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

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Glean AA, Ferguson SK, Holdsworth CT, Colburn TD, Wright JL, Fees AJ, Hageman KS, Poole DC, Musch TI. Effects of nitrite 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 (radiolabeled 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 (~2%) 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 herein, 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 isomers 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 upregulated when plasma NO2 concentration is increased either by dietary means (e.g., via beetroot juice) or direct venous or arterial NO2 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 (Iib + 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 NO2 vasodilates the human circulation, particularly during muscle contractions (10). Collectively, these results substantiate the role of circulating NO2 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 NO2 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 ligating 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 braided polyester suture. The thorax was then closed with 3-0 silk. Bupivacaine (1.5 mg/kg sc), ampicillin (50 mg/kg im), and buprenorphine (~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 3 h after 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 exercise in rats with CHF and CHF + NO2 conditions during submaximal locomotory exercise (n = 8). *P < 0.05 vs. CHF.
Effects of NO₂

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

<table>
<thead>
<tr>
<th>Muscle</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></td>
<td>CHF</td>
<td>CHF + NO₂</td>
</tr>
<tr>
<td>Ankle extensors</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 hallucis longus (71%)</td>
<td>53 ± 12</td>
<td>83 ± 11*</td>
</tr>
<tr>
<td>Ankle flexors</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>Knee extensors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vastus intermedius (4%)</td>
<td>436 ± 28</td>
<td>357 ± 36</td>
</tr>
<tr>
<td>Vastus medialis (82%)</td>
<td>176 ± 20</td>
<td>175 ± 24</td>
</tr>
<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 ± 7</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>Knee flexors</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>
</tr>
<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>Thigh adductors</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 IIb + IIId/x muscle fibers according to Delp and Duan (11). BF, blood flow; VC, vascular conductance. *P < 0.05 vs. CHF.
between the experimental conditions (P > 0.05 for all). Exercising blood lactate concentration, however, was significantly increased with NO₂⁻ infusion (CHF: 1.6 ± 0.2 mmol/l and CHF + NO₂⁻: 3.3 ± 0.4 mmol/l, P < 0.05).

Effects of NO₂⁻ 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 NO₂⁻ 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 NO₂⁻ infusion. In contrast, BF was lower in 2 and VC in 1 of the 28 muscle and muscle parts with NO₂⁻ infusion (Table 1). Nearly all of the muscles and muscle parts exhibiting greater BF and VC after NO₂⁻ 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. 2). BF in the stomach and small intestine decreased with NO₂⁻ 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 NO₂⁻ 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 NO₂⁻ 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 NO₂⁻ 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 NO₂⁻ therapy.

Impacts of NO₂⁻ 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 NO₂⁻ 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 NO₂⁻ 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 O₂ delivery (for a review, see Ref. 47), the beneficial impact of NO₂⁻ 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 NO₂⁻ 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 NO₃⁻ - NO₂⁻ -NO pathway observed in previous human and animal investigations (18, 19, 24, 29). The bases for this effect are likely the reduced O₂ delivery to utilization ratio and lower P₉₀/PH environment found within fast twitch muscles at rest and during contractions (40). This environment, while inhibitory to NOS function, potentiates the reduction of NO₂⁻ 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 NO₃⁻ supplementation with beetroot juice (18). It is possible that chronic exposure to an elevated NO₂⁻ concentration (e.g., days) may elicit a greater effect on skeletal muscle vascular function as this alters the expression of key Ca²⁺-handling proteins augmenting the contractile function of fast twitch, but not slow twitch, skeletal
modest improvements in vascular function may augment metab-
lolic control during exercise (35), an intervention in which BF
distributed preferentially to hypoxic tissues (i.e., type IIB +
ld/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 demon-
strated 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
the consequence 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
effect 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 intra-
arterial 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 glyc-
eryl 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 toler-
ance, 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 dem-
onstrated presently, these results highlight the therapeutic po-
tential 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 met-
abolic 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 ex-
pected. 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 micro-
vascular 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 reduc-
tions 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). How-
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 could also be considered a potential limitation of this investigation, our question and hypothesis relate specifically to the impact of NO2 infusion in CHF and whether it can improve exercising muscle BF and VC. Although 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 accompanied 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


REFERENCES


