EFFECTS OF NITRITE INFUSION ON SKELETAL MUSCLE VASCULAR CONTROL DURING EXERCISE IN RATS WITH CHRONIC HEART FAILURE

by

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Abstract

Chronic heart failure (CHF) reduces nitric oxide (NO) bioavailability and impairs skeletal muscle vascular control during exercise. Reduction of nitrite (NO_2^-) to NO may impact exercise-induced hyperemia particularly in muscles with pathologically-reduced O_2 delivery. We tested the hypothesis that NO_2^- 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. Following a >21 day recovery, mean arterial pressure (MAP, carotid artery catheter) and skeletal muscle BF (radiolabelled microspheres) were measured during treadmill exercise (20 $m \cdot min^{-1}$, 5% incline) with and without NO_2^- infusion. The myocardial infarct size (35 ± 3%) indicated moderate CHF. NO_2^- infusion increased total hindlimb skeletal muscle VC (CHF: 0.85 ± 0.09, CHF+NO_2^-: 0.93 ± 0.09 $ml \cdot min^{-1} \cdot 100g^{-1} \cdot mmHg^{-1}$, $p<0.05$) without changing MAP (CHF: 123 ± 4 mmHg, CHF+NO_2^-: 120 ± 4 mmHg, $p=0.17$). Total hindlimb skeletal muscle BF was not significantly different (CHF: 102 ± 7, CHF+NO_2^-: 109 ± 7 $ml \cdot min^{-1} \cdot 100g^{-1}$, $p>0.05$). 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 following NO_2^- infusion were comprised of ≥63% type IIb+IId/x muscle fibers. These data demonstrate that NO_2^- infusion can augment skeletal muscle vascular control during exercise in CHF rats. Given the targeted effects shown herein, a NO_2^--based therapy may provide an attractive "needs-based" approach for treatment of the vascular dysfunction in CHF.

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Introduction

Chronic heart failure (CHF) is characterized by a combination of central and peripheral circulatory dysfunction that ultimately impairs exercise tolerance and quality of life (29, 47). About 23 million individuals worldwide suffer from this disease (36), and whilst 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 (21, reviewed by 47). Though exercise rehabilitation represents an effective therapeutic modality for treating CHF (9), the reduced NO bioavailability in CHF is associated with 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 (19, 25) and thus have great potential to improve exercise tolerance and quality of life in this population.

NO is synthesized endogenously via three isoforms of nitric oxide synthase (NOS) as well as by the non-enzymatic reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and finally to NO (reviewed by 37). The contribution of the NO_3^--NO_2^--NO pathway can be upregulated when plasma [NO_2^-] is increased either by dietary means (e.g. via beetroot juice) or direct venous or arterial NO_2^- infusion (30, 37, 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, 32, 37, 47, 52).

Recently, several investigations have employed dietary NO_3^- as a means of increasing plasma [NO_2^-] and have shown beneficial effects on the exercise capacity of healthy (5-7, 40, 49, 50) and patient populations (31, 52). Mechanistic insights have revealed that NO_3^- supplementation increases exercising skeletal muscle blood flow (BF) (17), skeletal muscle microvascular partial pressure of O_2 (PO_2mv) (16, 18) and the rate of skeletal muscle force

development (24) particularly in muscles comprised of predominantly fast twitch fibers. This fiber type preferential effect is likely due to the lower PO_2/pH environment in contracting fast twitch muscle (IIb+IId/x fibers) (39), an environment compounded by CHF. Furthermore, supplementation with NO_2^- has been shown to reverse the arterial stiffness and endothelial dysfunction evident from aging animals (48) whilst direct infusion of NO_2^- vasodilates the human circulation, particularly during muscle contractions (10). Collectively, these results substantiate the role of circulating NO_2^- 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 this investigation was to test the hypotheses that 1) acute infusion of NO_2^- would increase total hindlimb skeletal muscle BF and vascular conductance (VC) during exercise in rats with CHF, and 2) there would be a positive correlation between the proportion of type IIb+IId/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 months of age, Charles River Laboratories, Wilmington, MA) were maintained at accredited animal facilities at Kansas State University on a 12:12-hr light-dark cycle with food (PMI Nutrition International LLC, Brentwood, MO) and water provided *ad libitum*. All procedures employed in this investigation were approved by the Institutional Animal Care and Use Committee of Kansas State University and conducted according to the National Research Council *Guide for the Care and Use of Laboratory Animals*.

Myocardial infarction protocol and treadmill acclimatization

Myocardial infarction (MI) was induced in rats (n=8) by surgically ligating the left main coronary artery (43). Briefly, rats were anaesthetized initially with a 5% isoflurane–O_2 mixture (Butler Animal Health Supply, Elk Grove Village, IL; Linweld, Inc., Dallas, TX) and maintained subsequently on ~2.5% isoflurane–O_2 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 2-0 gut and the skin was closed with 3-0 silk. Bupivacaine (1.5 $mg \cdot kg^{-1}$ subcutaneously), ampicillin (50 $mg \cdot kg^{-1}$ i.m.), and buprenorphine (~0.03 $mg \cdot kg^{-1}$ i.m.) were administered to reduce the risk of infection. After removing the rats from mechanical ventilation and anesthesia, the rats were monitored closely for ≥6 hours post-surgery. Following a minimum of 21 days of recovery for complete remodeling of necrotic myocardial tissue and 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 $m \cdot min^{-1}$ up a 5% incline; this approximate speed and incline have been shown to elicit ~65% of maximal oxygen uptake for these CHF animals (41-43).

Surgical instrumentation

On the day of the final experiment, rats were anaesthetized with a 5% isoflurane–O_2 mixture and maintained subsequently on ~3% isoflurane–O_2. Core temperature measured via rectal probe was 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 measurement 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 and Company, 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 described previously (43) for arterial blood sampling and sodium nitrite ($NaNO_2^-$) 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 terminated, and the rats were given a minimum of 60 min to recover (22). Following recovery, the 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 of heparinized saline. Two min following the infusion, the caudal artery catheter was connected to a 1-ml syringe chambered in a Harvard infusion/withdrawal pump (Model 907, Cambridge, MA), and the first bout of moderate, submaximal treadmill exercise was initiated up a 5% incline with speed progressing to 20 $m \cdot min^{-1}$ within the first 30 sec. 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 (^{57}Co or ^{85}Sr in random order; Perkin Elmer, Waltham, MA) were thoroughly mixed and agitated by sonication. At ~3 min running time, the carotid artery catheter was disconnected from the pressure transducer and, for the determination of regional BF, 0.5-0.6 × 10^6 radiolabeled microspheres (15 μm diameter, ~0.10 ml) were infused into the aortic arch in <10 sec 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 $ml \cdot min^{-1}$. Blood withdrawal was terminated 30 sec following the microsphere infusion, and ~0.3 ml of blood was sampled from the carotid artery catheter for the determination of blood [lactate], pH, PCO_2, PO_2, $\%O_2$ saturation, and hematocrit (Nova Stat Profile M, Nova Biomedical, Waltham, MA). Exercise was then terminated.

Following a minimum of 30 min of recovery, MAP and HR were recorded immediately before and after the bolus infusion of $NaNO_2^-$ (5 $mg \cdot kg^{-1}$; Sigma Chemical, St. Louis, MO) via the caudal artery catheter. In preliminary investigations, this dose has been shown to significantly raise plasma [NO_2^-] (2). Two min following $NaNO_2^-$ infusion, the exercise and microsphere infusion protocol (radio-labeled differently from the first) was repeated.

Determination of BF and VC

After the final exercise protocol had been completed, rats were euthanized via pentobarbital sodium overdose (≥50 $mg \cdot kg^{-1}$ 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 expressed as $ml \cdot min^{-1} \cdot 100$ g of $tissue^{-1}$. Adequate mixing of the microspheres was verified for each microsphere infusion as demonstrated by a <15% difference in BF to the right and left kidneys or to the right and left hindlimb musculature. VC was then calculated by normalizing BF to MAP measured at the time of microsphere infusion and expressed as $ml \cdot min^{-1} \cdot 100$ g of $tissue^{-1} \cdot mmHg^{-1}$. The LV, right ventricle (RV), and lungs were normalized to body weight, and MI size of the LV was measured via planimetry as described previously by our laboratory (19).

Statistical analyses

All results were compared between experimental conditions (CHF and CHF+NO_2^-) using paired Student's *t* test. Pearson product moment correlations determined for the percentage type IIb+IId/x muscle fibers according to Delp and Duan (11) and changes in skeletal muscle BF and VC. Values are presented as mean ± standard error (SEM). Statistical significance was accepted at $p<0.05$.

Results

Indices 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 (6600 mmHg $\cdot sec^{-1}$), lung weight-to-body mass ratio (4.76 ± 0.44 $mg \cdot g^{-1}$), and RV-to-body mass ratio (0.64 ± 0.03 $mg \cdot g^{-1}$) determined in these animals provided evidence that moderate CHF was present when compared to previous investigations studying varying degrees of MIs (42, 43).

Hemodynamic responses

There were no significant between-condition differences ($p>0.05$) in MAP at rest (CHF: 116 ± 6, CHF+NO_2^-: 117 ± 7 mmHg) or during exercise (CHF: 123 ± 4, CHF+NO_2^-: 120 ± 4 mmHg) or in exercising HR (CHF: 516 ± 11, CHF+NO_2^-: 517 ± 13 bpm). Furthermore, no differences in arterial PCO_2, PO_2, $\%O_2$ saturation, or hematocrit were found between the experimental conditions ($p>0.05$ for all). Exercising blood [lactate], however, was significantly increased with NO_2^- infusion (CHF: 1.6 ± 0.2, CHF+NO_2^-: 3.3 ± 0.4 $mmol \cdot L^{-1}$, $p<0.05$).

Effects of NO_2^- on BF and VC

Total hindlimb skeletal muscle BF during exercise was not different between conditions (Figure 1; $p=0.23$). However, total hindlimb skeletal muscle VC increased ~10% with NO_2^- infusion (Figure 1; $p<0.05$). BF in 6 (~21%) and VC in 8 (~29%) of the 28 individual hindlimb muscles and muscle parts investigated were significantly greater during exercise with NO_2^- infusion. In contrast, BF was lower in 2 and VC in 1 of the 28 muscle and muscle parts with NO_2^- infusion (Table 1). Nearly all of the muscles and muscle parts exhibiting greater BF and VC following NO_2^- infusion were comprised of ≥63% type IIb+IId/x muscle fibers. There was a

significant positive correlation for both ΔBF% and ΔVC% with the percentage of type IIb+IId/x fibers (Figure 2). BF in the stomach and small intestine decreased with NO_2^- infusion, but when BF was normalized for MAP, VC decreased only in the stomach (Table 2).

Figure 1 - Total exercising skeletal muscle BF and VC

Total hindlimb skeletal muscle BF (upper panel) and VC (lower panel) for CHF and CHF+NO_2^- conditions during submaximal locomotory exercise (*n*=8). **p*<0.05 vs. CHF.

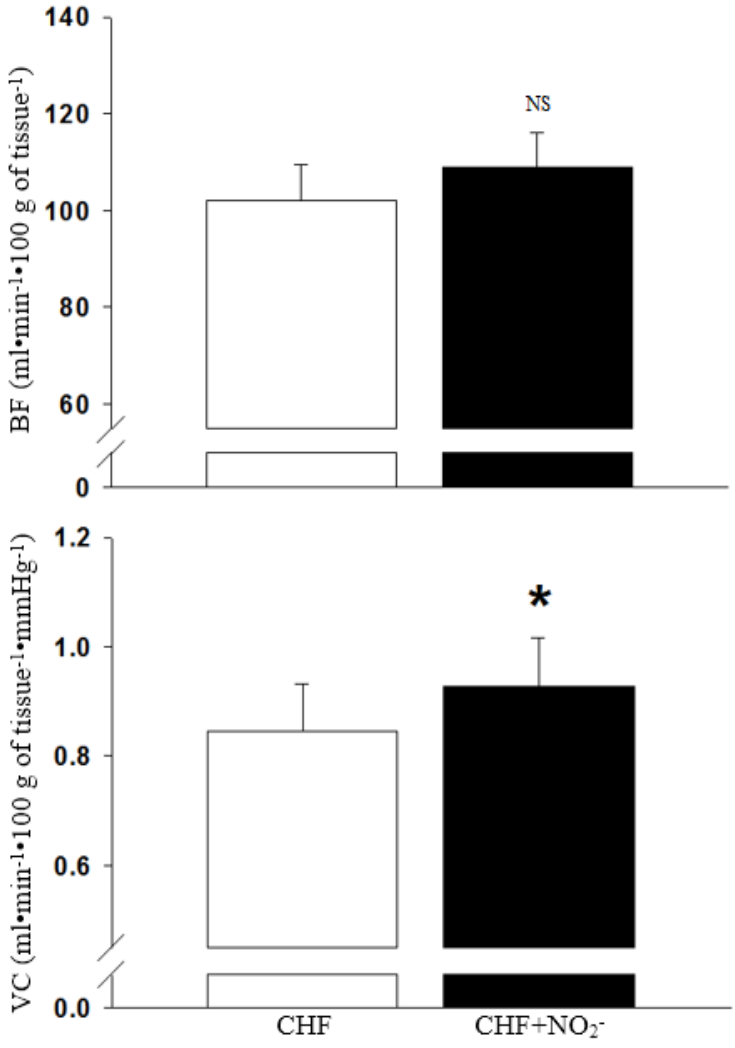


Table 1 - Exercising BF and VC in individual hindlimb skeletal muscles

Effects of NO_2^- infusion (5 mg·kg^{-1}) on exercising hindlimb BF (ml·min^{-1}·100 g of tissue^{-1}) and VC (ml·min^{-1}·100 g of tissue^{-1}·mmHg^{-1}) in CHF rats (*n*=8).

	BF		VC	
Muscle or muscle part (% type IIb+IId/x)	**CHF**	**CHF+NO_2^-**	**CHF**	**CHF+NO_2^-**
Ankle extensors				
Soleus (9%)	311 ± 24	309 ± 32	2.54 ± 0.20	2.61 ± 0.29
Plantaris (80%)	168 ± 21	217 ± 22*	1.42 ± 0.24	1.87 ± 0.26*
Gastrocnemius, red (14%)	410 ± 20	418 ± 36	3.38 ± 0.27	3.53 ± 0.33
Gastrocnemius, white (100%)	28 ± 8	48 ± 11*	0.24 ± 0.07	0.42 ± 0.11*
Gastrocnemius, mixed (91%)	137 ± 10	150 ± 13	1.13 ± 0.11	1.27 ± 0.14*
Tibialis posterior (73%)	93 ± 13	136 ± 21	0.76 ± 0.11	1.12 ± 0.15
Flexor digitorum longus (68%)	51 ± 12	75 ± 17	0.43 ± 0.10	0.66 ± 0.19
Flexor halicus longus (71%)	53 ± 12	83 ± 11*	0.46 ± 0.13	0.72 ± 0.14*
Ankle flexors				
Tibialis anterior, red (63%)	256 ± 17	316 ± 28*	2.11 ± 0.19	2.69 ± 0.32*
Tibialis anterior, white (80%)	80 ± 9	102 ± 14*	0.67 ± 0.09	0.87 ± 0.14*
Extensor digitorum longus (76%)	43 ± 8	50 ± 8	0.35 ± 0.07	0.43 ± 0.09
Peroneals (67%)	131 ± 14	145 ± 21	1.09 ± 0.14	1.24 ± 0.21
Knee extensors				
Vastus intermedius (4%)	436 ± 28	357 ± 36	3.55 ± 0.21	3.01 ± 0.31
Vastus medialis (82%)	176 ± 20	175 ± 24	1.49 ± 0.24	1.50 ± 0.22
Vastus lateralis, red (35%)	384 ± 53	294 ± 50*	3.11 ± 0.42	2.44 ± 0.39*
Vastus lateralis, white (100%)	27 ± 7	28 ± 5	0.23 ± 0.07	0.25 ± 0.05
Vastus lateralis, mixed (89%)	147 ± 16	140 ± 15	1.20 ± 0.14	1.17 ± 0.13
Rectus femoris, red (66%)	277 ± 26	285 ± 22	2.29 ± 0.27	2.41 ± 0.22
Rectus femoris, white (100%)	106 ± 12	111 ± 14	0.90 ± 0.14	0.94 ± 0.12
Knee flexors				
Biceps femoris anterior (100%)	29 ± 5	38 ± 6	0.24 ± 0.05	0.32 ± 0.05*
Biceps femoris posterior (92%)	69 ± 9	77 ± 7	0.58 ± 0.09	0.66 ± 0.07
Semitendinosus (83%)	39 ± 6	45 ± 7	0.31 ± 0.05	0.38 ± 0.06
Semimembranosus, red (72%)	96 ± 10	120 ± 12*	0.78 ± 0.08	1.00 ± 0.10*
Semimembranosus, white (100%)	21 ± 5	31 ± 6	0.17 ± 0.04	0.26 ± 0.05
Thigh adductors				
Adductor longus (5%)	321 ± 28	282 ± 23*	2.69 ± 0.36	2.41 ± 0.27
Adductor magnus & brevis (89%)	81 ± 13	77 ± 10	0.69 ± 0.14	0.66 ± 0.11
Gracilis (77%)	32 ± 7	33 ± 6	0.27 ± 0.06	0.28 ± 0.05
Pectineus (69%)	53 ± 12	52 ± 10	0.44 ± 0.10	0.45 ± 0.10

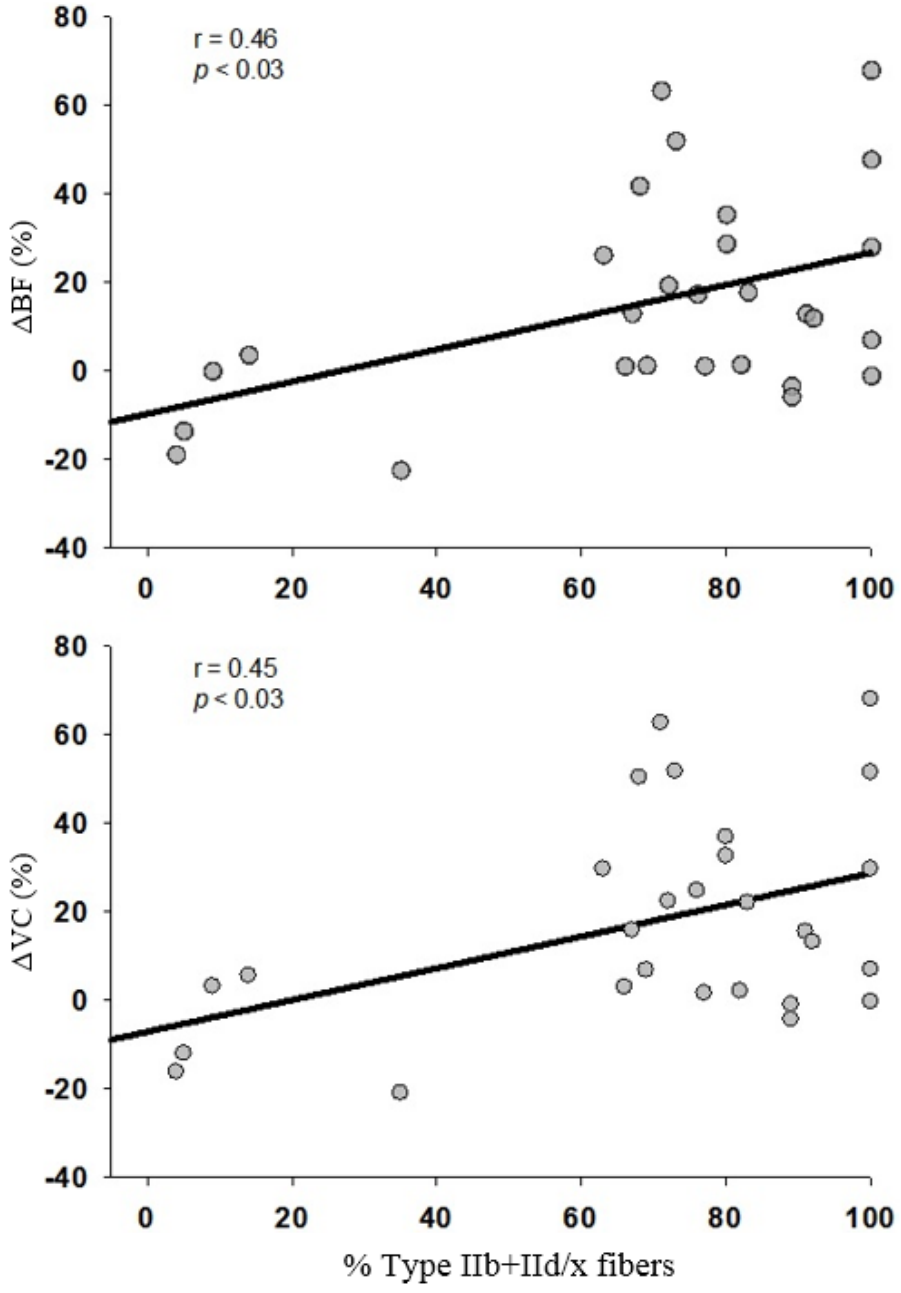
Table 2 - BF and VC in the kidneys and splanchnic organs

Effects of NO_2^- infusion (5 mg·kg^{-1}) on exercising BF (ml·min^{-1}·100 g of tissue^{-1}) and VC (ml·min^{-1}·100 g of tissue^{-1}·mmHg^{-1}) in the kidneys and select organs of the splanchnic region in CHF rats (*n*=8).

	BF		**VC**	
	CHF	**CHF+NO_2^-**	**CHF**	**CHF+NO_2^-**
Kidneys	424 ± 18	380 ± 60	3.48 ± 0.22	3.18 ± 0.48
Stomach	67 ± 5	36 ± 6*	0.54 ± 0.03	0.30 ± 0.05*
Adrenals	453 ± 54	430 ± 54	3.77 ± 0.56	3.65 ± 0.49
Spleen	117 ± 33	54 ± 16	0.92 ± 0.25	0.44 ± 0.14
Pancreas	147 ± 16	118 ± 25	1.20 ± 0.13	0.97 ± 0.21
Sm. intestine	357 ± 24	271 ± 55*	2.90 ± 0.17	2.22 ± 0.44
Lg. intestine	277 ± 38	177 ± 33	2.26 ± 0.29	1.45 ± 0.27
Liver**	22 ± 3	27 ± 5	0.18 ± 0.03	0.23 ± 0.05

Figure 2 - ΔBF and ΔVC vs. percent IIb+IId/x fibers

Relationship between the relative changes in total hindlimb BF and VC (ΔBF and ΔVC, respectively) with arterial NO_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).



Discussion

The principal original findings of this investigation were that 1) acute arterial NO_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 following NO_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_2^- administration can impact exercising vascular control in CHF with targeted effects in muscles comprised 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_2^- therapy.

Impacts of NO_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 intra-muscular BF during whole body dynamic exercise. In rats with CHF, arterial infusion of NO_2^- resulted in a ~10% increase in total hindlimb skeletal muscle VC during exercise. While total hindlimb skeletal muscle BF was not statistically elevated, 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_2^- in CHF. Given that CHF is hallmarked by exercise intolerance due, in part, to severe vascular endothelial dysfunction and impaired O_2 delivery (reviewed by 47), the beneficial impact of NO_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_2^- infusion were positively correlated with the percentage of type IIb+IId/x muscle fibers. The elevations in BF and VC observed in muscles comprised of ≥63% of fast twitch muscle fibers provide additional evidence for a fiber type-selective effect of the NO_3^--NO_2^--NO pathway observed in previous human and animal investigations (17, 18, 24, 28). The bases for this effect are likely the reduced O_2 delivery-to-utilization ratio (QO_2/VO_2) and lower PO_2/pH environment found within fast twitch muscles at rest and during contractions (39). This environment, while inhibitory to NOS function, potentiates the reduction of NO_2^- to NO (10, 14). In healthy rats, stronger fiber type correlations (r=0.74 and 0.71 for ΔBF and ΔVC, respectively) were found following 5 days of NO_3^- supplementation with beetroot juice (17). It is possible that chronic exposure to elevated [NO_2^-] (e.g. days) may elicit a greater effect on skeletal muscle vascular function as this alters expression of key calcium handling proteins augmenting contractile function of fast twitch, but not slow twitch, skeletal muscle (24). Given the tight relationship between QO_2 and VO_2 necessary to regulate skeletal muscle metabolic control during exercise (34), an intervention in which BF is distributed preferentially to hypoxic tissues (i.e. type IIb+IId/x fibers) could reduce overall fatigability thereby helping restore exercise tolerance in this population.

Inorganic NO_2^- treatment generally, but not always, reduces blood pressure in hypertensive patients (reviewed by 30). In contrast to our previous investigations in healthy rats (15, 17), NO_2^- did not reduce exercising MAP in CHF. These results are in agreement with Maher *et al*. (38) who demonstrated no change in the resting MAP of CHF patients following intra-arterial NO_2^- infusion. Furthermore, Ormerod *et al*. (45) demonstrated that, in patients with severe CHF, short-term $NaNO_2^-$ infusion increased venous capacitance and attenuated right atrial pressure while only modestly reducing blood pressure. The absent MAP effect herein might be the consequence of an impaired sensitivity to NO which is known to exist at the vascular and

platelet level in CHF (2). In addition, in CHF we expect increased NO scavenging via elevated reactive oxygen species (8) which may have ultimately tempered the effects of NO_2^- on MAP and 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 NO_2^- on MAP and skeletal muscle BF. Whether chronic administration of intra-arterial NO_2^- infusion impacts sympathetic nerve activity in CHF remain to be elucidated.

Clinical and therapeutic relevance

Current therapeutic strategies often employed 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 (reviewed by 13). Fortunately, this does not appear to be a concern with inorganic NO-based interventions (38) and, given the selective effects of NO_2^- infusion on skeletal muscle BF and VC demonstrated presently, these results highlight the therapeutic potential of this approach. In particular NO_2^--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 NO_2^- production, thus further promoting NO homeostasis.

Experimental considerations

The increased blood [lactate] found herein after NO_2^- infusion was not expected. An increase in skeletal muscle O_2 delivery would raise the O_2 pressure head within the

microvasculature (required for capillary-myocyte O_2 flux) as dictated by PO_2mv. This would be expected to improve metabolic control within these tissues by reducing the ADP/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 PO_2mv especially in CHF, it is possible that a rapid influx of NO_2^- and the consequential increase in BF within these muscles may have flushed lactate into the systemic circulation resulting in a brief elevation in blood [lactate]. This phenomenon is supported empirically by Williams *et al*. (51) who have demonstrated substantial increases in circulating [lactate] (as high as 20 $mmol \cdot L^{-1}$) in Weddell seals when their skeletal muscle BF was rapidly restored following deep-water dives. Future investigation of the blood [lactate] time course with NO_2^- would elucidate this presumption. An alternative explanation could be related to NO-induced changes in skeletal muscle glucose uptake, which has been shown to occur following infusion of the NO donor, sodium nitroprusside (33). A local shift to glycolytic metabolism accompanied by a rapid vasodilation within these tissues may account for the changes in blood [lactate] 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, 35). However, it is worth mentioning that the [lactate] following NO_2^- infusion reported herein are well within reasonable ranges of what has been observed previously in our laboratory at this running speed (15, 17, 26), particularly in rats with CHF (27). Nevertheless, future investigations into the effects of NO_2^- on the skeletal muscle PO_2mv at rest and during contractions will provide crucial insight into the metabolic basis for this effect.

Another potential limitation to the present investigation is that we reference 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 relative to fiber type (41), those

changes are only apparent in animals with severe left ventricular dysfunction (i.e. infarct size ~59% of LV endocardial circumference) (12). The indices of CHF presented herein, however, (MI size ~34%) 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.

Conclusions

This investigation is the first to examine the impact of acute NO_2^- infusion (5 $mg \cdot kg^{-1}$) 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 demonstrate the potential for NO_2^- to induce changes in vascular control during exercise. However, the elevation seen in exercising blood [lactate] raises further questions as to how NO_2^- impacts skeletal muscle metabolic control and thus warrants future investigation. Nonetheless, given the emerging evidence supporting the therapeutic potential of the NO_3^--NO_2^--NO pathway (1, 37, 52) these results highlight the ability for an acute NO_2^- 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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