Skeletal muscle microvascular oxygenation dynamics in heart failure: exercise training and nitric oxide-mediated function

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Hirai DM, Copp SW, Holdsworth CT, Ferguson SK, McCullough DJ, Behnke BJ, Musch TI, Poole DC. Skeletal muscle microvascular oxygenation dynamics in heart failure: exercise training and nitric oxide-mediated function. Am J Physiol Heart Circ Physiol 306: H690–H698, 2014. First published January 10, 2014; doi:10.1152/ajpheart.00901.2013.—Chronic heart failure (CHF) induces multiple alterations in cardiovascular structure and function that culminate in reduced exercise capacity and poor prognosis (8, 51, 52, 54). Derangements in the skeletal muscle O2 transport pathway are major features of this disease (54). Within the microcirculation, impaired hemodynamic control (35, 56) compromises the dynamic matching between O2 delivery and utilization (Q˙O2 and VO2, respectively) and reduces muscle microvascular oxygenation (PO2mv) during transitions in metabolic demand (3, 11, 14, 19, 45). As dictated by Fick’s law of diffusion, lower PO2mv reduces the driving pressure for blood-myocyte O2 flux and impairs oxidative metabolism and contractile performance (29, 64).

Impaired nitric oxide (NO)-mediated function is a hallmark of CHF (16, 32, 37). Notwithstanding the plethora of factors modulating muscle blood flow during exercise (including neural, mechanical, and humoral components), deterioration of NO-mediated function plays a key role in the blunted functional hyperemic response characteristic of CHF (28, 34). Accordingly, these perturbations contribute to the pathological PO2mv profiles (characterized by lower PO2mv and faster PO2mv fall; i.e., speeded kinetics) during muscle contractions in CHF (19). Endurance exercise training, on the other hand, is a nonpharmacological intervention capable of improving NO-mediated function in skeletal muscle of both healthy (21, 43) and CHF (24, 40, 66) individuals. Recent evidence indicates that exercise training improves muscle PO2mv (resulting in higher PO2mv and slower PO2mv kinetics) during muscle contractions in healthy young rats partly via NO-dependent mechanisms (26). Whether similar improvements in contracting muscle PO2mv occur, and, if so, whether they are mediated via enhanced NO signaling in CHF is unknown. Given the role of skeletal muscle dysfunction in the pathophysiology and limiting symptoms of CHF (8, 51, 52, 54) and the importance of PO2mv in setting the driving force for blood-myocyte O2 flux and metabolic control, a better understanding of the mechanistic basis for improving contracting muscle PO2mv with exercise training in CHF is imperative to the design of effective therapeutic treatments for this disease.

The purpose of the present investigation was to determine the effects of endurance exercise training on contracting muscle PO2mv and the contribution of NO to these responses in rats with matched central indexes of CHF. Based on the potential improvement in NO-mediated function following exercise training in CHF (24, 40, 66), we tested the hypotheses that 1) exercise training would elevate muscle PO2mv and slow PO2mv kinetics (i.e., resulting in higher microvascular oxygenation across the on-constractions transient); 2) increased NO levels [via the NO donor sodium nitroprusside (SNP)] would elevate PO2mv and slow PO2mv kinetics to a greater extent in sedentary compared with trained CHF rats; and 3) reduced NO levels [nonspecific NO synthase inhibition with N^5-nitro-L-arginine methyl ester (L-NAME)] would lower PO2mv and speed PO2mv kinetics to a greater extent in trained compared
with sedentary CHF rats during the transition from rest to contractions.

MATERIALS AND METHODS

A total of 26 male Sprague-Dawley rats (body mass: 346 ± 6 g; ~2 to 3 mo old; Charles Rivers Laboratories, Boston, MA) were used in the present investigation. Rats were maintained on a 12-h:12-h light/dark cycle with food and water provided ad libitum. All experimental procedures followed guidelines established by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Kansas State University.

Myocardial infarction was induced in all rats via ligation of the left main coronary artery as described in detail previously (14, 48–50). Briefly, rats were anesthetized with 5% isoflurane-O₂ mixture (Butler Animal Health Supply, Dublin, OH) and intubated for mechanical ventilation with a rodent respirator (model 680; Harvard Instruments, Holliston, MA) for the duration of the surgical procedure. The heart was accessed through a left thoracotomy in the fifth intercostal space and the left main coronary artery was ligated (6-0 silk suture) at ~1 to 2 mm distal to the edge of the left atrium. The incision was then closed, and ampicillin (50 mg/kg im) was injected locally to reduce the opportunity for infection. The analgesic agents bupivacaine (1.5 mg/kg sc) and buprenorphine (0.01–0.05 mg/kg im) were administered subsequently and anesthesia and mechanical ventilation discontinued. All rats were monitored closely for ~6 h for the development of arrhythmias and signs of undue stress (e.g., labored breathing). Rats were also monitored daily (e.g., appetite, weight loss/gain, gait, and posture) according to an intensive 10-day post-operative plan conducted in association with the university veterinary staff. The survival rate for the rats receiving the myocardial infarction operation was 88% (23 out of 26). At the end of the experimental protocol, one rat demonstrated no discernable damage to the left ventricle along with no changes in cardiac morphology or hemodynamic function and was therefore not included in the data analyses. Accordingly, results from the present investigation are presented below for a total of 22 animals. Each animal was allowed ~3 wk of recovery before initiation of any experimental procedure. Previous reports support that this rat myocardial infarction model induces central and peripheral vascular and microvascular dysfunction consonant with CHF (10, 48–50).

Rats were then familiarized with downhill running on a custom-built motor-driven treadmill over the course of a 1-wk period (5–10 min/day at a speed of 20 m/min and ~14% grade). After the familiarization phase, rats were assigned randomly to either sedentary (CHF; n = 11) or endurance exercise trained (CHF + EXT; n = 11) groups. Sedentary rats were confined to cage activities, whereas trained rats ran 5 days/ wk over 6 to 7 wk on the declined treadmill (~14% grade). All rats from the CHF + EXT group underwent the same training protocol, in which treadmill running duration and speed were increased progressively from 10 min at 25 m/min to 60 min at 35 m/min. This final workload was maintained for the final 3 to 4 wk. Downhill treadmill running was selected for the present investigation given that both level and uphill treadmill running do not recruit the rat spinotrapezius muscle (31). Previous work from our laboratory has demonstrated that downhill treadmill running specifically trains the rat spinotrapezius muscle and constitutes an effective model for whole-body exercise training (23, 26). Of note, downhill running as a model of exercise training requires faster treadmill speeds than conventional level or uphill running regimens to elicit equivalent training adaptations in rats with a similar degree of left ventricular dysfunction and CHF (48). CHF and CHF + EXT rats underwent subsequent experimental procedures (described below) after ~5 and 10 wk from the myocardial infarction surgery, respectively. This experimental design was used herein to match central cardiac function in an attempt to isolate peripheral vascular adaptations.

**Peak oxygen uptake measurements.** Peak oxygen uptake (VO₂peak) was measured in CHF and CHF + EXT rats during a downhill (~14% grade) running test performed in a metabolic chamber placed on the treadmill. As described previously (9, 23, 26), the speed was set initially to 25 m/min for 2 to 3 min and then increased progressively in a ramplike fashion by ~5–10 m/min until the rat was unable to keep pace with the treadmill belt or no further elevations in VO₂ were observed despite continued increases in treadmill speed. At this point of the test the VO₂peak was measured and recorded. Alterations in gait (e.g., lowering of the hindlimbs, dropping of the tail, and elevation of the snout) normally occurred immediately before termination of the test. Gas measurements were performed in real time via an inline O₂ analyzer (model S-3A/I; AEI Technologies; Pittsburgh, PA). The analyzer was calibrated with precision-mixed gases that spanned the expected range of gas concentrations based on previous studies. We have reported previously highly reproducible VO₂peak measurements using the aforementioned techniques and protocol (9).

**Surgical preparation.** Rats were anesthetized initially with a 5% isoflurane-O₂ mixture and maintained on 2% to 3% isoflurane-O₂ and placed on a heating pad to maintain core temperature at ~37°C to 38°C as measured via rectal probe. The right carotid artery was cannulated, and a 2-Fr catheter-tipped pressure transducer (Millar Instruments; Houston, TX) was advanced into the left ventricle for measurement of systolic and diastolic pressures and left ventricular ΔP/dt/dt (LV dp/dt). Upon completion of these measurements, the transducer was removed and the carotid artery was cannulated with a catheter (PE-10 connected to PE-50; Intra-Medic Tubing; Clay Adams Brand, Sparks, MD) for continuous monitoring of mean arterial pressure (MAP; Digi-Med BPA model 200; Louisville, KY) and infusion of the phosphorescent probe palladium meso-tetra-(4-carboxyphenyl)porphyrin dendrimer (R2; 15 mg/kg; Oxygen Enterprises, Philadelphia, PA). The tail (caudal) artery was cannulated (PE-10 connected to PE-50) for blood sampling and infusion of anesthetic agents. Blood from the tail catheter was sampled at the end of the experimental protocol for determination of arterial gases, pH, and systemic hematocrit (Nova Stat Profile M; Waltham, MA).

Isoflurane-O₂ mixture inhalation was discontinued progressively after catheter placement procedures, and rats were kept under anesthesia with pentobarbital sodium administered intravenously to effect. The level of anesthesia was monitored frequently via the blink and toe-pinch reflexes and supplemented as necessary. Overlying skin and fascia from the midventral region of the rat were reflected surgically to expose the left anterior descending coronary artery. Myocardial blood flow was measured with a microsphere probe (model microsphere 8.5; Biomedical Sciences, San Diego, CA) (17). Myocardial blood flow was calculated by injecting radioactive microspheres (1.8 μm; Microparticles, Cockeysville, MD) through a catheter inserted into the left atrium. A second catheter was advanced into the left ventricle for measurement of coronary sinus blood flow. At the end of each experiment, rats were sacrificed by potassium chloride injection (170–200 mg/kg im) and the heart excised for histological examination. Myocardial infarction surgery, respectively. This experimental design demonstrated that the superfusion protocol used herein (see below) does not exert residual (i.e., post-washout) SNP alteration...
and/or impairment of skeletal muscle blood flow, VO₂ or PO₂mv responses either at rest or during contractions (27). The spinotrapezius was superfused with each solution (average flow rate of ~1.5 ml/min) for a total time of 3 min, followed by ~3 min incubation period to allow resting muscle PO₂mv to stabilize. Subsequently, electrical stimulation (1 Hz, ~7 V, 2-ms pulse duration) of the muscle was evoked via a stimulator (model s48; Grass Technologies, Quincy, MA) for 3 min. This stimulation protocol evokes an approximately four- to fivefold increase in blood flow together with approximately six- to sevenfold increase in metabolic rate (which corresponds to ~30% spinotrapezius VO₂peak) above resting with either minor or no alterations in blood pH that are consistent with moderate intensity exercise (4, 27). The muscle was then allowed to recover for ~25 min before the next condition was initiated (stimulation parameters were held constant). During the recovery period following the SNP condition, the muscle was superfused at an average flow rate of ~1.5 ml/min with Krebs-Henseleit to wash out SNP. A >30-min recovery period between consecutive bouts (i.e., 3 min off-transition, ~25 min passive recovery, 3 min superfusion, ~3 min incubation) was used herein to prevent any priming and drug-ordering effects that could confound the experimental interpretation of the PO₂mv responses to muscle contractions (5, 20). We have reported previously that the spinotrapezius preparation exhibits reproducible PO₂mv responses from three contraction bouts separated by ~30 min (26). At the end of the experimental protocol, rats were euthanized with intra-arterial pentobarbital sodium overdose (>50 mg/kg). For each rat, the lungs and heart were dissected, weighed, and normalized to body mass. Myocardial infarct size was determined via planimetry as described in detail previously (19).

Spinotrapezius muscle PO₂mv measurement. PO₂mv was measured by phosphorescence quenching using a frequency domain phosphorimeter (PMOD 5000; Oxygen Enterprises, Philadelphia, PA). As described in detail previously (4), this technique applies the Stern-Volmer relationship (59), which describes quantitatively the O₂ dependence of the phosphorescent probe (R2) via the following equation:

\[ PO₂mv = \frac{[(\tau′/\tau) - 1]}{(k_Q \times \tau′)} \]

where kQ is the quenching constant and τ and τ′ are the phosphorescence lifetimes in the absence of O₂ and the ambient O₂ concentration, respectively. The phosphor R2 (τ′ = 601 μs and kQ = 409 mmHg⁻¹/l at pH 7.4 and temperature ~38°C (41)) was infused ~15 min before initiation of the first muscle contraction. R2 contains Pd-porphyrin cores that bind to biological macromolecules (primarily albumin in blood plasma) (67). This facilitates its uniform distribution in the plasma and provides a signal corresponding to the volume-weighted O₂ pressure in the microvascular compartment (primarily the PO₂ within capillaries, which volumetrically constitutes the major intramuscular space) (53). Restriction of the R2 probe to the microvascular space is also promoted by its negative charge (55). The common end of the bifurcated light guide was positioned ~2–4 mm superficial to the dorsal surface of the exposed spinotrapezius muscle. The phosphorometer modulates sinusoidal excitation frequencies between 100 Hz and 20 kHz and allows phosphorescence lifetime measurements from 10 μs to ~2.5 ms. The excitation light (524 nm) was focused on a randomly selected area of ~2 mm diameter of exposed muscle and has a penetration depth of ~500 μm. PO₂mv was measured continuously and recorded at 2-s intervals throughout the duration of the experimental protocol.

Analysis of muscle PO₂mv kinetics. The kinetics of PO₂mv were described by nonlinear regression analysis using the Marquardt-Levenberg algorithm (SigmaPlot 11.2; Systat software, San Jose, CA) for the onset of contractions. Transient PO₂mv responses were fit with either a one- or two-component model as follows.

One-component:

\[ PO₂mv(t) = PO₂mv(0) - PO₂mv(1) \left( 1 - e^{-\left( -t/TD \right)} \right) \]

Two-component:

\[ PO₂mv(t) = PO₂mv(0) - PO₂mv(1) \left( 1 - e^{-\left( -t/TD \right)} \right) + PO₂mv(2) \left( 1 - e^{-\left( -t/TD_1 \right)} \right) \]

where PO₂mv(0) is the PO₂mv at a given time t; PO₂mv(1,2) correspond to the precontracting resting PO₂mv; TD and TD₁ are the time constants (i.e., time to 63% of the response) for each component. Goodness of fit was determined using three criteria: the coefficient of determination, sum of squared residuals, and visual inspection.

The mean response time (MRT) was used to describe the overall dynamics of the PO₂mv fall following the onset of muscle contractions:

\[ MRT = TD + \tau \]

where TD and τ are defined above. The MRT analysis was limited to the first component of the PO₂mv response given that inclusion of an emergent second component underestimates the actual speed of the PO₂mv fall for the onset of contractions (26).

Movement of the light guide and/or animal was avoided to monitor the same sampling site in a given animal throughout all experimental conditions. However, alteration of the PO₂mv measurement plane (e.g., due to deep sighs of the animal, etc.) during muscle contractions precluded kinetic curve fitting in some instances. Therefore, PO₂mv kinetics from the present investigation are given for the following animal numbers under the specified conditions: CHF control (10/11), CHF SNP (10/11), CHF l-NAME (10/11), CHF + EXT control (11/11), CHF + EXT SNP (7/11) and CHF + EXT l-NAME (11/11).

Citrate synthase measurement. The activity of the mitochondrial enzyme citrate synthase (a marker of oxidative capacity) from the spinotrapezius and select individual hindlimb muscles or muscle parts (soleus, red gastrocnemius, and white gastrocnemius) was measured (soleus, red gastrocnemius, and white gastrocnemius) was measured by 10.220.33.5 on October 23, 2017 http://ajpheart.physiology.org/ Downloaded from

### Table 1. Morphological and hemodynamic characteristics of CHF and CHF + EXT rats

<table>
<thead>
<tr>
<th>CHF</th>
<th>CHF + EXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDP, mmHg</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>LV dp/dt, mmHg/s</td>
<td>6,300 ± 347</td>
</tr>
<tr>
<td>RV, mg</td>
<td>350 ± 23</td>
</tr>
<tr>
<td>RV/body mass, mg/g</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td>Lung, mg</td>
<td>2,606 ± 304</td>
</tr>
<tr>
<td>Lung/body mass, mg/g</td>
<td>5.55 ± 0.75</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>38 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVEDP, left ventricular end-diastolic pressure; LV dp/dt, left ventricular Δpressure/Δtime; RV, right ventricle; CHF, chronic heart failure; EXT, endurance exercise trained.
RESULTS

Morphological and hemodynamic variables revealed impaired ventricular function consistent with the presence of a substantial myocardial infarction and LV wall destruction in both groups (Table 1). CHF and CHF + EXT rats showed similar central indexes of moderate compensated heart failure (Table 1; \( p > 0.05 \) for all).

The greater body weight in CHF + EXT compared with CHF rats (CHF: 479 ± 11; CHF + EXT: 541 ± 10 g; \( p < 0.05 \)) can be ascribed to the experimental design used herein, i.e., endpoint was \( 0.05 \) and 10 weeks post-myocardial infarction for CHF and CHF + EXT rats, respectively. CHF + EXT rats had higher \( \text{VO}_2 \text{peak} \) than CHF rats (CHF: 63 ± 2; CHF + EXT: 68 ± 2 ml kg\(^{-1}\) min\(^{-1} \); \( p < 0.05 \)). Citrate synthase activity was higher in the soleus (CHF: 29.0 ± 1.0; CHF + EXT: 36.9 ± 1.7 mmol g\(^{-1}\) min\(^{-1} \)) and red gastrocnemius (CHF: 39.4 ± 1.4; CHF + EXT: 55.1 ± 1.3 mmol g\(^{-1}\) min\(^{-1} \)) and spinotrapezius (CHF: 19.7 ± 0.7; CHF + EXT: 21.6 ± 0.8 mmol g\(^{-1}\) min\(^{-1} \)) muscles from CHF + EXT rats (\( p < 0.05 \) for all). Citrate synthase activity from the white gastrocnemius was not different between CHF and CHF + EXT (10.6 ± 0.8 and 12.2 ± 1.0 mmol g\(^{-1}\) min\(^{-1} \), respectively; \( p > 0.05 \)).

No differences in relative spinotrapezius muscle mass were found between CHF and CHF + EXT (0.89 ± 0.03 and 0.94 ± 0.02 mg/g, respectively; \( p > 0.05 \)).

There were no differences in arterial \( \text{O}_2 \) saturation (CHF: 90.9 ± 1.3; CHF + EXT: 93.8 ± 0.6%), \( \text{pH} \) (CHF: 7.42 ± 0.01; CHF + EXT: 7.43 ± 0.01) or systemic hematocrit (CHF: 34.3 ± 1.3; CHF + EXT: 36.0 ± 0.6%) between groups (\( p > 0.05 \) for all).

**Effects of exercise training on muscle \( \text{PO}_{2\text{mv}} \) in CHF.** MAP was not different in CHF compared with CHF + EXT rats either before or after Krebs-Henseleit superfusion (Table 2; \( p > 0.05 \) for all). The stimulation protocol used herein did not evoke significant changes in MAP in CHF or CHF + EXT rats in any of the distinct superfusion conditions (\( p > 0.05 \) for all). The mean difference in MAP between the onset and offset of contractions was as follows: CHF control: 2 ± 1; CHF SNP: 8 ± 2; CHF l-NAME: 2 ± 1; CHF + EXT control: 0 ± 3; CHF + EXT SNP: 6 ± 2; CHF + EXT l-NAME: −1 ± 1 mmHg.

Although resting and contracting steady-state \( \text{PO}_{2\text{mv}} \) [\( \text{PO}_{2\text{mv(BL)}} \) and \( \text{PO}_{2\text{mv(IS)}} \), respectively] were not different between groups (Fig. 1 and Table 3; \( p > 0.05 \) for both), exercise training induced significant differences in the time course of \( \text{PO}_{2\text{mv}} \) following the onset of contractions under the control condition (Figs. 1–4 and Table 3). Specifically, the overall speed of \( \text{PO}_{2\text{mv}} \) fall during contractions (MRT and \( T_{0.3} \)) was markedly slowed in CHF + EXT compared with CHF rats (Fig. 4 and Table 3; \( p < 0.05 \) for all).

Table 2. *Mean arterial pressure (expressed in mmHg) pre- and postsuperfusion of Krebs-Henseleit (control), SNP, and L-NAME in CHF and CHF + EXT rats*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SNP</th>
<th>L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>CHF</td>
<td>105 ± 3</td>
<td>104 ± 3</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>CHF + EXT</td>
<td>115 ± 7</td>
<td>115 ± 7</td>
<td>114 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. SNP, sodium nitroprusside. Significantly different from: *CHF post- \( N^\alpha\)-nitro-l-arginine methyl ester (l-NAME) superfusion.

**Effects of altered NO on muscle \( \text{PO}_{2\text{mv}} \) in sedentary and trained CHF rats.** SNP superfusion did not change MAP in either CHF or CHF + EXT rats (Table 2; \( p > 0.05 \) for both). Relative to the control condition, SNP elevated \( \text{PO}_{2\text{mv(BL)}} \) and \( \text{PO}_{2\text{mv(IS)}} \) in both groups to similar values (Figs. 1 and 2 and Table 3; \( p < 0.05 \) for all). No differences in the amplitude of
the \( \Delta PO_{2mv} \) response following the onset of contractions (\( \Delta_1 PO_{2mv} \) and \( \Delta_\text{Total} PO_{2mv} \)) were observed between CHF and CHF + EXT rats with SNP (Table 3; \( p > 0.05 \) for all). SNP slowed MRT, \( T_{63} \), and \( \tau_1 \) in both groups such that CHF + EXT rats continued to display slower \( PO_{2mv} \) kinetics when compared with their sedentary counterparts (Fig. 4 and Table 3; \( p < 0.05 \) for all). It is important to note that the absolute and relative changes promoted by SNP in MRT, \( T_{63} \), and \( \tau_1 \) were not different when comparing CHF and CHF + EXT rats (data not shown; \( p > 0.05 \)).

\( \text{L-NAME} \) superfusion did not change MAP in either CHF or CHF + EXT rats (Table 2; \( p > 0.05 \) for both). Relative to the control condition, \( \text{L-NAME} \) had no effects on \( PO_{2mv(BL)} \) in either group but decreased \( PO_{2mv(SS)} \) only in CHF + EXT rats (Figs. 1 and 2 and Table 3; \( p < 0.05 \)). No differences in the amplitude of the \( PO_{2mv} \) response following the onset of contractions (\( \Delta_1 PO_{2mv} \), \( \Delta_2 PO_{2mv} \), and \( \Delta_{\text{Total}} PO_{2mv} \)) were observed between CHF and CHF + EXT rats with \( \text{L-NAME} \) (Table 3; \( p > 0.05 \) for all). Notably, \( \text{L-NAME} \) did not alter the time course of \( PO_{2mv} \) fall at the onset of contractions (i.e., \( T_{D1}, \tau_1, MRT \), and \( T_{63} \)) in CHF or CHF + EXT rats when compared with the control condition (Figs. 3 and 4 and Table 3).

### Table 3. Spinotrapezius muscle \( PO_{2mv} \) kinetics following the onset of contractions under control, SNP, and L-NAME conditions in CHF and CHF + EXT rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CHF + EXT</th>
<th>SNP</th>
<th>CHF + EXT</th>
<th>L-NAME</th>
<th>CHF + EXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>( PO_{2mv(BL)} ), mmHg</td>
<td>24.3 ± 2.3</td>
<td>22.6 ± 1.9</td>
<td>33.7 ± 2.3*</td>
<td>35.3 ± 2.0*</td>
<td>23.5 ± 2.4†</td>
<td>21.3 ± 1.0†</td>
</tr>
<tr>
<td>( \Delta_1 PO_{2mv} ), mmHg</td>
<td>10.3 ± 1.0</td>
<td>9.5 ± 1.1</td>
<td>8.8 ± 0.8</td>
<td>10.4 ± 2.1</td>
<td>11.9 ± 1.6</td>
<td>11.6 ± 1.0</td>
</tr>
<tr>
<td>( \Delta_2 PO_{2mv} ), mmHg</td>
<td>1.9 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>8.8 ± 0.8</td>
<td>10.4 ± 2.1</td>
<td>2.4 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>( \Delta_{\text{Total}} PO_{2mv} ), mmHg</td>
<td>8.8 ± 1.0</td>
<td>8.7 ± 1.0</td>
<td>24.8 ± 2.2*</td>
<td>24.9 ± 2.2*</td>
<td>9.7 ± 1.3</td>
<td>10.6 ± 0.8</td>
</tr>
<tr>
<td>( PO_{2mv(SS)} ), mmHg</td>
<td>15.5 ± 1.8</td>
<td>13.9 ± 1.2</td>
<td>4.0 ± 1.7*</td>
<td>5.7 ± 2.1†‡</td>
<td>13.7 ± 1.2†</td>
<td>10.7 ± 0.6†‡</td>
</tr>
<tr>
<td>( TD_1 ), s</td>
<td>8.9 ± 1.5</td>
<td>12.6 ± 1.3†‡</td>
<td>39.0 ± 6.5*</td>
<td>49.8 ± 8.3‡</td>
<td>6.7 ± 0.7†‡</td>
<td>11.9 ± 2.1†‡</td>
</tr>
<tr>
<td>( TD_2 ), s</td>
<td>45.7 ± 5.7</td>
<td>83.3 ± 10.5</td>
<td>50.2 ± 7.1†‡</td>
<td>55.0 ± 7.1♭‡</td>
<td>35.4 ± 4.7</td>
<td>60.5 ± 15.5</td>
</tr>
<tr>
<td>( \tau_1 ), s</td>
<td>11.9 ± 1.3</td>
<td>19.7 ± 3.0#</td>
<td>39.0 ± 6.5*</td>
<td>49.8 ± 8.3‡</td>
<td>10.1 ± 1.4†‡</td>
<td>19.1 ± 2.9†#</td>
</tr>
<tr>
<td>( \tau_2 ), s</td>
<td>41.2 ± 12.6</td>
<td>57.0 ± 9.9</td>
<td>0.8 ± 1.0</td>
<td>1.3 ± 1.0</td>
<td>59.3 ± 9.1</td>
<td>38.0 ± 13.5</td>
</tr>
<tr>
<td>( T_{63} ), s</td>
<td>20.4 ± 1.2</td>
<td>31.2 ± 2.3*</td>
<td>42.2 ± 7.7*</td>
<td>55.0 ± 7.1†‡</td>
<td>17.4 ± 1.6†‡</td>
<td>31.3 ± 3.4†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( PO_{2mv(BL)} \) resting \( PO_{2mv} \), \( \Delta_1 PO_{2mv} \), amplitude of the first component; \( \Delta_2 PO_{2mv} \), amplitude of the second component; \( \Delta_{\text{Total}} PO_{2mv} \), overall amplitude regardless of one- or two-component model fit; \( PO_{2mv(SS)} \), contracting steady-state \( PO_{2mv} \); \( TD_1 \), time delay for the first component; \( TD_2 \), time delay for the second component; \( \tau_1 \), time constant for the first component; \( \tau_2 \), time constant for the second component; \( T_{63} \), time to reach 63% of the primary amplitude as determined independent of modeling procedures. The one-component exponential model was used to analyze the \( PO_{2mv} \) kinetics in the following conditions: CHF control (2/10), CHF SNP (10/10), CHF L-NAME (1/10), CHF + EXT control (7/10), CHF + EXT SNP (7/7), CHF + EXT L-NAME (5/11).

Significantly different from: *control within group; †SNP within group; ‡CHF within superfusion condition; #P = 0.05–0.1 vs. CHF within condition.

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**Fig. 2.** Spinotrapezius muscle \( PO_{2mv} \) responses from representative CHF and CHF + EXT rats under sodium nitroprusside (SNP) and N\(^\text{ω}\)-nitro-L-arginine methyl ester (L-NAME) superfusion conditions. Time zero denotes the onset of muscle contractions. Note that SNP slowed \( PO_{2mv} \) kinetics to a greater extent in CHF + EXT compared with CHF rats. L-NAME did not alter \( PO_{2mv} \) kinetics in either group but reduced the contraction steady-state \( PO_{2mv} \) only in CHF + EXT rats when compared with control. Therefore, CHF + EXT rats continued to exhibit slower \( PO_{2mv} \) kinetics than their sedentary counterparts during the L-NAME condition. \( PO_{2mv} \) kinetics parameters are shown in Table 3 and Fig. 4.

**Fig. 3.** Mean relative spinotrapezius muscle \( PO_{2mv} \) responses in CHF and CHF + EXT rats under control, SNP, and L-NAME superfusion conditions. Time zero denotes the onset of muscle contractions. SE bars were omitted for clarity. Note slower \( PO_{2mv} \) kinetics in CHF + EXT compared with CHF rats during the control condition. SNP slowed \( PO_{2mv} \) kinetics in CHF + EXT compared with CHF rats, whereas L-NAME had no effects on \( PO_{2mv} \) kinetics in either group relative to the control condition. \( PO_{2mv} \) kinetics parameters are shown in Table 3 and Fig. 4.
3; \ p > 0.05 \text{ for all). Thus CHF + EXT rats exhibited slower PO_{mv} kinetics (i.e., TD, MRT, and T_{63}) than their sedentary counterparts during the L-NAME condition (Fig. 4 and Table 3; \ p<0.05 \text{ for all}).

**DISCUSSION**

The present investigation demonstrates that endurance exercise training fundamentally changes the microvascular oxygenation profile (i.e., slows PO_{mv} kinetics) following the onset of contractions in the spinotrapezius muscle of CHF rats. Relative to the control condition, increased NO with SNP slowed PO_{mv} kinetics in both groups (such that CHF + EXT rats had greater MRT of the PO_{mv} fall), whereas decreasing NO bioavailability with L-NAME had no effects on PO_{mv} kinetics in either group. Contrary to our hypothesis, enhanced NO-mediated function may not be obligatory for training-induced improvements in contracting muscle microvascular oxygenation in CHF.

**Exercise training and muscle microvascular oxygenation in CHF.** Skeletal muscle PO_{mv} kinetics reflect the dynamic matching between QO$_2$ and VO$_2$ within the microvascular space during transitions in metabolic demand (4). Therefore, slowed PO_{mv} kinetics following the onset of contractions in CHF + EXT rats (Figs. 1–4 and Table 3) indicates an improved QO$_2$/VO$_2$ matching secondary to faster adjustment in muscle QO$_2$ compared with CHF rats (14). Importantly, enhanced microvascular QO$_2$ responses across the rest-contraction transient after exercise training oppose the preadations of CHF on muscle conductive and diffusive microvascular QO$_2$ [i.e., mainly reduced red blood cell flux (f_{RBC}) and proportion of red blood cell-flowing capillaries] (35, 56) and reduce fractional O$_2$ extraction (thus increasing effectively the pressure gradient driving O$_2$ from the blood into the myocyte). Moreover, improvements in contracting muscle microvascular QO$_2$ are crucial to support a faster rate of oxidative phosphorylation (i.e., faster VO$_2$ kinetics; as suggested by higher spinotrapezius citrate synthase activity seen herein and reported previously) (46, 58) following completion of an exercise training program in CHF individuals. At any given submaximal contractile activity, these training-induced adaptations in microvascular O$_2$ transport would be expected to attenuate perturbations of the intracellular milieu (e.g., changes in ADP, PCR, and Cr concentrations), reduce the rate of anaerobic glycolysis and reliance on finite energy sources, and contribute to increased exercise tolerance as documented previously (29, 30, 64, 65), thus providing a mechanistic basis for these effects.

The improvement (slowing) in muscle PO_{mv} kinetics seen herein after training derives principally from adaptations in peripheral microvascular control given that no differences in central indexes of heart failure were observed between CHF and CHF + EXT groups (Table 1). This finding is consistent with our experimental design and with the notion that training-induced adaptations to left ventricular pump function (i.e., improved systolic volume and cardiac output) are a function of training frequency, intensity, and duration, such that central cardiac adaptations in CHF rats might only be observed following high-intensity sprint training regimens (47).

The peripheral adaptations observed herein are also consistent with the fact that CHF patients exhibit substantial plasticity within their skeletal muscle O$_2$ transport pathway (conductive and diffusive components) in response to exercise training (17) and the major role of skeletal muscle dysfunction in the pathophysiology of CHF (8, 51, 52, 54). As discussed previously (26, 54), there is an interdependence between muscle conductive and diffusive O$_2$ transport components setting fractional O$_2$ extraction (57): O$_2$ extraction = 1 − e^{-DO_2/Q_m}$, where DO$_2$ is muscle O$_2$ diffusing capacity (dictated largely by capillary hematocrit and the volume density of red blood cell-flowing capillaries) (18, 22), $\beta$ is the slope of the O$_2$ dissociation curve in the physiologically relevant range, and Q$_m$ is muscle blood flow. Because $\beta$ is unlikely to be affected appreciably by exercise training, alterations in O$_2$ extraction (and thus PO$_{mv}$) will depend primarily on the $DO_2/Q_m$ ratio (57). It is interesting to note that although CHF induces peripheral microvascular dysfunction via impairments in both muscle DO$_2$ (reduced proportion of red blood cell-flowing capillaries) and Q$_m$ (reduced f_{RBC}) (35, 56), exercise training can promote beneficial adaptations in both DO$_2$ and Q$_m$ (60). In this sense, slower PO$_{mv}$ kinetics following the onset of contractions in CHF + EXT rats (Figs. 1–4 and Table 3) is evidence for a lower $DO_2/Q_m$ ratio (i.e., reduced fractional O$_2$ extraction) and suggests that adaptations in conductive (Q$_m$; mainly faster f_{RBC} kinetics) rather than diffusive O$_2$ transport are of relatively greater importance in improving contracting muscle microvascular oxygenation in this disease.

**Effects of altered NO on PO$_{mv}$ kinetics in CHF.** NO and its derivatives are important modulators of skeletal muscle vascular control (63). Impairments in NO-mediated function contribute to diminished functional hyperemia (28, 34) and temporal QO$_2$/VO$_2$ mismatch (19) during muscle contractions in CHF. Conversely, exercise training potentially ameliorates NO-induced vasodilation in CHF (24, 40, 66) and improves (slows) contracting muscle PO$_{mv}$ kinetics in healthy individuals (26). We therefore hypothesized initially that endurance exercise training would enhance muscle microvascular oxygenation following the onset of contractions (i.e., slow PO$_{mv}$ kinetics) in CHF partly via improved NO-mediated function. Surprisingly, SNP slowed the overall dynamics of the PO$_{mv}$ fall (mean response time; MRT) in CHF and CHF + EXT rats, whereas L-NAME did not alter MRT in either group (Fig. 4). Despite the small but significant reduction in the contracting
steady-state $PO_{2mv}$ [$PO_{2mv}^{SS}$] in CHF + EXT rats with 1-NAME (Table 3), our results suggest that improved NO-mediated function is not obligatory for training-induced enhancement of muscle $PO_{2mv}$ kinetics following contractions onset in rats with CHF.

These responses contrast markedly with those seen previously in healthy rats, in which SNP slowed $PO_{2mv}$ kinetics to a greater extent in sedentary animals and 1-NAME abolished the differences in $PO_{2mv}$ kinetics between sedentary and trained animals that were evident during the control condition (26). It is therefore crucial to appreciate that in trained CHF skeletal muscle, enhanced NO-mediated function may not be obligatory to slow $PO_{2mv}$ kinetics (and consequently to increase the pressure head for $O_2$ diffusion) at a time when $V_{O2}$ is rising at its fastest rate and improve muscle $O_2$ supply and oxidative function following contractions onset (4, 14, 29, 54, 64).

Potential mechanisms underlying slowed $PO_{2mv}$ kinetics with training in CHF. Although endurance exercise training improves muscle $PO_{2mv}$ kinetics (potentially via a speeding of the $f_{RBC}$ response as discussed above) following the onset of contractions in both healthy (26) and CHF (present investigation) rats, enhanced NO signaling does not appear to be intrinsic to training-induced adaptations in muscle microvascular $O_2$ exchange in CHF + EXT (Figs. 1–4 and Table 3). Despite evidence supporting an improvement in NO-induced vasodilation in CHF after exercise training (24, 40, 66), it is interesting to note that Lindsay et al. (39) reported no effects of training on acetylcholine-induced relaxation of aortic rings in CHF rats and Yi et al. (68) found preserved prostaglandin-induced relaxation of coronary arteries from trained CHF dogs. Together with the fact that impairments in NO-mediated function with CHF might be compensated by increased contribution of other vasodilators such as endothelium-derived hyperpolarizing factors (EDHF) (33, 42), these data highlight the redundancy and synergism of mechanisms regulating muscle $O_2$ delivery (25) and suggest that exercise training may alter the relative contribution of distinct mechanisms governing microvascular oxygenation in CHF.

Among the myriad of training-induced adaptations that might enhance contracting muscle $PO_{2mv}$ kinetics in CHF + EXT are improved neurohumoral (e.g., ↓ catecholamines, arginine vasopressin, angiotensin II, endothelin-1), autonomic (↓ sympathetic tone and ↑ vagal tone), and inflammatory (e.g., ↓ tumor necrosis factor-$\alpha$, interleukin-6) responses; reduced oxidative stress (e.g., ↑ superoxide dismutase, catalase, glutathione peroxidase); and structural alterations (↑ capillarity) (7, 12, 15, 51, 52, 54). Training might also produce alterations in the regional distribution of $Q_{tot}$ (49) and attenuate spatial heterogeneities in contracting muscle microvascular oxygenation (36). These and other potential adaptations may shift the balance between vasodilators and vasoconstrictors in favor of the former without obligatory improvements in NO-mediated function (e.g., ↑ EDHF and ↓ endothelin-dependent contracting factors, respectively) and thus improve muscle $Q_O_2/V_O_2$ matching (slow $PO_{2mv}$ kinetics) during transitions in metabolic demand in trained CHF individuals. Future studies designed to address the relative contribution of the abovementioned factors in improving skeletal muscle $PO_{2mv}$ kinetics following exercise training in CHF will be valuable.

Clinical implications. Resolution of the mechanisms underlying the improvements in muscle microvascular oxygenation following exercise training in CHF is crucial to the development of effective therapeutic strategies targeting this disease. The current results carry important clinical implications for CHF populations by demonstrating that enhanced NO-mediated function may not be obligatory for slowed $PO_{2mv}$ kinetics across the rest-contractions transient after training. Moreover, endurance exercise training might constitute a more powerful intervention than some contemporary pharmacological therapies (e.g., angiotensin-converting enzyme inhibitors and $\beta$-adrenergic blockers), which have been reported not to abrogate microvascular oxygenation deficits during muscle contractions in CHF patients (61).

Experimental considerations. As mentioned above, downhill treadmill running was used herein as a model of endurance exercise training given that this paradigm effectively trains the rat spinotrapezius muscle (as evidenced by slowed $PO_{2mv}$ kinetics, greater citrate synthase activity, and resistance to fatigue) and induces training adaptations such as increased whole-body $V_{O2peak}$ and hindlimb muscle citrate synthase activity (present results; refs. 23, 26). In fact, the effects of downhill training on whole-body $V_{O2peak}$ and muscle oxidative enzyme capacity observed in the current investigation are similar to those reported previously by Musch et al. (48) in male CHF rats with similar left ventricular infarct size and LVEDP subjected to uphill training (10% grade).

It could be argued that reduced driving pressure (MAP) with SNP compared with 1-NAME in CHF rats (Table 2) potentially constrains blood flow dynamics and influences $PO_{2mv}$ kinetics during metabolic transitions. However, previous reports from our laboratory (6) indicate that this effect is negligible or nonexistent when MAP is greater than $\sim$70 mmHg as herein.

Further experimental considerations involving the utilization of downhill treadmill running as a model of endurance exercise training, differences in vascular adaptations to training based on muscle fiber type composition, and potential muscle damage following eccentric exercise (downhill running) have been discussed in detail previously (26).

Conclusions

The current novel findings indicate that endurance exercise training is a powerful nonpharmacological treatment capable of improving muscle microvascular oxygenation (resulting in slowed $PO_{2mv}$ kinetics) during metabolic transitions in CHF. Contrary to what was found previously in healthy skeletal muscle (26), enhanced NO-mediated function may not be obligatory for these training-induced microvascular adaptations in CHF.

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