Effects of nitrate infusion on skeletal muscle vascular control during exercise in rats with chronic heart failure

Angela A. Glean, Scott K. Ferguson, Clark T. Holdsworth, Trenton D. Colburn, Jennifer L. Wright, Alex J. Fees, Karen S. Hageman, David C. Poole, and Timothy I. Musch

Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas; and Department of Kinesiology, Kansas State University, Manhattan, Kansas

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Glean AA, Ferguson SK, Holdsworth CT, Colburn TD, Wright JL, Fees AJ, Hageman KS, Poole DC, Musch TI. Effects of nitrate infusion on skeletal muscle vascular control during exercise in rats with chronic heart failure. Am J Physiol Heart Circ Physiol 309: H1354–H1360, 2015. First published September 14, 2015; doi:10.1152/ajpheart.00421.2015.—Chronic heart failure (CHF) reduces nitric oxide (NO) bioavailability and impairs skeletal muscle vascular control during exercise. Reduction of NO2 to NO may impact exercise-induced hyperemia, particularly in muscles with pathologically reduced O2 delivery. We tested the hypothesis that NO2 infusion would increase exercising skeletal muscle blood flow (BF) and vascular conductance (VC) in CHF rats with a preferential effect in muscles composed primarily of type IIb IId/x fibers. CHF (coronary artery ligation) was induced in adult male Sprague-Dawley rats. After a >21-day recovery, mean arterial pressure (MAP; carotid artery catheter) and skeletal muscle BF (radioindicated microspheres) were measured during treadmill exercise (20 m/min, 5% incline) with and without NO2 infusion. The myocardial infarct size (35 ± 3%) indicated moderate CHF. NO2 infusion increased total hindlimb skeletal muscle VC (CHF: 0.85 ± 0.09 ml·min⁻¹·100 g⁻¹·mmHg⁻¹ and CHF + NO2: 0.93 ± 0.09 ml·min⁻¹·100 g⁻¹·mmHg⁻¹, P < 0.05) without changing MAP (CHF: 123 ± 4 mmHg and CHF + NO2: 120 ± 4 mmHg, P = 0.17). Total hindlimb skeletal muscle BF was not significantly different (CHF: 102 ± 7 and CHF + NO2: 109 ± 7 ml·min⁻¹·100 g⁻¹·mmHg⁻¹, P > 0.05). BF increased in 6 (~21%) and VC in 8 (~29%) of the 28 individual muscles and muscle parts. Muscles and muscle portions exhibiting greater BF and VC after NO2 infusion comprised ~63% type IIb + IId/x muscle fibers. These data demonstrate that NO2 infusion can augment skeletal muscle vascular control during exercise in CHF rats. Given the targeted effects shown here, a NO2-based therapy may provide an attractive “needs-based” approach for treatment of the vascular dysfunction in CHF.

nitrate; nitric oxide; blood flow; disease

NEW & NOTEWORTHY

Chronic heart failure results in impaired skeletal muscle O2 delivery and thus exercise tolerance due, in part, to reduced nitric oxide (NO) bioavailability. Here, we demonstrate the vascular effects of NO2 (a NO storage pool) infusion on skeletal muscle vascular function during exercise in rats with chronic heart failure.

CHRONIC HEART FAILURE (CHF) is characterized by a combination of central and peripheral circulatory dysfunction that ultimately impairs exercise tolerance and quality of life (30, 47). About 23 million individuals worldwide suffer from this disease (37), and while central cardiac dysfunction is fundamental to the etiology of CHF, the peripheral vascular impairments induced by CHF are of paramount importance. CHF-induced vascular dysregulation is thought to be due, in part, to reduced nitric oxide (NO)-mediated function (Ref. 20; for a review, see Ref. 47). Although exercise rehabilitation represents an effective therapeutic modality for treating CHF (9), reduced NO bioavailability in CHF is associated with an impaired ability to perform exercise and/or even complete daily physical activities. Consequently, interventions that increase NO bioavailability may ameliorate the skeletal muscle vascular dysfunction evident in this disease (20, 25) and thus have great potential to improve exercise tolerance and quality of life in this population.

NO is synthesized endogenously via three isofoms of NO synthase (NOS) as well as by the nonenzymatic reduction of NO3 to NO2 and, finally, to NO (for a review, see Ref. 38). The contribution of the NO3-NO2-NO pathway can be up-regulated when plasma NO3 concentration is increased either by dietary means (e.g., via beetroot juice) or direct venous or arterial NO3 infusion (31, 38, 44). Given the attenuation of the NOS-mediated pathway of NO production in CHF, it has been proposed that an exogenous source of NO may serve as a novel therapy aimed at restoring peripheral vascular function (1, 33, 38, 47, 52).

Recently, several investigations have used dietary NO3 as a means of increasing plasma NO2 concentration and have shown beneficial effects on the exercise capacity of healthy (5–7, 41, 49, 50) and patient (32, 52) populations. Mechanistic investigations have revealed that NO3 supplementation increases exercising skeletal muscle blood flow (BF) (18), skeletal muscle microvascular PO2 (17, 19), and the rate of skeletal muscle force development (24), particularly in muscles composed of predominantly fast twitch fibers. This fiber type preferential effect is likely due to the lower PO2/pH environment in contracting fast twitch muscle (IId + IId/x fibers) (40), an environment exacerbated by CHF. Furthermore, supplementation with NO3 has been shown to reverse the arterial stiffness and endothelial dysfunction evident in aged animals (48), whereas direct infusion of NO3 vasodilates the human circulation, particularly during muscle contractions (10). Collectively, these results substantiate the role of circulating NO3 and provide a compelling therapeutic option to counteract the impaired NOS function and thus potentially reverse the peripheral vascular dysfunction found in CHF.

Therefore, the purpose of the present investigation was to test the hypotheses that 1) acute infusion of NO3 would increase total hindlimb skeletal muscle BF and vascular con-
ductance (VC) during exercise in rats with CHF and 2) there would be a positive correlation between the proportion of type IIb + IIId/x muscle fibers and the increases in muscle BF and VC found in the individual muscle or muscle parts of the hindlimb locomotor muscles of the rat.

METHODS

Animal care and selection. Male Sprague-Dawley rats (−3 mo of age, Charles River Laboratories, Wilmington, MA) 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. The rat chow (PMI Nutrition, Brentwood, MO) had caloric contributions of 29.8% protein, 13.4% fat, and 56.8% carbohydrate. All procedures used in this investigation were approved by the Institutional Animal Care and Use Committee of Kansas State University and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Myocardial infarction protocol and treadmill acclimatization. Myocardial infarction (MI) was induced in rats (n = 21) by surgically ligation the left main coronary artery (43). Briefly, rats were anesthetized initially with a 5% isoflurane-O2 mixture (Butler Animal Health Supply, Elk Grove Village, IL, and Linveld, Dallas, TX) and maintained subsequently on ~2.5% isoflurane-O2 and then intubated and mechanically ventilated with a rodent respirator (Harvard model 680, Harvard Instruments, Holliston, MA) for the duration of the surgical procedure. A left thoracotomy was performed to expose the heart through the fifth intercostal space, and the left main coronary artery was ligated 1–2 mm distal to the edge of the left atrium with a 6-0 absorbing 0.03 mg/kg im) were administered to reduce the risk of infection. After rats were removed from mechanical ventilation and anesthesia, they were monitored closely for 24 h post surgery. After a minimum of 21 days of recovery for complete remodeling of necrotic myocardial tissue and the development of compensated CHF (21, 43), all rats were familiarized with running on a custom-built, motor-driven treadmill for ~5 min daily for 5 consecutive days. All rats ran at a speed of 20 m/min up a 5% incline; this approximate speed and incline have been shown to elicit ~65% of maximal O2 uptake for these CHF animals (42).

Surgical instrumentation. On the day of the final experiment, rats were anesthetized with a 5% isoflurane-O2 mixture and maintained subsequently on ~3% isoflurane-O2. Core temperature was measured via a rectal probe and maintained at ~37–38°C by a surgical heating pad. The right carotid artery was isolated and cannulated for the advancement of a 2-Fr catheter-tipped pressure transducer (Millar Instruments, Houston, TX) into the left ventricle (LV) for measurements of systolic and diastolic pressures, LV end-diastolic pressure (LVEDP), and the rate of LV pressure rise over time (LV dp/dt). The 2-Fr pressure transducer was removed, and the artery was recannulated with a catheter (PE-10 connected to PE-50, Intra-Medic polyethylene tubing, Clay Adams Brand, Becton Dickinson, Sparks, MD) for the measurement of mean arterial pressure (MAP) and heart rate (HR) and the infusion of radiolabeled microspheres (DigiMed BPA model 200, Louisville, KY; see below). A second catheter was placed in the caudal (tail) artery as previously described (43) for arterial blood sampling and NaNO2 infusion. Both catheters were tunneled subcutaneously through the dorsal aspect of the cervical region and exteriorized via a puncture wound in the skin. The incisions were then closed, anesthesia was terminated, and the rats were given a minimum of 60 min to recover (22). After recovery, rats were placed on the motor-driven treadmill, and the carotid artery catheter was connected to a pressure transducer (Gould Statham P23ID, Valley View, OH) maintained at the same height as the animal. Rats were given a stabilization period of ~15 min before the final experimental protocol was initiated while HR and MAP were monitored continuously using the carotid artery catheter.

Exercise protocol and measurement of hindlimb skeletal muscle BF. Before exercise was initiated, each rat was infused with a bolus vehicle of ~0.5 ml heparinized saline. Two minutes after the infusion, the caudal artery catheter was connected to a 1-ml syringe chambered in a Harvard infusion/withdrawal pump (model 907), and the first bout of moderate treadmill exercise was initiated up a 5% incline with speed progressing to 20 m/min within the first 30 s. The rat continued at this speed for another 2.5 min until a total time of 3 min was reached. During this time, radiolabeled microspheres (57Co or 85Sr in random order, Perkin-Elmer, Waltham, MA) were thoroughly mixed by a vortex agitator (Fisher Scientific, Hampton, NH). For the determination of regional BF, the carotid artery catheter was disconnected from the pressure transducer at ~3 min running time, and 0.5–0.6 × 106 radiolabeled microspheres (15-μm diameter, ~0.10 ml) were infused into the aortic arch in <10 s followed immediately by a 0.5 ml saline flush. 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 after the microsphere infusion, and ~0.3 ml blood was sampled from the carotid artery catheter for the determination of blood lactate concentration, pH, PO2, and hematocrit (Nova Stat Profile M, Nova Biomedical, Waltham, MA). Exercise was then terminated, and a second withdrawal of ~0.8 ml blood was taken from the carotid artery for the determination of plasma NO2 concentration. For plasma NO2 concentration, blood was drawn into heparinized tubes and rapidly centrifuged at 5,000 g at 4°C for 6 min. Plasma was then extracted and frozen immediately at −80°C for later analysis.

Fig. 1. Total hindlimb skeletal muscle blood flow (BF; bottom) and vascular conductance (VC; top) for congestive heart failure (CHF) and CHF + NO2 conditions during submaximal locomotory exercise (n = 8). *P < 0.05 vs. CHF.
−80°C for later analysis via chemiluminescence as previously described (16–19).

After a minimum of 30 min of recovery, MAP and HR were recorded immediately before and after the bolus infusion of NaNO2 (5 mg/kg, Sigma Chemical, St. Louis, MO) via the caudal artery catheter. In a preliminary investigation, this dose was shown to significantly raise plasma NO2− concentration. Two minutes after NaNO2 infusion, the exercise and microsphere infusion protocol (radiolabeled differently from the first) was repeated.

**Determination of BF and VC.** After the final exercise protocol had been completed, rats were euthanized via a pentobarbital sodium overdose (≥50 mg/kg administered into the carotid artery catheter). The thorax of each rat was opened, and accurate placement of the carotid artery catheter was confirmed before the heart, lungs, select internal organs, and 28 individual muscles and muscle parts of the hindlimb were excised. Upon removal, tissues were blotted, weighed, and placed promptly into counting vials. The radioactivity of each tissue was determined with a gamma scintillation counter (Packard Auto Gamma Spectrometer model 5230, Downers Grove, IL). Tissue BF was then calculated using the reference sample method (43) and normalizing BF to MAP measured at the time of microsphere infusion. BF was then calculated using the reference sample method (43) and normalizing BF to MAP measured at the time of microsphere infusion.

**Indexes of CHF.** The total body mass of the eight CHF rats that completed the investigation was 445 ± 17 g. The MI size (34.6 ± 3.2% of LV area), LVEDP (18 ± 1 mmHg), LV dP/dt (6,600 mmHg/s), lung weight-to-body mass ratio (4.76 ± 0.44 mg/g), and right ventricle-to-body mass ratio (0.64 ± 0.03 mg/g) determined in these animals provided evidence that moderate CHF was present compared with a previous investigation studying varying degrees of MIs (46). Abovementioned, rats were euthanized via a pentobarbital sodium overdose (≥50 mg/kg administered into the carotid artery catheter). The thorax of each rat was opened, and accurate placement of the carotid artery catheter was confirmed before the heart, lungs, select internal organs, and 28 individual muscles and muscle parts of the hindlimb were excised. Upon removal, tissues were blotted, weighed, and placed promptly into counting vials. The radioactivity of each tissue was determined with a gamma scintillation counter (Packard Auto Gamma Spectrometer model 5230, Downers Grove, IL). Tissue BF was then calculated using the reference sample method (43) and normalizing BF to MAP measured at the time of microsphere infusion. BF was then calculated using the reference sample method (43) and normalizing BF to MAP measured at the time of microsphere infusion.

**Table 1. Effects of NO2− infusion (5 mg/kg) on exercising hindlimb BF and VC in CHF rats**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>BF, ml·min−1·100 g tissue−1</th>
<th>VC, ml·min−1·100 g tissue−1·mmHg−1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ankle extensors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus (9%)</td>
<td>311 ± 24</td>
<td>309 ± 32</td>
</tr>
<tr>
<td>Plantaris (80%)</td>
<td>168 ± 21</td>
<td>217 ± 22*</td>
</tr>
<tr>
<td>Gastrocnemius, red (14%)</td>
<td>410 ± 20</td>
<td>418 ± 36</td>
</tr>
<tr>
<td>Gastrocnemius, white (100%)</td>
<td>28 ± 8</td>
<td>48 ± 11*</td>
</tr>
<tr>
<td>Gastrocnemius, mixed (91%)</td>
<td>137 ± 10</td>
<td>150 ± 13</td>
</tr>
<tr>
<td>Tibialis posterior (73%)</td>
<td>93 ± 13</td>
<td>136 ± 21</td>
</tr>
<tr>
<td>Flexor digitorum longus (68%)</td>
<td>51 ± 12</td>
<td>75 ± 17</td>
</tr>
<tr>
<td>Flexor halicus longus (71%)</td>
<td>53 ± 12</td>
<td>83 ± 11*</td>
</tr>
<tr>
<td><strong>Ankle flexors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis anterior, red (63%)</td>
<td>256 ± 17</td>
<td>316 ± 28*</td>
</tr>
<tr>
<td>Tibialis anterior, white (80%)</td>
<td>80 ± 9</td>
<td>102 ± 14*</td>
</tr>
<tr>
<td>Extensor digitorum longus (76%)</td>
<td>43 ± 8</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Peroneals (67%)</td>
<td>131 ± 14</td>
<td>145 ± 21</td>
</tr>
<tr>
<td><strong>Knee extensors</strong></td>
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<tr>
<td>Vastus intermedius (4%)</td>
<td>436 ± 28</td>
<td>357 ± 36</td>
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<tr>
<td>Vastus medialis (82%)</td>
<td>176 ± 20</td>
<td>175 ± 24</td>
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<tr>
<td>Vastus lateralis, red (35%)</td>
<td>384 ± 53</td>
<td>294 ± 50*</td>
</tr>
<tr>
<td>Vastus lateralis, white (100%)</td>
<td>27 ± 5</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>Vastus lateralis, mixed (89%)</td>
<td>147 ± 16</td>
<td>140 ± 15</td>
</tr>
<tr>
<td>Rectus femoris, red (66%)</td>
<td>277 ± 26</td>
<td>285 ± 22</td>
</tr>
<tr>
<td>Rectus femoris, white (100%)</td>
<td>106 ± 12</td>
<td>111 ± 14</td>
</tr>
<tr>
<td><strong>Knee flexors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps femoris anterior (100%)</td>
<td>29 ± 5</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>Biceps femoris posterior (92%)</td>
<td>69 ± 9</td>
<td>77 ± 7</td>
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<tr>
<td>Semitendinosus (83%)</td>
<td>39 ± 6</td>
<td>45 ± 7</td>
</tr>
<tr>
<td>Semimembranosus, red (72%)</td>
<td>96 ± 10</td>
<td>120 ± 12*</td>
</tr>
<tr>
<td>Semimembranosus, white (100%)</td>
<td>21 ± 5</td>
<td>31 ± 6</td>
</tr>
<tr>
<td><strong>Thigh adductors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adductor longus (5%)</td>
<td>321 ± 28</td>
<td>282 ± 23*</td>
</tr>
<tr>
<td>Adductor magnus &amp; brevis (89%)</td>
<td>81 ± 13</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>Gracilis (77%)</td>
<td>32 ± 7</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>Pectineus (69%)</td>
<td>53 ± 12</td>
<td>52 ± 10</td>
</tr>
</tbody>
</table>

Data are mean ± SE; n = 8 rats with congestive heart failure (CHF). Values in parentheses indicate the percentage of type Ila + IIa/x muscle fibers according to Delp and Duan (11). BF, blood flow; VC, vascular conductance. *P < 0.05 vs. CHF.

**Statistical analyses.** All results were compared between experimental conditions (CHF and CHF + NO2−) using a paired Student’s t-test. Pearson product moment correlations determined the percent type IIb/x muscle fibers according to Delp and Duan (11) and changes in skeletal muscle BF and VC. Values are presented as means ± SE. Statistical significance was accepted at P < 0.05.

**RESULTS**

**Indexes of CHF.** The total body mass of the eight CHF rats that completed the investigation was 445 ± 17 g. The MI size (34.6 ± 3.2% of LV area), LVEDP (18 ± 1 mmHg), LV dP/dt (6,600 mmHg/s), lung weight-to-body mass ratio (4.76 ± 0.44 mg/g), and right ventricle-to-body mass ratio (0.64 ± 0.03 mg/g) determined in these animals provided evidence that moderate CHF was present compared with a previous investigation studying varying degrees of MIs (46).

**Hemodynamic responses.** NaNO2 infusion significantly raised plasma NO2− concentration compared with the CHF condition (CHF: 0.4 ± 0.01 μM and CHF + NO2−: 108.1 ± 10 μM, P < 0.01). There were no significant between-condition differences (P > 0.05) in MAP at rest (CHF: 116 ± 6 mmHg and CHF + NO2−: 117 ± 7 mmHg) or during exercise (CHF: 123 ± 4 mmHg and CHF + NO2−: 120 ± 4 mmHg) or in exercising HR (CHF: 516 ± 11 beats/min and CHF + NO2−: 517 ± 13 beats/min). Furthermore, no differences in arterial Pco2, Po2, percent O2 saturation, or hematocrit were found.
between the experimental conditions (P > 0.05 for all). Exercising blood lactate concentration, however, was significantly increased with NO\textsubscript{2} infusion (CHF: 1.6 ± 0.2 mmol/l and CHF + NO\textsubscript{2}: 3.3 ± 0.4 mmol/l, P < 0.05).

Effects of NO\textsubscript{2} on BF and VC. Total hindlimb skeletal muscle BF during exercise was not different between conditions (P = 0.23; Fig. 1). However, total hindlimb skeletal muscle VC increased ~10% with NO\textsubscript{2} infusion (P < 0.05; Fig. 1). BF in 6 (~21%) and VC (~29%) in 8 of the 28 individual hindlimb muscles and muscle parts investigated were significantly greater during exercise with NO\textsubscript{2} infusion. In contrast, BF was lower in 2 and VC in 1 of the 28 muscle and muscle parts with NO\textsubscript{2} infusion (Table 1). Nearly all of the muscles and muscle parts exhibiting greater BF and VC after NO\textsubscript{2} infusion were composed of ≥63% type IIb + IId/x muscle fibers. There was a significant positive correlation for both percent change in BF and percent change in VC with the percentage of type IIb + IId/x fibers (Fig. 1). BF in the stomach and small intestine decreased with NO\textsubscript{2} infusion, but when BF was normalized for MAP, VC decreased only in the stomach (Table 2).

**DISCUSSION**

The principal original findings of this investigation were that 1) acute arterial NO\textsubscript{2} infusion resulted in a significant increase in total hindlimb skeletal muscle VC during exercise with increases in BF to specific muscles and muscle parts and 2) there was a positive correlation between the changes in BF and VC after NO\textsubscript{2} infusion and the percentage of type IIb + IId/x muscle fibers found in the 28 muscles or muscle parts examined. These results suggest that acute NO\textsubscript{2} administration can impact exercising vascular control in CHF with targeted effects in muscles composed of less oxidative fast twitch muscle fibers. Since CHF results in compromised NOS function, particularly in slow twitch muscles, patients with this disease may rely more heavily on fatigable fast twitch muscle recruitment even at moderate intensities of exercise and thus may benefit from NO\textsubscript{2} therapy.

Impacts of NO\textsubscript{2} infusion on skeletal muscle BF, VC, and MAP during exercise. One of the strengths of the present investigation lies in the radiolabeled microsphere technique used to measure both inter- and intramuscular BF during whole body dynamic exercise. In rats with CHF, arterial infusion of NO\textsubscript{2} resulted in an ~10% increase in total hindlimb skeletal muscle VC during exercise. While total hindlimb skeletal muscle BF was not statistically elevated at this intensity of exercise, the significant increase in BF to 6 and VC in 8 of the 28 individual muscles and muscle portions suggests a selective vasodilatory role for NO\textsubscript{2} in CHF, particularly in major locomotory muscles such as the plantaris and tibialis anterior. Given that CHF is hallmarked by exercise intolerance due, in part, to severe vascular endothelial dysfunction and impaired O\textsubscript{2} delivery (for a review, see Ref. 47), the beneficial impact of NO\textsubscript{2} infusion on the vascular function of several primary hindlimb locomotory muscles may improve metabolic control within these tissues and could ultimately result in improved tolerance to exercise.

Furthermore, and consistent with our original hypothesis, the changes in BF and VC elicited by NO\textsubscript{2} infusion were positively correlated with the percentage of type IIb + IId/x muscle fibers. The elevations in BF and VC observed in muscles composed of ≥63% fast twitch muscle fibers provide additional evidence for a fiber type-selective effect of the NO\textsubscript{2} - NO\textsubscript{3} - NO pathway observed in previous human and animal investigations (18, 19, 24, 29). The bases for this effect are likely the reduced O\textsubscript{2} delivery to utilization ratio and lower PO\textsubscript{2}/pH environment found within fast twitch muscles at rest and during contractions (40). This environment, while inhibitory to NOS function, potentiates the reduction of NO\textsubscript{2} to NO (10, 14). In healthy rats, stronger fiber type correlations (r = 0.74 and 0.71 for the change in BF and change in VC, respectively) were found after 5 days of NO\textsubscript{3} supplementation with beetroot juice (18). It is possible that chronic exposure to an elevated NO\textsubscript{2} concentration (e.g., days) may elicit a greater effect on skeletal muscle vascular function as this alters the relationship between the relative changes in total hindlimb BF (ΔBF; top) and VC (ΔVC; bottom) with arterial NO\textsubscript{2} infusion during submaximal locomotory exercise and the percentage of type IIb + IId/x fibers found in the individual muscles and muscle parts of the rat (n = 8) hindlimb according to Delp and Duan (11).
exercise based, such as cardiac rehabilitation programs. Even would likely compliment other CHF interventions that are effects of NO2 elevated ROS (8), which may have ultimately tempered the addition, in CHF, we expect increased NO scavenging via the consequence of an impaired sensitivity to NO that is known blood pressure. The absence of a MAP effect herein might be state may have tempered the effects of acute NO2 effects of NO2 inorganic NO-based interventions (39), and, given the selective organic NO-based interventions is the development of toler-

Conclusions. In 11002 healthy rats rather than from rats with CHF (11). While CHF muscle (24). Given the tight relationship between O2 delivery and O2 utilization necessary to regulate skeletal muscle metabolic control during exercise (35), an intervention in which BF is distributed preferentially to hypoxic tissues (i.e., type IIb + IId/x fibers) could reduce overall fatigability, thereby helping to restore exercise tolerance in this population.

Inorganic NO2 treatment generally, but not always, reduces blood pressure in hypertensive patients (for a review, see Ref. 31). In contrast to our previous investigations in healthy rats (16, 18), NO2 did not reduce exercising MAP in CHF. These results are in agreement with Maher et al. (39), who demonstrated no change in resting MAP of CHF patients after intra-arterial NO2 infusion. Furthermore, Ormerod et al. (45) demonstrated that, in patients with severe CHF, short-term NaNO2 infusion increased venous capacitance and attenuated right atrial pressure while only modestly reducing arterial blood pressure. The absence of a MAP effect herein might be due to the consequences of an impaired sensitivity to NO that is known to exist at the vascular and platelet level in CHF (2). In addition, in CHF, we expect increased NO scavenging via elevated ROS (8), which may have ultimately tempered the effects of NO2 on MAP and potentially skeletal muscle BF. It is also possible that the compromised pumping capacity of the heart and resultant sympathetic hyperactivity found in the CHF state may have tempered the effects of acute NO2 on MAP and skeletal muscle BF. Whether chronic administration of intrarterial NO2 infusion impacts sympathetic nerve activity in CHF remains to be elucidated.

Clinical and therapeutic relevance. Current therapeutic strategies often used to ameliorate the complications of CHF include treatment with organic vasodilator drugs such as glyceryl trinitrate (nitroglycerine) and a combination of diuretic-based interventions. Both of these strategies are aimed at reducing afterload and allowing stroke volume of the heart to increase (4). A well-documented limitation to the use of organic NO-based interventions is the development of tolerance, which ultimately limits efficacy (for a review, see Ref. 13). Fortunately, this does not appear to be a concern with inorganic NO-based interventions (39), and, given the selective effects of NO2 infusion on skeletal muscle BF and VC demonstrated presently, these results highlight the therapeutic potential of this approach. In particular, NO2-based therapies would likely compliment other CHF interventions that are exercise based, such as cardiac rehabilitation programs. Even modest improvements in vascular function may augment metabolic control during exercise, thereby improving adherence to exercise-based rehabilitation programs (1), which in and of themselves would upregulate NOS function and endogenous NO2 production, thus further promoting NO homeostasis.

Experimental considerations. The increased blood lactate concentration found herein after NO2 infusion was not expected. An increase in skeletal muscle O2 delivery would raise the O2 pressure head within the microvasculature (required for capillary-myocyte O2 flux), as dictated by microvascular PO2. This would be expected to improve metabolic control within these tissues by reducing the ADP-to-ATP ratio, glycolysis/glycogenolysis stimulation, and thus lactate production within these tissues (23). Given the markedly lower BF in fast twitch muscle at rest and during exercise and their decreased microvascular PO2, especially in CHF, it is possible that a rapid influx of NO2 and the consequential increase in BF within these muscles may have flushed lactate into the systemic circulation, resulting in a brief elevation in arterial lactate concentration. This phenomenon is supported empirically by Williams et al. (51), who demonstrated substantial increases in circulating lactate concentration (as high as 20 mmol/l) in Weddell seals when their skeletal muscle BF was rapidly restored after deep-water dives. Future investigation of the blood lactate concentration time course with NO2 would elucidate this concept. An alternative explanation could be related to NO-induced changes in skeletal muscle glucose uptake, which has been shown to occur after infusion of the NO donor sodium nitroprusside (34). Thus, a local shift to glycolytic metabolism accompanied by a rapid vasodilation within these tissues may account for the changes in blood lactate concentration seen herein. In addition, the small reductions in BF to muscles comprised predominantly of slow twitch fibers (e.g., red portion of the vastus lateralis) may have also stimulated increased lactate production as these muscles often require the highest absolute BF during exercise (3, 36). However, it is worth mentioning that the lactate concentrations after NO2 infusion reported herein are well within reasonable ranges of what has been observed previously in our laboratory at this running speed (16, 18, 27), particularly in rats with CHF (28). Nevertheless, future investigations into the effects of NO2 on skeletal muscle microvascular PO2 at rest and during contractions will provide crucial insights into the metabolic basis for this effect.

One potential limitation to the present investigation is the reference of fiber type composition of skeletal muscles from healthy rats rather than from rats with CHF (11). While CHF does impact skeletal muscle biochemistry and histology rela-
tive to fiber type, those changes are only apparent in animals with severe LV dysfunction (i.e., infarct size of ~59% of the LV endocardial circumference) (12). The indexes of CHF presented herein, however (MI size: ~35%), are consistent with moderate CHF (46). Therefore, we do not suspect the fiber type composition of our moderate CHF rats to be different from that of the referenced healthy rats. Although the lack of a healthy control group was not used, BF and VC values during exercise in healthy control animals are abundant in the literature (18, 26). Thus, the death of additional animals to serve as controls herein is not ethically warranted.

As mentioned above, a strength of the present investigation lies in the techniques used to measure skeletal muscle BF and VC during whole body dynamic exercise. While it seems logical to postulate that NO2 infusion impacted skeletal muscle VC via changes in vascular smooth muscle function (presumably due to increased NO bioavailability), it must be acknowledged this was not directly measured. To this point, however, the efficacy of elevated NO2 concentration on reducing vessel tension in the isolated rat aorta has been demonstrated by Cosby et al. (10) and thus supports the present findings. Furthermore, our laboratory has recently shown that NO2 infusion restores vascular function in the face of NOS blockade elicited via N-nitro-l-arginine methyl ester (15).

Conclusions. This investigation is the first to examine the impact of acute NO2 infusion (5 mg/kg) on exercise-induced hyperemia in rats with CHF. The augmented total hindlimb skeletal muscle VC accompanying with preferential increases in BF in muscles and muscle portions comprised predominantly of fast twitch muscle fibers demonstrates the potential for NO2 to induce changes in vascular control during exercise. However, the elevation seen in exercising blood lactate concentration raises further questions as to how NO2 impacts skeletal muscle metabolic control and thus warrants future investigation. Nonetheless, given the emerging evidence supporting the therapeutic potential of the NO3-NO2-NO pathway (1, 38, 52), these results highlight the ability for an acute NO2 intervention to selectively augment skeletal muscle BF distribution during exercise in CHF. Ultimately, a therapy in which exercise, dietary, and pharmacological interventions are combined may provide the most efficacious means of restoring functionality and improving quality of life within the CHF population.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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