Skeletal muscle vascular control during exercise: impact of nitrite infusion during nitric oxide synthase inhibition in healthy rats

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Abstract

The nitric oxide synthase (NOS) independent pathway of nitric oxide (NO) production in which nitrite (NO_2^-) is reduced to NO may have therapeutic applications for those with cardiovascular diseases in which the NOS pathway is downregulated. We tested the hypothesis that NO_2^- infusion would reduce mean arterial pressure (MAP) and increase skeletal muscle blood flow (BF) and vascular conductance (VC) during exercise in the face of NOS blockade via L-NAME. Following infusion of L-NAME (10 mg · kg^{-1}: L-NAME), male Sprague-Dawley rats (3-6 months, n=8) exercised without (L-NAME) and after infusion of sodium NO_2^- (7 mg · kg^{-1}:, L-NAME + NO_2^-). MAP and hindlimb skeletal muscle BF (radiolabeled microsphere infusions) were measured during submaximal treadmill running (20 m · min^{-1}, 5% grade). Across group comparisons were made with a published control dataset (n=11). Relative to L-NAME, NO_2^- infusion significantly reduced MAP (P<0.03). The lower MAP in L-NAME+NO_2^- was not different from healthy control animals (control: 137 ± 3 L-NAME: 157 ± 7, L-NAME + NO_2^-: 136 ± 5 mmHg). Also, NO_2^- infusion significantly increased VC when compared to L-NAME (P<0.03), ultimatly negating any significant differences from control animals (control: 0.78 ± 0.05, L-NAME: 0.57 ± 0.03, L-NAME + NO_2^-; 0.69 ± 0.04 ml · min^{-1} · 100 g^{-1} · $mmHg^{-1}$) with no apparent fiber type preferential effect. Overall hindlimb BF was decreased significantly by L-NAME: however, in L-NAME+NO_2^- BF improved to a level not significantly different from healthy controls (control: 108 ± 8, L-NAME: 88 ± 3, L-NAME + NO_2^-: 94 ± 6 ml · min^{-1} · 100 g^{-1}, P=0.38 L-NAME vs. L-NAME + NO_2^-). Individuals with diseases that impair NOS activity, and thus vascular function, may benefit from a NO_2^- based therapy in which NO bioavailability is elevated in a NOS-independent manner.

Key words: nitric oxide; nitrate; blood flow

Abbreviations list: ANOVA, analysis of variance; BF, blood flow; CHF, chronic heart failure; LSD, least significant difference; MAP, mean arterial pressure; NO, nitric oxide; NO_2^-, nitrite; NO_3^-, nitrate; NOS, nitric oxide synthase; O_2, oxygen; PO_2mv, microvascular partial pressure of oxygen; QO_2, oxygen delivery; VC, vascular conductance; $\dot{V}O_2$, oxygen uptake.

Introduction

The cardiovascular response to exercise is characterized by a multitude of neural, humoral and mechanical components serving to elevate cardiac output and redistribute blood flow (BF), and thus O_2 delivery (QO_2), to contracting myocytes. Of the humoral regulators, the ubiquitous signaling molecule nitric oxide (NO) plays a fundamental role in the hyperemic response to exercise and, as a result, its bioavailability is key to elicit the changes in QO_2 necessary to meet the rapidly rising O_2 demand ($\dot{V}O_2$) of the skeletal muscle (reviewed by [1]). Indeed, disease states hallmarked by reduced NO bioavailability (i.e. chronic heart failure, CHF, reviewed by [2]) demonstrate a robust disruption in spatial and temporal skeletal muscle QO_2, resulting in perturbed metabolic function and compromised exercise tolerance.

NO is synthesized endogenously in a reaction catalyzed by the NO synthase (NOS) family of enzymes or the one-step reduction of nitrite (NO_2^-) to NO; the latter being a NOS-independent pathway (reviewed by [3]). Recent evidence from murine models suggests that the bioactivity of NO_2^- may be upregulated via ingestion of nitrate (NO_3^-) rich food stuffs (i.e. beetroot juice), thus likely elevating NO bioavailability (following the reduction of NO_3^- to NO_2^- and finally NO) resulting in improved skeletal muscle vascular, metabolic [4-6], and contractile [7] function. These results extend to humans as several laboratories have demonstrated ergogenic effects of dietary NO_3^- supplementation in healthy [8-13] and diseased [14-17] populations. Interestingly, while these studies employ a dietary means of increasing endogenous [NO_2^-], vasoactivity of the directly infused anion is evident in humans [18-21] and animals [22-25] suggesting that bolus delivery may afford an expedited method of augmenting vascular and metabolic control *in vivo*.

Bearing in mind the beneficial impacts of dietary NO_3^- supplementation on exercise performance, and the vascular effects of NO_2^- infusion highlighted above it is logical to consider

that direct infusion with NO_2^- may also impact skeletal muscle vascular control during exercise. Furthermore, when considering that NO_2^- reduction to NO is potentiated in low PO_2 and/or pH environments [18], bioactivity of NO_2^- may be further facilitated (or relied upon) when NOS function is reduced or completely abolished and O_2 transport is impaired (as is the case in many pathological conditions). If direct NO_2^- infusion augments exercising skeletal muscle vascular function independent of NOS, NO_2^- therapy could emerge as an attractive means of restoring NO bioavailability in various cardiovascular diseases in which NOS function is compromised.

Despite these prospects, there are no investigations into the effects of NO_2^- infusion on exercising skeletal muscle vascular control under conditions of NOS blockade. Therefore, the purpose of this investigation was to determine the impact(s) of NO_2^- infusion on skeletal muscle vascular control during exercise in rats with NOS blockade elicited via L-NAME. We tested the hypothesis that, relative to the L-NAME condition, treatment with NO_2^- would restore exercising mean arterial pressure (MAP) and total exercising hindlimb skeletal muscle BF and vascular conductance (VC) to values observed in healthy young-adult rats (with intact NOS function).

Methods

Ethical approval

All procedures employed in this investigation were approved by the Institutional Animal Care and Use Committee of Kansas State University and were conducted under the guidelines established by *The Journal of Physiology* [26]. Sixteen young adult male Sprague-Dawley rats (~3 months of age, Charles River Laboratories, Wilmington, MA, USA) were maintained at accredited animal facilities at Kansas State University on a 12:12-hr light-dark cycle with food and water provided *ad libitum*. All rats were familiarized with running on a custom-built motor-driven treadmill for 5 min · day^{-1} at a speed of 20 m · min^{-1} up a 5% grade for ~5 days. In an effort to minimize the unnecessary utilization of additional animals, control BF, VC, blood gas, [lactate], and plasma [NO_2^-]/[NO_3^-] values reported herein represent animals from recently published work (n=11, [27]) and followed the same experimental procedures as detailed below.

Surgical instrumentation

On the day of the experiment, rats were anaesthetized initially with a 5% isoflurane-O_2 mixture and maintained subsequently on 3% isoflurane/O_2 mixture. A catheter (PE-10 connected to PE-50, Intra-Medic polyethylene tubing, Clay Adams Brand, Becton, Dickinson and Company, Sparks, MD, USA) was placed in the ascending aorta via the right carotid artery. A second catheter was surgically placed in the caudal (tail) artery as described previously [28]. 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 closed, anesthesia was terminated and the rats were given a minimum of 60 min to recover [29].

L-NAME infusion

Rats were then placed on the treadmill and, following a ~5 minute resting period, N^G-nitro-L arginine methyl ester (10 mg · kg^{-1}, L-NAME; *n*=8, Sigma Chemical, St. Louis, MO, USA) was administered to each rat via the caudal artery catheter to inhibit NOS. This dose has been used extensively in our laboratory and has demonstrated inhibition of NOS via attenuation of acetylcholine induced reductions in MAP [30,31].

Exercise protocol and measurement of hindlimb skeletal muscle BF

Following L-NAME infusion, the caudal artery catheter was connected to a 1 ml syringe chambered in a Harvard infusion/withdrawal pump (model 907, Cambridge, MA, USA) and the carotid artery catheter was connected to a pressure transducer (Gould Statham P23ID, Valley View, OH, USA) maintained at the same height as the animal. Approximately 3 min post-L-NAME infusion, exercise was initiated and treadmill speed was increased progressively over a ~30 s period to a speed of 20 m · min^{-1} (5% grade, ~60% $\dot{V}O_2$max; [32]). The rats continued to exercise for another 2.5 min until a total time of 3 min was reached. At 3 min the Harvard pump was activated and withdrawal was initiated at a rate of 0.25 ml · min^{-1}. Simultaneously, HR and MAP were measured and recorded. The carotid artery catheter was then disconnected from the pressure transducer and 0.5-0.6 × 10^6 15 μm diameter radiolabeled microspheres (^{57}Co or ^{85}Sr in random order; Perkin Elmer, Waltham, MA, USA) were infused into the aortic arch for determination of regional BF (L-NAME condition). Following the microsphere infusion, ~0.3 ml of blood was sampled from the carotid artery catheter for the determination of blood [lactate] (Nova Stat Profile M, Nova Biomedical, Waltham, MA, USA) and exercise was terminated.

NO_2^- infusion

Following a 30 min recovery period a bolus infusion of sodium NO_2^- (7 mg · kg^{-1} body mass, L-NAME + NO_2^-; *n*=8, Sigma Chemical, St. Louis, MO, USA) was

administered to each rat via the caudal artery catheter. The exercise and microsphere infusion protocols (radio-labeled differently from the first) were then repeated (condition L-NAME + NO_2^-).

Blood sampling and measurement of plasma [NO_3^-] and [NO_2^-]

Immediately following microsphere infusion but prior to the termination of exercise, a ~0.3 ml blood sample was drawn from the carotid artery catheter for determination of blood pH, PO_2, and $\%O_2$ saturation (Nova Stat Profile M, Nova Biomedical, Waltham, MA, USA). For plasma [NO_3^-] and [NO_2^-], following the termination of exercise ~0.8 ml of blood was drawn into heparinized tubes and rapidly centrifuged at 5000 g at 4°C for 6 minutes. Plasma was then extracted and frozen immediately at -80°C for later analysis via chemiluminescence as described previously [4,5,27,33].

Determination of BF and VC

Rats were euthanized via pentobarbital sodium overdose (≥50 mg · kg^{-1}). The thorax of each rat was opened and accurate placement of the carotid artery catheter was confirmed before the internal organs and 28 individual muscles and muscle parts of the hindlimb were excised.

Radioactivity of each tissue was determined with a gamma scintillation counter (Packard Auto Gamma Spectrometer, model 5230, Downers Grove, IL, USA). Tissue BF was then calculated using the reference sample method [28] and expressed as ml · min^{-1} · $100g^{-1}$. VC was then calculated by normalizing BF to MAP and expressed as ml · min^{-1} · $100g^{-1}$ · $mmHg^{-1}$.

Statistical analysis

Results were compared among (control vs. L-NAME and control vs. L-NAME + NO_2^-) and within (L-NAME vs. L-NAME + NO_2^-) groups using *a priori* unpaired and paired one-tail

Student's *t* tests, respectively, corrected for multiple comparisons. Values are expressed as mean ± SEM.

Results

MAP, HR, plasma [NO_3^-] and [NO_2^-] and blood gases

Relative to control, post NO_2^- infusion plasma [NO_2^-] (control: 0.17 ± 0.2, L-NAME + NO_2^-: 306.8 ± 38.7 µMol, $P<0.01$) and [NO_3^-] (control: 17.8 ± 1, L-NAME + NO_2^-: 152.5 ± 35 µMol, $P<0.01$) were significantly elevated. Relative to control, MAP was significantly higher in the L-NAME condition (Figure 1, $P<0.03$). Following NO_2^- infusion, MAP was reduced significantly when compared to the L-NAME condition ($P<0.03$). Exercising MAP was not different between control and L-NAME+NO_2^- groups ($P=0.36$). Relative to the control and L-NAME+NO_2^- conditions, exercising HR was significantly lower in the L-NAME condition (control: 528 ± 12, L-NAME: 493 ± 37, L-NAME + NO_2^-: 520 ± 33 beats · min^{-1}, $P<0.01$).

There were no differences in arterial PO_2, PCO_2, or $\%O_2$ saturation during exercise. Arterial blood [lactate] during exercise was greater following NO_2^- infusion (3.8 ± 0.5 mM) compared to control (2.7 ± 0.4 mM) and L-NAME only (2.1 ± 0.3 mM) conditions, ($P<0.016$).

BF and VC

L-NAME significantly reduced exercising total hindlimb skeletal muscle BF and VC (Figure 2, $P<0.03$). Following NO_2^- infusion total hindlimb skeletal muscle VC was restored to levels observed in control rats (Figure 2, $P<0.03$ L-NAME vs. L-NAME+NO_2^-, $P>0.10$ control vs. L-NAME+NO_2). There were no differences in total hindlimb skeletal muscle BF during exercise in L-NAME vs. L-NAME + NO_2^- or control vs. L-NAME + NO_2^- conditions (Figure 2 bottom panel, $P>0.03$).

Relative to control, L-NAME treated rats had lower BF in 5 and VC in 15 of the 28 individual hindlimb muscles and muscle parts, whereas this was the case for only 3 muscles (BF and VC) in the L-NAME+NO_2^- condition (Table 1, $P<0.03$ for all). Moreover, following NO_2^-

infusion, VC in 19 of the 28 individual hindlimb muscles and muscle parts was increased significantly when compared to the L-NAME condition ($P<0.03$, Table 1).

Relative to control, BF and VC were lower in the adrenals and pancreas while VC was lower in the kidneys, stomach, and small intestine in rats treated with L-NAME ($P<0.03$, Table 2). Following NO_2^- infusion, renal and adrenal BF and VC were lower when compared to control animals while renal and adrenal BF was reduced when compared to L-NAME ($P<0.03$, Table 2).

4. Discussion

The principal original finding of this investigation is that, in the face of NOS blockade, NO_2^- infusion restored exercising MAP and hindlimb skeletal muscle VC to levels observed in young-adult healthy rats with intact NOS function. While NO_2^- infusion did not increase BF when compared to the L-NAME condition, it did abolish the lower BF induced by L-NAME. Elevations in VC and reductions in MAP could serve to reduce afterload and thus reduce the work of the heart during exercise. These results demonstrate that NO_2^- may serve as a powerful modulator of vascular control *in vivo*, independent of NOS function and thus may hold promising therapeutic potential, particularly in diseases with impaired NOS function and chronically elevated MAP.

Effects of inorganic NO_2^- infusion on skeletal muscle BF and VC and MAP

An abundance of research has focused on defining the vasoactive/cardioprotective role(s) of NO_2^- with many studies suggesting that the reduction of NO_2^- to NO compliments the well understood NOS pathway of NO production, particularly when NOS function becomes uncoupled or otherwise impaired (reviewed by [34,35]). The vascular responses to NO_2^- infusion presented herein support this notion. Similar to what has been reported previously in our laboratory [36,37], infusion with the comprehensive NOS blocker L-NAME increased MAP ~15% and decreased skeletal muscle VC ~26% during exercise. Consistent with our hypothesis, infusion with NO_2^- ($7 mg \cdot kg^{-1}$) restored MAP and VC to levels similar to those observed in healthy control animals. One potential explanation for these effects of NO_2^- could be the lower PO_2/pH environment present within the skeletal muscle following NOS inhibition [33]. Such environments facilitate (or uninhibit) NO_2^- reduction to NO *in vivo* [18,38], which may allow local

NO_2^- to support the blood-myocyte PO_2 gradient (via $\uparrow QO_2$ and microvasculature PO_2, PO_2mv) that, when compromised, leads to tissue hypoxia and exacerbates intracellular perturbations [39].

One striking aspect of this investigation, in which acute NO_2^- infusion was employed, was that the augmented skeletal muscle VC was observed in muscles and muscle parts that span the full spectrum of fast and slow twitch fibre types (Table 1). This is in contrast to investigations utilizing short-term dietary NO_3^- supplementation as a means of increasing circulating [NO_2^-]. Specifically, there is a fibre type preferential effect of dietary NO_3^- supplementation as rats given NO_3^- rich beetroot juice for 5 days exhibited elevated skeletal muscle BF and VC exclusively in muscles and muscle portions comprised of $\geq 66\%$ type IIb + d/x muscle fibres [27]. Moreover, beetroot juice elevates PO_2mv during muscle contractions in the gastrocnemius (fast twitch) but not soleus (slow twitch) muscles [33]. The substantial array of muscles and muscle portions exhibiting a vasoactive response to NO_2^- infusion herein suggests that the fibre type preferential effects observed following dietary NO_3^- supplementation may be conferred via changes in protein expression which require a longer period of elevated NO_2^- exposure to manifest. This idea is supported by evidence from Hernandez, Schiffer, Ivarsson, Cheng, Bruton, Lundberg, Weitzberg, Westerblad [7] in which the improvements in fast twitch skeletal muscle force production evoked by NO_3^- supplementation were attributed to elevations in calcium handling proteins (i.e. calsequestrin 1 and the dihydropyridine receptor) which were present following multiple days of dietary NO_3^- supplementation.

Additionally, the discrepancies in the vascular responses to NO_3^- vs. NO_2^- treatment could be related to the relative impacts of NOS inhibition in fast vs. slow twitch muscles. Skeletal muscles comprised predominantly of slow twitch fibres demonstrate the greatest deficits in BF and VC following L-NAME infusion [36] likely due to a greater expression of endothelial

NOS (eNOS) within these tissues [40]. These slow twitch muscles may exhibit much greater BF and $\dot{V}O_2$ than their fast twitch counterparts both at rest and during exercise (~100% greater for both BF and $\dot{V}O_2$ [41]). Consequently, NOS inhibition may have crippled O_2 delivery in these muscles sufficiently enough to produce an environment ripe for NO_2^- bioactivation (i.e. very low PO_2 and pH). This effect could place more emphasis on NO_2^- as the primary source of NO in these specific tissues when vascular function is impaired, as it is in many disease states [42]. In this regard, the spatial changes in VC seen following NO_2^- infusion herein may mimic closely what would be observed in individuals with diseases that compromise NOS function. However, these questions require further investigation using specific models of vascular disease.

Clinical and Therapeutic implications

In healthy individuals eNOS is the primary endogenous source for NO_2^- and NO [43]. Endothelial dysfunction becomes evident early on in many diseases including CHF (reviewed by [2]) and peripheral artery disease (reviewed by [44]) and thus likely limits vascular and metabolic function via attenuated NO production from both NOS dependent and independent pathways [43,45]. As evidenced by Hirai *et al.* [46,47], reduced NO from NOS dramatically impairs the matching of skeletal muscle QO_2 to $\dot{V}O_2$ such that superfusion of L-NAME in the contracting rat spinotrapezius muscle transforms the healthy PO_2*mv* profile into one resembling CHF [46]. In this regard, the blockade of NOS induced by L-NAME infusion performed in the present investigation presents a challenge that mimics the consequences of CHF, and potentially other diseases. Therefore, from the present findings, a therapy in which systemic [NO_2^-] is elevated (via endogenous or exogenous sources) may provide beneficial vascular responses independent of NOS function. Even small improvements in vascular function may enhance metabolic control

during dynamic exercise; potentially improving adherence to rehabilitation programs [35], which in-and-of themselves would upregulate eNOS function and endogenous NO_2^- production.

Experimental considerations and Potential limitations

A surprising result of the present investigation was the rise in exercising blood [lactate] following NO_2^- infusion (~41% and 81% greater vs. control and L-NAME respectively). Lower levels of NO may act as a useful brake on mitochondrial activity via competitive binding to complex IV of the respiratory chain [48]. In contrast, high concentrations of NO have been associated with adverse effects on cell respiration via nitrosylation of mitochondrial electron chain complexes, specifically complex I [49]. In addition NO works to inhibit complex IV (cytochrome oxidase) thereby reducing cellular O_2 consumption. Both of these effects may prove beneficial in certain environments or situations when O_2 delivery becomes reduced as reductions in tissue $\dot{V}O_2$ work to extend the PO_2 gradient across a larger tissue area, effectively sharing the available O_2 [50]. However, in the current study it is possible that the rate of NO_2^- reduction to NO became high enough to overwhelm mitochondrial respiration, thus leading to impaired oxidative metabolism and an increased reliance on glycolytic means of ATP production. In addition, while the current dose of NO_2^- raised plasma [NO_3^-] to levels very similar to what has been reported following dietary NO_3^- supplementation in humans [9,14] and animals [5,27] the plasma [NO_2^-] were much greater than that achieved via NO_3^- supplementation, and thus may have contributed to the aforementioned effect on metabolism. In this regard a comprehensive dose-response relationship will need to be determined before NO_2^- can be used as an effective therapeutic.

Furthermore, considering that NOS was acutely inhibited in the present investigation, the impacts of NO_2^- infusion may differ when administered to specific models of vascular diseases

that have been developed chronically, as this would more closely mimic specific etiologies. Additionally, due to the relatively long half-life and bioactivity of L-NAME metabolites (~20 hours in rats [51]) the experimental design was limited to a fixed sequence and therefore, an ordering effect cannot be ruled out. Future investigations in which NO_2^- is employed in healthy control animals would also provide further insight into the bioactivity of NO_2^- in animals with intact NOS function and could shed light on how a NO_2^- based intervention may impact healthy cardiovascular function.

Conclusions

These data highlight the potential for NO_2^- to act independently of NOS and improve skeletal muscle vascular control during exercise. Considering the multiple cardiovascular diseases that impair NOS function, therapies that increase [NO_2^-] may result in improved skeletal muscle vascular control during exercise. However, the NO_2^- induced changes in blood [lactate] seen during exercise herein suggests that the reduction of NO_3^- to NO_2^-, accomplished via facultative anaerobes in the mouth following dietary NO_3^- consumption, may provide the controlled release of NO_2^- needed to elicit the most beneficial vascular and metabolic changes during exercise. It is anticipated that future investigations into the vascular impacts of both NO_2^- and NO_3^- based therapies will provide crucial insight into the potential benefits, and limitations, of both interventions.

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Author Contributions

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All authors have approved the final version of the manuscript.

Disclosures

None

Table 1 Effects NO_2^- infusion (7 mg · kg^{-1}) on exercising hindlimb skeletal muscle BF (ml · min^{-1} · $100g^{-1}$) and VC (ml · min^{-1} · $100g^{-1}$ · $mmHg^{-1}$) in rats with NOS blockade (L-NAME).

	BF			VC		
	Control	**L-NAME**	**L-NAME+NO_2^-**	**Control**	**L-NAME**	**L-NAME+NO_2^-**
Ankle extensors						
Soleus (9%)	295 ± 42	242 ± 71	285 ± 36	2.14 ± 0.30	1.56 ± 0.17	2.06 ± 0.23†
Plantaris (80%)	207 ± 15	144 ± 8*	173 ± 15	1.50 ± 0.10	0.93 ± 0.06*	1.27 ± 0.08†
Gastrocnemius, red (14%)	452 ± 44	333 ± 59	362 ± 65	3.27 ± 0.30	2.18 ± 0.02*	2.63 ± 0.44†
Gastrocnemius, white (100%)	42 ± 7	26 ± 3	37 ± 4†	0.30 ± 0.05	0.17 ± 0.02*	0.27 ± 0.03†
Gastrocnemius, mixed (91%)	149 ± 12	120 ± 5	141 ± 8	1.08 ± 0.08	0.77 ± 0.04*	1.04 ± 0.04†
Tibialis posterior (73%)	118 ± 17	81 ± 12	91 ± 13	0.85 ± 0.12	0.51 ± 0.07*	0.66 ± 0.09†
Flexor digitorum longus (68%)	99 ± 14	60 ± 7*	69 ± 9	0.71 ± 0.09	0.38 ± 0.04*	0.51 ± 0.06†
Flexor halicus longus (71%)	75 ± 10	68 ± 8	99 ± 14†	0.54 ± 0.06	0.44 ± 0.06	0.74 ± 0.11†
Ankle flexors						
Tibialis anterior, red (63%)	343 ± 35	209 ± 10*	219 ± 20*	2.48 ± 0.23	1.36 ± 0.10*	1.62 ± 0.14*
Tibialis anterior, white (80%)	119 ± 14	83 ± 6*	89 ± 12	0.86 ± 0.09	0.54 ± 0.05*	0.66 ± 0.09†
Extensor digitorum longus (76%)	54 ± 7	75 ± 20	77 ± 17	0.39 ± 0.05	0.50 ± 0.14	0.57 ± 0.13†
Peroneals (67%)	128 ± 11	72 ± 14*	91 ± 13*	0.93 ± 0.08	0.46 ± 0.09*	0.67 ± 0.09*†
Knee extensors						
Vastus intermedius (4%)	359 ± 39	257 ± 25	302 ± 39	2.60 ± 0.27	1.66 ± 0.17*	2.20 ± 0.25†
Vastus medialis (82%)	114 ± 18	137 ± 13	144 ± 14	0.82 ± 0.12	0.89 ± 0.08	1.06 ± 0.08†
Vastus lateralis, red (35%)	388 ± 43	310 ± 35	281 ± 25	2.82 ± 0.29	2.02 ± 0.26	2.08 ± 0.52
Vastus lateralis, white (100%)	33 ± 5	26 ± 8	31 ± 7	0.24 ± 0.03	0.16 ± 0.04	0.23 ± 0.04†
Vastus lateralis, mixed (89%)	167 ± 21	123 ± 12	127 ± 13	1.22 ± 0.14	0.81 ± 0.09*	0.94 ± 0.09†
Rectus femoris, red (66%)	224 ± 33	181 ± 15	204 ± 17	1.62 ± 0.23	1.17 ± 0.10	1.50 ± 0.11†
Rectus femoris, white (100%)	101 ± 13	81 ± 7	91 ± 8	0.73 ± 0.09	0.52 ± 0.05	0.67 ± 0.06†
Knee flexors						
Biceps femoris anterior (100%)	50 ± 8	33 ± 4	36 ± 4	0.36 ± 0.05	0.21 ± 0.03*	0.27 ± 0.03†
Biceps femoris posterior (92%)	79 ± 8	65 ± 3	71 ± 5	0.58 ± 0.06	0.42 ± 0.02*	0.53 ± 0.04†
Semitendinosus (83%)	56 ± 6	34 ± 3*	37 ± 4*	0.40 ± 0.04	0.22 ± 0.02*	0.28 ± 0.03*
Semimembranosus, red (72%)	119 ± 14	86 ± 7	83 ± 14	0.87 ± 0.09	0.56 ± 0.05*	0.62 ± 0.11
Semimembranosus, white (100%)	33 ± 6	38 ± 7	40 ± 11	0.24 ± 0.04	0.25 ± 0.05	0.30 ± 0.09
Thigh adductors						
Adductor longus (5%)	315 ± 38	263 ± 26	231 ± 31†	2.28 ± 0.26	1.71 ± 0.21	1.68 ± 0.22
Adductor magnus & brevis (89%)	83 ± 8	80 ± 7	80 ± 9	0.60 ± 0.05	0.52 ± 0.05	0.60 ± 0.06
Gracilis (77%)	42 ± 4	37 ± 4	34 ± 5	0.30 ± 0.03	0.24 ± 0.02	0.26 ± 0.04
Pectineus (69%)	54 ± 8	40 ± 6	46 ± 11	0.39 ± 0.06	0.25 ± 0.03	0.34 ± 0.08

Data are mean ± SEM. Values in parentheses indicate % type IIb + d/x according to Delp & Duan (1996).
Control: n=11, L-NAME: n=8, L-NAME + NO_2^-: n=8. *P<0.03 vs. control. †P<0.03 vs. L-NAME.

Table 2. Effects of NO_2^- infusion (7 mg · kg^{-1}) on exercising BF (ml · min^{-1} · $100g^{-1}$) and VC (ml · min^{-1} · $100g^{-1}$ · $mmHg^{-1}$) in the kidneys and organs of the splanchnic region.

	BF			VC		
	Control	L-NAME	L-NAME + NO_2	Control	L-NAME	L-NAME + NO_2
Kidney	421 ± 42	338 ± 28	267 ± 31*†	3.05 ± 0.28	2.22 ± 0.25*	1.96 ± 0.22*
Stomach	67 ± 13	38 ± 3	35 ± 4	0.49 ± 0.10	0.25 ± 0.02*	0.25 ± 0.03
Adrenals	353 ± 72	128 ± 17*	100 ± 66*	2.87 ± 0.44	0.85 ± 0.14*	0.72 ± 0.15*
Spleen	61 ± 14	102 ± 21	48 ± 7†	0.44 ± 0.10	0.68 ± 0.16	0.35 ± 0.06
Pancreas	110 ± 15	72 ± 8*	93 ± 22	0.80 ± 0.11	0.47 ± 0.06*	0.67 ± 0.15
Sm. intestine	240 ± 27	177 ± 24	211 ± 26	1.74 ± 0.18	1.17 ± 0.19*	1.55 ± 0.17
Lg. intestine	127 ± 16	123 ± 20	140 ± 42	0.92 ± 0.10	0.82 ± 0.15	1.01 ± 0.28
Liver**	16 ± 4	15 ± 2	13 ± 3	0.12 ± 0.02	0.10 ± 0.01	0.09 ± 0.02

Data are mean ± SEM. **Indicates arterial, not portal, BF and VC. Control: n=11, L-NAME: n=8, L-NAME + NO_2^-: n=8. *P<0.03 vs. control. †P<0.03 vs. L-NAME.

Figure captions

Figure 1. Exercising MAP, systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP) values for control, L-NAME and L-NAME+NO_2^- conditions. *P<0.03 vs. control, #P<0.03 vs. L-NAME. Note: control values represented are from previously published data.

Figure 2. Total hindlimb skeletal muscle BF and VC for control, L-NAME and L-NAME+NO_2^- conditions in rats during submaximal locomotory exercise. *P<0.03 vs. control, #P<0.03 vs. L-NAME. Note: control values represented are from previously published data.

Figure 1.

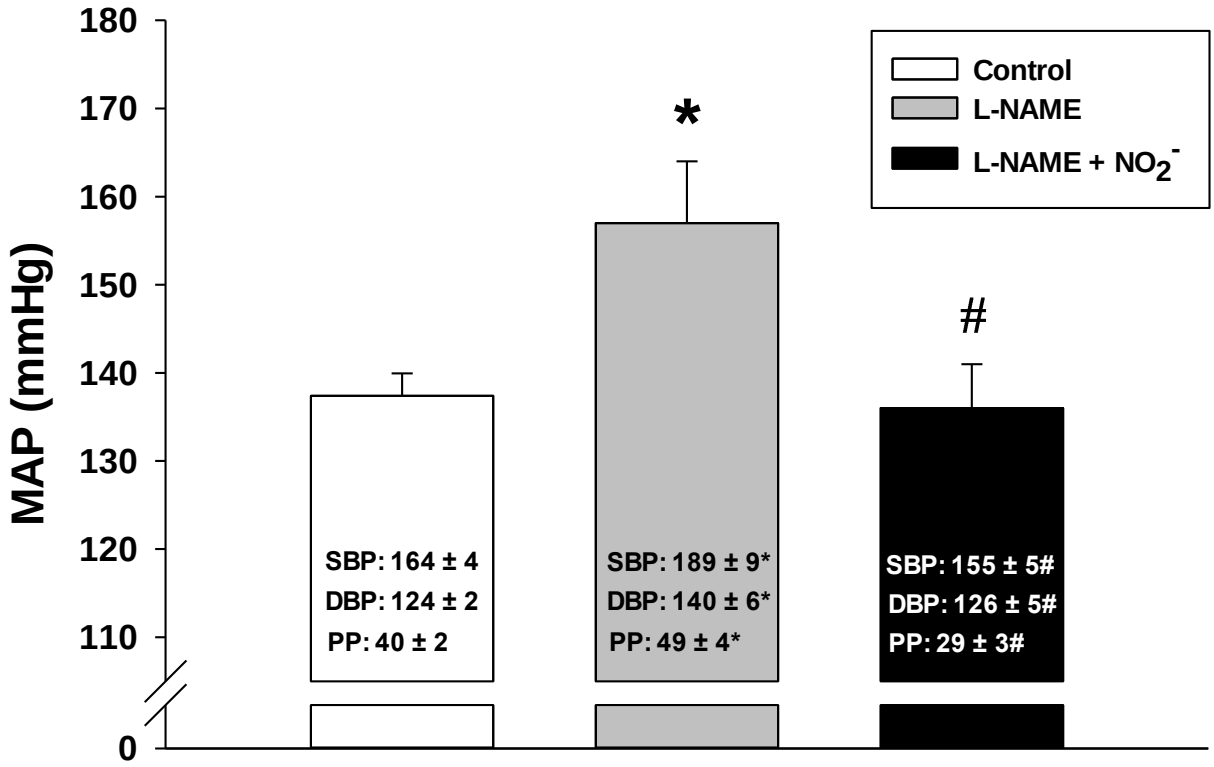
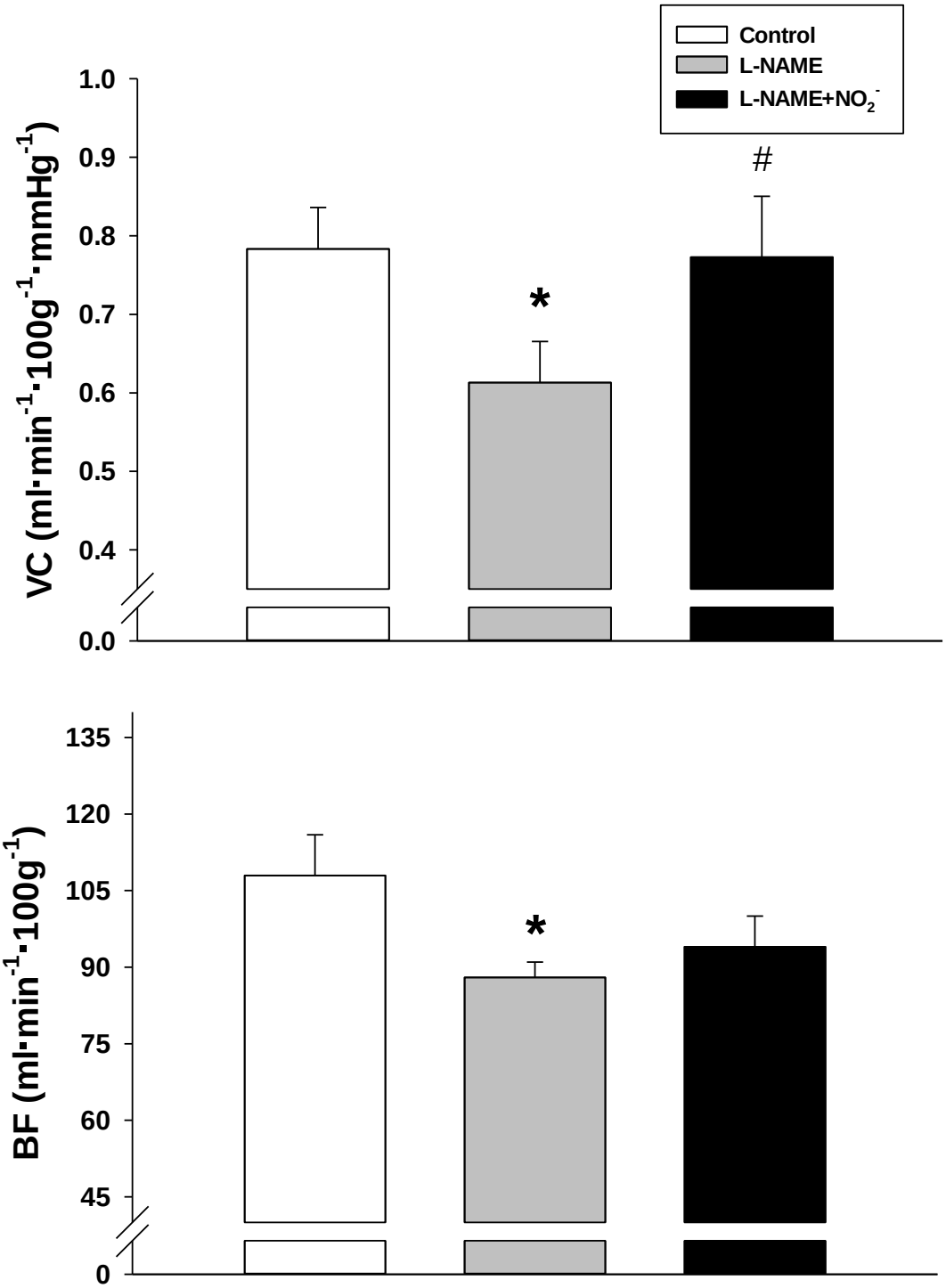

MAP (mmHg)
180
170
160
150
140
130
120
110
0
Control
L-NAME
L-NAME + NO_2^-
*
#
SBP: 164 ± 4
DBP: 124 ± 2
PP: 40 ± 2
SBP: 189 ± 9*
DBP: 140 ± 6*
PP: 49 ± 4*
SBP: 155 ± 5#
DBP: 126 ± 5#
PP: 29 ± 3#

Figure 2.


Control
L-NAME
L-NAME+NO2-
VC (ml·min-1·100g-1·mmHg-1)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.0
*
#
BF (ml·min-1·100g-1)
135
120
105
90
75
60
45
0
*

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