$) with normalized lung weight in older CHF rats (Fig. 5B). One data point was determined to be an influential outlier (depicted as a star in Fig. 5B). Without this influential outlier, the correlation between diaphragm BF and normalized lung weight was still significant ($r=0.98$; $p<0.01$). In older CHF rats, hindlimb BF was negatively related ($r=0.93$; $p=0.02$) to diaphragm BF during submaximal exercise (Fig. 6). No such relationships ($p>0.38$) were present in the older healthy rats.

4. Discussion

4.1 Major findings

The primary novel findings of this investigation are that 1) diaphragm BF and VC are greater in older CHF compared to older healthy rats during submaximal exercise, 2) submaximal exercise diaphragm BF was positively related, while hindlimb BF was negatively related to normalized lung weight in older CHF, and 3) a negative relationship exists between submaximal exercise diaphragm and hindlimb BF in older CHF. These findings suggest that in older individuals with CHF the diaphragm commands a higher BF response during submaximal exercise than in health at the expense of hindlimb BF.

4.2 CHF and diaphragm BF

CHF is associated with pulmonary abnormalities and an exaggerated ventilatory response (Sullivan et al, 1988; Agostoni et al, 2003; Myers et al, 1992) and consequently greater work of breathing (Cross et al, 2012) and tension-time index (Mancini et al, 1992) during submaximal exercise. In accordance, we found that diaphragm BF and VC were higher in older CHF compared to older healthy rats during submaximal exercise. Our submaximal exercise diaphragm BF (i.e. 257 mL/min/100g) in older CHF is lower than reported in young CHF rats (356-483 mL/min/100g; Musch, 1993) likely due to the reduced speed and substantially lower grade used in the present study (20 m/min, 5% grade) compared to the previous study (28 m/min, 20% grade). Our CHF diaphragm BFs are in line with maximal exercise diaphragm BFs in healthy rats (283-304 mL/min/100g; Smith et al, 2017) and ponies (265-325 mL/min/100g; Manohar, 1986; 1990), yet lower than those reported in rats exercising supra-maximally (360 mL/min/100g; Poole et al, 2000) and maximal-intensity human knee-extensor BF (385 mL/min/100g; Richardson et al, 1993). Furthermore, we found a greater heterogeneity in respiratory muscle BF and VC in CHF such that diaphragm BF and VC were higher in older CHF than older healthy rats, while no differences were present in intercostal or transversus abdominis BF or VCs during exercise. The higher

exercise diaphragm BF as well as the relationship between diaphragm BF and normalized lung weight is consistent with previous studies observing a link between pulmonary congestion and elevated diaphragm BF in young rats (Musch, 1993) and work of breathing in CHF patients (Cross et al, 2012). Furthermore, pulmonary congestion in CHF contributes to both the greater resistive work of breathing via (primary and secondary mechanisms) increasing airway resistance (Cabanès et al, 1989; Sasaki et al, 1990) and elastic work of breathing (Cross et al, 2012).

4.3 CHF and hindlimb BF

In CHF, the heightened sympathoexcitation impairs skeletal muscle BF during exercise in humans (Sullivan et al, 1989; Zelis et al, 1974) and animals (Musch & Terrell, 1992; Hirai et al, 1995). Exaggerated inspiratory/expiratory muscle metaboreflexes, pressor reflexes, and peripheral chemoreceptor sensitivity as well as enhanced humoral factors contribute to the sympathetically-mediated vasoconstriction in CHF (Poole et al, 2012). Consistent with these previous studies (Musch & Terrell, 1992; Hirai et al, 1995; Sullivan et al, 1989; Zelis et al, 1974), we found that hindlimb BF was reduced with CHF compared to healthy older rats during submaximal exercise. Furthermore, muscle BF abnormalities occur with CHF such that muscle BF is constrained to the highly oxidative muscles during exercise (Musch & Terrell, 1992; Hirai et al, 1995). Interestingly, we found that BF to the diaphragm, a highly oxidative muscle 60%-75% Type I/IIa (Delp & Duan, 1996; Eddinger et al.1985; Lieberman et al, 1973), was not attenuated. A plausible explanation for this latter finding is the α_1 -adrenergic receptors in the diaphragm, compared to the white and red portions of the gastrocnemius, have been found to be less sensitive to norepinephrine (Aaker & Laughlin, 2002) suggesting the heightened sympathoexcitation in CHF reduces BF to the hindlimb to a greater extent than the diaphragm.

Previous studies present compelling evidence demonstrating that derangements in the pulmonary system contribute to exercise intolerance in CHF. For example, unloading the inspiratory muscles improves exercise tolerance in patients with CHF (Mancini et al, 1997; O'Donnell et al, 1999; Borghi-Silva et al, 2008). High inspiratory muscle work and concomitant accumulation of metabolites leads to a metaboreflex arising from the inspiratory muscles resulting in sympathetically-mediated vasoconstriction in health (Smith et al, 2016; Smith et al, 2017; St Croix et al, 2000) that is exacerbated with CHF (Chiappa et al, 2008). Furthermore, the inspiratory muscle metaboreflex is tonically active during submaximal exercise in CHF (Olson et al, 2010; Miller et al, 2007), while maximal exercise is required to activate the inspiratory muscle metaboreflex in health (Harms et al, 1997). Specifically, unloading the inspiratory muscles resulted in increased leg BF and VC during submaximal exercise in CHF (Olson et al, 2010; Miller et al, 2007). It is important to note that unloading the inspiratory muscles in CHF also leads to increased stroke volume and cardiac output via reduced left ventricular afterload, which contributes to the increased leg BF (Miller et al, 2007; Olson et al, 2010). The relationships in the current study suggest that the high work of breathing observed in CHF results in elevated diaphragm BF compromising locomotor muscle BF during submaximal exercise. The present findings contrast previous reports (Vogiatzis et al, 2009) suggesting that respiratory muscle, specifically intercostal, BF is compromised conserving locomotor muscle BF during maximal exercise in healthy

humans. It is important to note that respiratory mechanics differ between rats and humans. At rest, rats have greater lung and chest wall compliance and less resistance compared to humans (Crosfill & Widdicombe, 1961). It remains to be determined whether unloading the work of breathing during submaximal exercise results in amelioration of the diaphragm BF response in CHF in addition to the increased leg BF and VC previously reported (Miller et al, 2007; Olson et al, 2010).

4.4 Experimental considerations

Several experimental considerations are warranted in the present study. First, ventilation and respiratory mechanics were not directly measured during exercise. However, it has previously been found in humans (Sullivan et al, 1988) and rats (Musch et al, 1993) that arterial carbon dioxide partial pressure and thus alveolar ventilation were not different during exercise in healthy individuals versus CHF patients, while ventilation was greater due to factors such as increased physiological dead space (Sullivan et al, 1988). Second, respiratory mechanics would have been valuable to confirm that work of breathing was greater in older CHF compared to older healthy rats consistent with findings in humans (Cross et al, 2012). However, the greater normalized lung weight supports the notion that CHF rats endured a higher work of breathing.

4.5 Conclusions

This is the first study to demonstrate that diaphragm BF and VC is accentuated during submaximal exercise in older CHF compared to healthy rats. Furthermore, we found in older CHF rats that the high diaphragm BF relates proportionally to the compromised hindlimb BF during submaximal exercise. Future studies are designed to determine if interventions such as altering the work of breathing, exercise training, and/or inspiratory muscle training can “normalize” the diaphragm BF response during exercise in CHF are indicated.

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References

- Aaker A, Laughlin MH. Diaphragm arterioles are less responsive to α 1-adrenergic constriction than gastrocnemius arterioles. *J Appl Physiol*. 2002; 92:1808–1816. [PubMed: 11960928]
- Agostoni P, Cattadori G, Bianchi M, Wasserman K. Exercise-induced pulmonary edema in heart failure. *Circulation*. 2003; 108:2666–2671. [PubMed: 14581402]
- Armstrong RB, Hayes DA, Delp MD. Blood flow distribution in rat muscles during preexercise anticipatory response. *J Appl Physiol*. 1989; 67:1855–1861. [PubMed: 2600019]
- Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart disease and stroke statistics-2017 Update: A report from the American Heart Association. *Circulation*. 2017; 135:e146–e603. [PubMed: 28122885]
- Borghi-Silva A, Carrascosa C, Oliveira CC, Barroco AC, Berton DC, Vilaca D, Lira-Filho EB, Ribeiro D, Nery LE, Nader JA. Effects of respiratory muscle unloading on leg muscle oxygenation and blood volume during high-intensity exercise in chronic heart failure. *Am J Physiol Heart Circ Physiol*. 2008; 294:H2465–H2472. [PubMed: 18375714]
- Bronson, R. Cross-sectional pathology of aging rodents. In: Harrison, D., Caldwell, NJ., editors. *Genetic Effects on Aging II*. Telford: 1990. p. 279-357.

- Cabanes LR, Weber SN, Matran R, Regnard J, Richard MO, Degeorges ME, Lockhart A. Bronchial hyperresponsiveness to methacholine in patients with impaired left ventricular function. *N Engl J Med*. 1989; 320:1317–1322. [PubMed: 2541334]
- Chiappa GR, Roseguini BT, Vieira PJC, Alves CN, Tavares A, Winkelmann ER, Ferlin EL, Steine R, Ribeiro JP. Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure. *J Am Coll Cardiol*. 2008; 51:1663–1671. [PubMed: 18436118]
- Crosfill ML, Widdicombe JG. Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. *J Physiol*. 1961; 158:1–14. [PubMed: 13696595]
- Cross TJ, Sabapathy S, Beck KC, Morris NR, Johnson BD. The resistive and elastic work of breathing during exercise in patients with chronic heart failure. *Eur Respir J*. 2012; 39:1449–1457. [PubMed: 22034652]
- Delp MD, Duan C. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. *J Appl Physiol*. 1996; 80(1):261–270. [PubMed: 8847313]
- Eddinger TJ, Moss RL, Cassens RG. Fiber number and type composition in extensor digitorum longus, soleus, and diaphragm muscles with aging in Fisher 344 rats. *J Histochem Cytochem*. 1985; 33:1033–1041. [PubMed: 2931475]
- Flaim SF, Nellis SH, Toggart EJ, Drexler H, Kanda K, Newman ED. Multiple simultaneous determinations of hemodynamics and flow distribution in conscious rats. *J Pharmacol Methods*. 1984; 11:1–39. [PubMed: 6546957]
- Ferguson DW, Berg WJ, Sander JS. Clinical and hemodynamic correlates of sympathetic nerve activity in normal humans and patients with heart failure: evidence from direct microneurographic recordings. *J Am Coll Cardiol*. 1990; 16:1125–1134. [PubMed: 2229759]
- Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nিকেle GA, Nelson WB, Dempsey JA. Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol*. 1997; 82(5):1573–1583.
- Hirai DM, Copp SW, Hageman KS, Poole DC, Musch TI. Aging altered the contribution of nitric oxide to regional muscle hemodynamic control at rest and during exercise in rats. *J Appl Physiol*. 2011; 111:989–998. [PubMed: 21757576]
- Hirai T, Zelis R, Musch TI. Effects of nitric oxide synthase inhibition on the muscle blood flow response to exercise in rats with heart failure. *Cardiovas Res*. 1995; 30:469–476.
- Ishise S, Pegram BL, Yamamoto J, Kitamura Y, Frohlich ED. Reference sample microsphere method: cardiac output and blood flows in conscious rat. *Am J Physiol*. 1980; 239(4):H443–H449. [PubMed: 7425136]
- Koch DW, Leuenberger UA, Proctor DN. Augmented leg vasoconstriction in dynamically exercising old men during acute sympathetic stimulation. *J Physiol*. 2003; 551:337–344. [PubMed: 12824451]
- Larkin LM, Halter JB, Supiano MA. Effect of aging on rat skeletal muscle BAR function in male Fischer 344 X Brown Norway rats. *Am J Physiol Regul Integr Comp Physiol*. 1996; 270:R462–468.
- Lieberman DA, Faulkner JA, Craig A, Maxwell L. Performance and histochemical composition of guinea pig and human diaphragm. *J Appl Physiol*. 1973; 34:233–237. [PubMed: 4265565]
- Lipman RD, Chrisp CE, Hazzard DG, Bronson RT. Pathologic characterization of Brown Norway, Brown Norway X Fischer 344, and Fischer 344 X Brown Norway rats with relation to age. *J Gerontol*. 1996; 51:B54–B59.
- Mancini D, Donchez L, Levine S. Acute unloading of the work of breathing extends exercise duration in patients with heart failure. *J Am Coll Cardiol*. 1997; 29(3):590–596. [PubMed: 9060898]
- Mancini DM, Henson D, LaManca J, Levine S. Respiratory muscle function and dyspnea in patients with chronic congestive heart failure. *Circulation*. 1992; 86:909–918. [PubMed: 1516204]
- Manohar M. Vasodilator reserve in respiratory muscles during maximal exertion in ponies. *J Appl Physiol*. 1986; 60(5):1571–1577. [PubMed: 3710976]
- Manohar M. Inspiratory and expiratory muscle perfusion in maximally exercised ponies. *J Appl Physiol*. 1990; 68(2):544–548. [PubMed: 2318766]

- Miller JD, Smith CA, Hemaue SJ, Dempsey JA. The effects of inspiratory intrathoracic pressure production on the cardiovascular response to submaximal exercise in health and chronic heart failure. *Am J Physiol Heart Circ Physiol*. 2007; 292:H580–H592. [PubMed: 16997896]
- Musch TI. Elevated diaphragmatic blood flow during submaximal in rats with chronic heart failure. *Am J Physiol*. 1993; 265:H1721–H1726. [PubMed: 8238585]
- Musch TI, Eklund KE, Hageman KS, Poole DC. Altered regional blood flow responses to submaximal exercise in older rats. *J Appl Physiol*. 2004; 96:81–88. [PubMed: 12959955]
- Musch TI, Terrell JA. Skeletal muscle blood flow abnormalities in rats with a chronic myocardial infarction: rest and exercise. *Am J Physiol*. 1992; 31:H411–H419.
- Myers J, Salleh A, Buchanan N, Smith D, Neutel J, Bowes E, Froelicher VF. Ventilatory mechanisms of exercise intolerance in chronic heart failure. *Am Heart J*. 1992; 124(3):710–719. [PubMed: 1514499]
- O'Donnell DE, D'Arsigny C, Raj S, Abdollah H, Webb KA. Ventilatory assistance improves exercise endurance in stable congestive heart failure. *Am J Respir Crit Care Med*. 1999; 160:1804–1811. [PubMed: 10588589]
- Olson TP, Joyner MJ, Dietz NM, Eisenach JH, Curry TB, Johnson BD. Effects of respiratory muscle work on blood flow distribution during exercise in heart failure. *J Physiol*. 2010; 588:2487–2501. [PubMed: 20457736]
- Poole DC, Hirai DM, Copp SW, Musch TI. Muscle oxygen transport and utilization in heart failure: implications for exercise (in)tolerance. *Am J Physiol Heart Circ Physiol*. 2012; 302:H1050–H1063. [PubMed: 22101528]
- Poole DC, Sexton WL, Behnke BJ, Ferguson CS, Hageman KS, Musch TI. Respiratory muscle blood flows during physiological and chemical hyperpnea in the rat. *J Appl Physiol*. 2000; 88:186–194. [PubMed: 10642380]
- Richardson RS, Poole DC, Knight DR, Kurdak SS, Hogan MC, Grassi B, Johnson EC, Kendrick KF, Erickson BK, Wagner PD. High muscle blood flow in man: is maximal O₂ extraction compromised? *J Appl Physiol*. 1993; 75(4):1911–1916. [PubMed: 8282650]
- Sasaki F, Ishizaki T, Mifune J, Fujimura M, Nishioika S, Miyabo S. Bronchial hyperresponsiveness in patients with chronic congestive chronic heart failure. *Chest*. 1990; 97:534–539. [PubMed: 2306955]
- Smith JR, Alexander AA, Hammer SM, Didier KD, Kurti SP, Broxterman RM, Barstow TJ, Harms CA. Cardiovascular consequences of the inspiratory muscle metaboreflex: effects of age and sex. *Am J Physiol Heart Circ Physiol*. 2017; 312(5):H1013–H1020. [PubMed: 28235792]
- Smith JR, Broxterman RM, Hammer SM, Alexander AA, Didier KD, Kurti SP, Barstow TJ, Harms CA. Sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex. *Am J Physiol Regul Integr Comp Physiol*. 2016; 311(5):R574–R581. [PubMed: 27488888]
- Smith JR, Hageman KS, Harms CA, Poole DC, Musch TI. Respiratory muscle blood flow during exercise: effects of sex and ovarian cycle. *J Appl Physiol*. 2017; 122(4):918–924. [PubMed: 28126910]
- St Croix CM, Morgan BJ, Wetter TJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *J Physiol*. 2000; 529:493–504. [PubMed: 11101657]
- Sullivan MJ, Higginbotham MB, Cobb FR. Increased exercise ventilation in patients with chronic heart failure: intact ventilatory control despite hemodynamic and pulmonary abnormalities. *Circulation*. 1988; 77(3):552–559. [PubMed: 3342486]
- Sullivan MJ, Knight JD, Higginbotham MB, Cobb FR. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation*. 1989; 80:769–781. [PubMed: 2791242]
- Vogiatzis I, Athanasopoulos D, Habazettl H, Kuebler WM, Wagner H, Roussos C, Wagner PD, Zakynthinos S. Intercoastal muscle blood flow limitation in athletes during maximal exercise. *J Physiol*. 2009; 587(14):3665–3677. [PubMed: 19451206]
- Zelis R, Longhurst J, Capone RJ, Mason DT. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation*. 1974; 50:137–143. [PubMed: 4835259]

Highlights

- Chronic heart failure (CHF) disproportionately afflicts older adults
- We examined if older CHF rats had elevated diaphragm BF during exercise
- Older CHF had greater diaphragm BF and lower hindlimb BF compared to healthy rats
- Submaximal exercise hindlimb BF was negatively related to diaphragm BF in CHF rats 1.

Figure. 1. Respiratory muscle BF at rest and during submaximal exercise

Diaphragm (A), intercostal (B), and transversus abdominis (C) BF at rest and during submaximal exercise in older healthy (white bar) and CHF (black bar) rats. Diaphragm, intercostal, and transversus abdominis BF increased ($p < 0.02$) from rest to submaximal exercise. Older CHF had greater ($p < 0.01$) diaphragm BF compared to healthy rats during submaximal exercise. *, significantly different from rest. †, significant different from healthy.

Figure. 2. Change in diaphragm BF from rest to submaximal exercise

Mean and individual changes in diaphragm BF for older healthy (white circles) and CHF (black circles) rats from rest to submaximal exercise. Older CHF had a greater increase ($p < 0.01$) in diaphragm BF compared to healthy rats. †, significant different from healthy.

Figure. 3. Respiratory muscle VCs at rest and during submaximal exercise

Diaphragm (A), intercostal (B), and transversus abdominis (C) BFs at rest and during submaximal exercise in older healthy (white bar) and CHF (black bar) rats. Older CHF had higher ($p < 0.01$) diaphragm VC compared to healthy rats during submaximal exercise. *, significantly different from rest. †, significant different from healthy.

Figure. 4. Hindlimb BF and VC at rest and during submaximal exercise

Hindlimb BF (A) and VC (B) at rest and during submaximal exercise in older healthy (white bar) and CHF (black bar) rats. During submaximal exercise, hindlimb BF was lower ($p=0.04$) for older CHF than healthy rats. *, significantly different from rest. †, significant different from healthy.

Figure. 5. Relationship between diaphragm and hindlimb BF's vs. normalized lung weight in CHF

The relationships between submaximal exercise diaphragm (A) and hindlimb (B) BF's versus normalized lung weight in older CHF rats. There was a significant relationship between diaphragm BF and normalized lung weight ($r=0.94$; $p<0.01$) as well as between hindlimb BF and normalized lung weight ($r=-0.91$; $p=0.03$).

Figure. 6. Relationship between diaphragm and hindlimb BF during submaximal exercise in CHF

The relationship between diaphragm and hindlimb BF during submaximal exercise in older CHF. There was a relationship between diaphragm and hindlimb BF ($r=-0.93$; $p=0.02$).

Table 1
Body weight and heart morphometrics measured in anesthetized rats

	Older Healthy	Older CHF
Body weight (g)	503 ± 17	495 ± 12
LVEDP (mmHg)	12 ± 1	23 ± 3 [†]
LV dP/dt (mmHg/s)	5,686 ± 186	5,200 ± 148 [†]
LV wt/body wt (mg/g)	2.00 ± 0.08	2.03 ± 0.07
RV wt/body wt (mg/g)	0.45 ± 0.01	0.89 ± 0.04 [†]
Lung wt/body wt (mg/g)	3.62 ± 0.12	8.15 ± 0.37 [†]

Values are mean±SE. LVEDP, left ventricular end-diastolic pressure; LV dp/dt, left ventricular developed pressure; LV, left ventricle; RV, right ventricle; wt, weight.

[†]p<0.05 vs. older healthy

Table 2
MAP and HR at rest and during submaximal exercise

	MAP (mmHg)	HR (beats/min)
Older Healthy		
Rest	130 ± 2	419 ± 5
Exercise	134 ± 2	457 ± 6 [*]
Older CHF		
Rest	102 ± 6 [‡]	350 ± 13 [‡]
Exercise	111 ± 6 ^{*‡}	400 ± 11 ^{*‡}

Values are mean ± SE. MAP, mean arterial pressure; HR, heart rate.

^{*}p<0.05 vs. rest;

[‡]p<0.05 vs. older healthy