SODIUM NITRITE IMPACTS THE PERIPHERAL CONTROL OF CONTRACTING SKELETAL MUSCLE MICROVASCULAR OXYGEN PRESSURE IN HEALTHY RATS

by

TRENTON DAVID COLBURN

B.S., Kansas State University, 2016

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Kinesiology
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Major Professor
Dr. Timothy I. Musch
Copyright

TRENTON DAVID COLBURN

2016
Abstract

Exercise intolerance characteristic of diseases such as chronic heart failure (CHF) and diabetes is associated with reduced nitric oxide (NO) bioavailability from nitric oxide synthase (NOS), resulting in an impaired microvascular $O_2$ driving pressure ($PO_2mv$: $O_2$ delivery – $O_2$ utilization) and metabolic control. Infusions of the potent NO donor sodium nitroprusside augment NO bioavailability yet decrease mean arterial pressure (MAP) thereby reducing its potential efficacy for patient populations. To eliminate or reduce hypotensive sequellae $NO_2^-$ was superfused onto the spinotrapezius muscle. It was hypothesized that local $NO_2^-$ administration would elevate resting $PO_2mv$ and slow $PO_2mv$ kinetics (increased $\tau$: time constant, MRT: mean response time) following the onset of muscle contractions. In 12 anesthetized male Sprague-Dawley rats, $PO_2mv$ of the circulation-intact spinotrapezius muscle was measured by phosphorescence quenching during 180 s of electrically-induced twitch contractions (1 Hz) before and after superfusion of NaNO$_2$ (30 mM). $NO_2^-$ superfusion elevated resting $PO_2mv$ (CON: $28.4 \pm 1.1$ vs $NO_2^-$: $31.6 \pm 1.2$ mmHg, P $\leq 0.05$), $\tau$ (CON: $12.3 \pm 1.2$ vs $NO_2^-$: $19.7 \pm 2.2$ s, P $\leq 0.05$) and MRT (CON: $19.3 \pm 1.9$ vs $NO_2^-$: $25.6 \pm 3.3$ s, P $\leq 0.05$). Importantly, these effects occurred in the absence of any reduction in MAP ($103 \pm 4$ vs $105 \pm 4$ mmHg, pre- and post-superfusion respectively; P $> 0.05$). These results indicate that $NO_2^-$ supplementation delivered to the muscle directly through $NO_2^-$ superfusion enhances the blood-myocyte driving pressure of oxygen without compromising MAP at rest and following the onset of muscle contraction. This strategy has substantial clinical utility for a range of ischemic conditions.
# Table of Contents

List of Figures ........................................................................................................................................ v
List of Tables .......................................................................................................................................... vi
Acknowledgements ............................................................................................................................ vii
Introduction ........................................................................................................................................ 1
Methods .............................................................................................................................................. 4
    Surgical Preparation ......................................................................................................................... 4
    Experimental Protocol ..................................................................................................................... 5
    PO$_2$-mv Measurement and Curve-fitting ....................................................................................... 6
    Statistical Analysis ........................................................................................................................ 7
Results .................................................................................................................................................. 8
    Microvascular Oxygen Pressures (PO$_2$-mv) .............................................................................. 8
    Central Hemodynamics and Blood Gases ...................................................................................... 8
Discussion ......................................................................................................................................... 13
    Microvascular Oxygen Pressure (PO$_2$-mv) Dynamics .............................................................. 13
    Potential Therapeutic Significance ............................................................................................... 15
    Experimental Considerations ....................................................................................................... 16
    Conclusion ..................................................................................................................................... 16
References ........................................................................................................................................... 18
List of Figures

Figure 1. NaNO₂ Superfusion vs. Intra-arterial Infusion on PO₂mv........................................ 10
Figure 2. NaNO₂ Superfusion vs. Intra-arterial Infusion on MAP ............................................. 11
Figure 3. Microvascular oxygen pressure (PO₂mv) during NaNO₂ superfusion ......................... 11
Figure 4. PO₂mv and MAP during contractions, and PO₂mv difference between NaNO₂ and Control .......................................................... 12
List of Tables

Table 1. PO$_2$mv parameters of the spinotrapezius muscle at rest and following the onset of contractions under control and NaNO$_2$ conditions .................................................................................................. 9
Acknowledgements

I would like to thank K. Sue Hageman for her superb technical assistance. Additionally, I am thankful for the incessant mentoring and support of Drs. Timothy I. Musch and David C. Poole as well as Drs. Clark T. Holdsworth and Scott K. Ferguson, and Jesse C. Craig.

Financial support for this research was provided by American Heart Association Midwest Affiliate (10GRNT4350011) and NIH (HL-108328) awards to David C. Poole.
Introduction

Sustained muscle contractions require a robust and appropriate hyperemic response that is contingent upon arteriolar vasodilation increasing muscle O$_2$ delivery (\(\dot{Q}_O_2\)) in proportion to the elevated O$_2$ demands (\(\dot{V}_O_2\)). It is also important to recognize that the instantaneous \(\dot{Q}_O_2/\dot{V}_O_2\) ratio sets microvascular O$_2$ pressures (\(P_{O_2}^{mv}\)) that are crucial for blood-myocyte O$_2$ flux and also the intracellular O$_2$ which influences metabolic control (29, 50).

Within the spectrum of vasoactive mediators for the exercise hyperemia nitric oxide (NO) is a key player. Thus, blockade of the endogenous endothelial NO synthase (eNOS) and/or neuronal NOS (nNOS) systems reduces exercising muscles(s) blood flow and \(\dot{Q}_O_2\) (8, 28) lowering \(P_{O_2}^{mv}\) (22) and impairing function. In diseases such as chronic heart failure dysfunction of the NOS system reduces NO bioavailability with the resultant decrease of \(P_{O_2}^{mv}\), especially in the transient phase immediately following the onset of contractions, slows \(\dot{V}_O_2\) kinetics and contributes to muscle dysfunction characteristic of chronic heart failure (CHF) (15, 21, 26, 44).

Given the reduced capacity for endogenous NO production in disease, possibly as a consequence of tissue hypoxia and elevated reactive oxygen species which constrain NOS function, there has been substantial interest in providing exogenous NO precursors such as sodium nitroprusside (SNP) and inorganic nitrate/nitrite (\(NO_3^-/NO_2^-\)) (37, 54). A particularly attractive feature of the \(NO_3^-/NO_2^-\) pathway is that the pathological muscle hypoxia found in CHF and other patients promotes \(NO_2^-\) reduction to NO and hence enhances \(\dot{Q}_O_2\) locally without compromising systemic blood pressure as might be expected for systemic vasodilators such as SNP or hydralazine, for example (51, 52). Recently, beetroot (BR) juice has been advocated as an \(NO_3^-/NO_2^-\) source that reduces mean arterial pressure, enhances muscle \(\dot{Q}_O_2\) and \(P_{O_2}^{mv}\), and
improves muscle contractile function and efficiency (19, 20, 33). Unfortunately, after absorption in the gut the NO$_3^-$-NO$_2^-$ reduction requires salivary gland NO$_3^-$ secretion and the participation of commensal bacteria in the oral cavity before resorption into the blood stream as circulating NO$_2^-$. These steps typically take hours to raise plasma [NO$_2^-$] (53) and can be blocked by mouthwash-induced bactericide (39).

Given these limitations the utility of increasing vascular [NO$_2^-$] more directly has been considered (38). However the results have been controversial with up to 36 µmol/min NO$_2^-$ infused into the forearms of healthy subjects proving ineffective (34). Arguing that hypoxia is crucial for the NO$_2^-$-NO reduction Maher and colleagues (38) subsequently demonstrated that direct arterial infusions into the resting forearm of subjects breathing 12% O$_2$ (estimated arterial PO$_2$ 48-55 mmHg) induced a robust arterial vasodilation. These are precisely the conditions extant in the microvasculature of contracting muscles. Specifically, in the rat during exercise muscle PO$_2$mv falls to ~20 mmHg in muscles comprised predominantly of slow twitch fibers or ~10 mmHg in fast twitch muscles (4, 40). Moreover, we have demonstrated that, when NO bioavailability is reduced by CHF (23) or NOS blockade (18), NO$_2^-$ infusions induce a robust increase in muscle Q̇O$_2$ that is especially pronounced in fast twitch muscles.

Because enhanced NO bioavailability has the potential to both elevate Q̇O$_2$ and reduce ŴO$_2$ direct measurements of PO$_2$mv are necessary to evaluate the efficacy of NO$_2^-$ treatment to enhance blood-myocyte O$_2$ flux. Moreover, because ŴO$_2$ demands change most rapidly within the first minute or so of contractions we argue that assessing the efficacy of NO$_2^-$ to raise PO$_2$mv across this interval with high temporal fidelity is crucial. In the rat spinotrapezius muscle we therefore tested the hypothesis that local NO$_2^-$ superfusion under normoxic conditions would
elevate PO$_{2\,mv}$ significantly and, importantly, would do so in the absence of systemic hypotension.
Methods

Twelve male Sprague-Dawley rats (mass = 421 ± 23 g, Charles River Laboratories, Wilmington, MA) were used in this investigation. Rats were provided food and water *ad libitum* while housed in a 12/12 hr light-dark cycle facility at Kansas State University. All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University and conducted according to National Institute of Health Guidelines.

*Surgical Preparation*

Rats were initially anesthetized with a 5% isoflurane-O₂ mixture and subsequently maintained on 3% isoflurane-O₂. Following cannulation of the carotid artery, a catheter (PE-10 connected to PE-50, Intra-Medic polyethylene tubing, Clay Adams Brand, Becton, Dickinson and Company, Sparks, MD) was inserted into the carotid artery for measurement of mean arterial pressure (MAP) and heart rate (HR), and infusion of the phosphorescent probe (see below). A second catheter was inserted into the caudal artery for the infusion of pentobarbital sodium anesthesia and arterial blood sampling. Upon closing the incisions for the carotid and caudal catheters, rats were transitioned to pentobarbital sodium anesthesia and concentrations of isoflurane were removed. The level of anesthesia was regularly monitored via toe pinch and palpebral reflex; with anesthesia supplemented as necessary. Rats were placed on a heating pad to maintain a core temperature of ~38°C (measured via rectal probe). Incisions were then made to carefully expose the right spinotrapezius muscle with overlying skin and fascia reflected such that the integrity of the neural and vascular supply was maintained (2). Using 6-0 silk sutures, silver wire electrodes were secured to the rostral (cathode) and caudal (anode) regions of the muscle. Exposed muscle tissue was superfused with warmed (38 °C) Krebs-Henseleit
bicarbonate buffered solution equilibrated with 5% CO₂-95% N₂. Surrounding exposed tissue was covered with Saran wrap (Dow Brands, Indianapolis, IN) to ensure that superfused solutions were confined to the spinotrapezius. The spinotrapezius muscle was selected based on its mixed muscle fiber-type composition and citrate synthase activity, which resembles the quadriceps muscle in humans (13, 35).

**Experimental Protocol**

The phosphorescent probe palladium meso-tetra (4 carboxyphenyl)tetrabenzoporphyrin dendrimer (G2: 15-20 mg kg⁻¹ dissolved in 0.4 ml saline) was infused via the carotid artery catheter. Following a brief stabilization period (~10 min), the common end of the light guide of a frequency domain phosphorimeter (PMOD 5000, Oxygen Enterprises, Philadelphia, PA) was positioned ~2–4 mm superficial to the dorsal surface of the exposed right spinotrapezius muscle as described previously (2). Fields containing large vessels were avoided in order to ensure that the measurements obtained were of principally capillary blood. PO₂mv was measured at rest and during the 180 s contraction protocol (1 Hz, ~6V, 2 ms pulse duration) via phosphorescence quenching (see below) and recorded at 2 s intervals. Muscle contractions were elicited via a Grass stimulator (model S88, Quincy, MA). Following the contraction period, blood samples were taken for analysis and PO₂mv was monitored to ensure that microvascular control was preserved and values returned to baseline. After a 30 min stabilization period NaNO₂ (30 mM in 3.0 ml Krebs-Henseleit bicarbonate buffered solution) was continuously superfused for 3 min along the exposed muscle. This NaNO₂ dose was chosen due to the curved nature of the spinotrapezius muscle spanning the rib cage. Once the superfused solution is applied, the portion that does not diffuse into the muscle runs down the Saran wrap and onto a waste collection site.
Approximately 1 min following superfusion, after PO\textsubscript{2mv} stabilized, the aforementioned contraction protocol was repeated. Rats were then euthanized via pentobarbital sodium overdose (≥50 mg/kg administered into the carotid artery catheter).

**PO\textsubscript{2mv} Measurement and Curve-fitting**

PO\textsubscript{2mv} was calculated using the Stern-Volmer relationship. Direct measurement of phosphorescence lifetime utilized in the following equation yields PO\textsubscript{2mv} (45);

\[ PO_{2mv} = \frac{\tau^* / \tau - 1}{k_Q \times \tau^*} \]

Where \( k_Q \) is the quenching constant and \( \tau^* \) and \( \tau \) are the phosphorescence lifetimes in the absence of O\textsubscript{2} and at the ambient O\textsubscript{2} concentration, respectively. For G2, \( k_Q \) is 273 mmHg\textsuperscript{-1}s\textsuperscript{-1} and \( \tau^* \) is 251 μs (16). Since the G2 phosphorescent probe binds to blood proteins, G2 is evenly distributed throughout plasma and compartmentalized to the vascular space (16, 43). Therefore, in vivo, the phosphorescence lifetimes are determined directly by O\textsubscript{2} pressure because \( k_Q \) and \( \tau^* \) do not change appreciably over the physiological ranges of temperature and pH (16, 45).

Using computer software (SigmaPlot 11.0, Systat Software, San Jose, CA), PO\textsubscript{2mv} responses were curve-fitted from the collected PO\textsubscript{2mv} data points. Curves were fit using either one-component or two-component models described below:

One component:
\[ PO_{2mv(t)} = PO_{2mv(BL)} - \Delta PO_{2mv} (1 - e^{-(t - TD)/\tau}) \]

Two component:
\[ PO_{2mv(t)} = PO_{2mv(BL)} - \Delta_1 PO_{2mv} (1 - e^{-(t - TD_1)/\tau_1}) + \Delta_2 PO_{2mv} (1 - e^{-(t - TD_2)/\tau_2}) \]

Where \( PO_{2mv(t)} \) represents the PO\textsubscript{2mv} at any given time \( t \), \( PO_{2mv(BL)} \) corresponds to the pre-contracting resting baseline PO\textsubscript{2mv}, \( \Delta_1 \) and \( \Delta_2 \) are the amplitudes for the first and second component, respectively, \( TD_1 \) and \( TD_2 \) are the time delays for each component, and \( \tau_1 \) and \( \tau_2 \) are
the time constants (i.e., time to reach 63% of the final response value) for each component. Appropriate fits were determined using the following criteria: 1) the coefficient of determination, 2) sum of the squared residuals, 3) visual inspection and analysis of the model fits to the data and the residuals. To provide an index of the overall principal kinetics response, the first and second components were used to calculate MRT_1 and MRT_2, respectively. MRT was calculated using the following equations:

One component: MRT_1 = TD_1 + \tau_1

Two component: MRT_2 = TD_2 + \tau_2

Where TD_1, TD_2, \tau_1 and \tau_2 are as described above.

**Statistical Analysis**

Data are presented as means ± SE. Preliminary data were compared between (Superfusion vs. IA Infusion) and within groups using two-way repeated measures ANOVA (MAP) with Tukey’s post hoc analyses. Study results were compared within (Pre- vs. Post-Superfusion) groups using one-way repeated measures ANOVA (MAP and HR) or paired 1- and 2- tail Student’s t-tests as appropriate for a priori directional hypotheses (blood gases, blood [lactate], and PO_2mv kinetics parameters). Significance was accepted at P ≤ 0.05.
Results

Preliminary data (n=3) in Figures 1 and 2 indicated that NaNO₂ (30 mM) during a 3-min superfusion protocol elevated PO₂mv without significantly changing MAP. Bolus intra-arterial (IA) infusion (7 mg kg⁻¹) decreased MAP significantly and transiently decreased PO₂mv within the initial 3-min.

Microvascular Oxygen Pressures (PO₂mv)

Within 3-min superfusion of NaNO₂ PO₂mv was significantly elevated (PO₂mvBL; CON: 28.4 ± 1.1 mmHg vs. NO₂⁻: 31.6 ± 1.2; P ≤ 0.05) (Table 1, Figure 3). Following the onset of contractions, NO₂⁻ exhibited a significantly larger PO₂mv amplitude (CON: 10.5 ± 0.9 vs. NO₂⁻: 12.7 ± 0.8 mmHg; P ≤ 0.05), slower τ (CON: 12.3 ± 1.2 vs. NO₂⁻: 19.7 ± 2.2 s; P ≤ 0.05), and increased MRT (CON: 19.3 ± 1.9 vs. NO₂⁻: 25.6 ± 3.3 s; P ≤ 0.05) (Figure 4A). As shown in Figure 4B, the difference in driving pressure between conditions continued for the first 40 seconds of electrically-stimulated contractions.

Central Hemodynamics and Blood Gases

Superfusion of NaNO₂ did not significantly alter MAP or HR from pre-superfusion values (MAP: 103 ± 4 vs. 105 ± 4 mmHg, HR: 330 ± 10 vs. 321 ± 11 bpm, pre- and post- respectively; both P > 0.05). Likewise, there was no significant difference in MAP or HR between CON and NO₂⁻ conditions following the contraction protocol (P > 0.05). Finally, there were no significant differences in %O₂ saturation (CON: 91.5 ± 1.5 vs. NO₂⁻: 93.1 ± 0.7%), PCO₂ (CON: 42 ± 2 vs. NO₂⁻: 41 ± 2 mmHg), or blood [lactate] (CON: 1.6 ± 0.1 vs. NO₂⁻: 1.6 ± 0.2 mM) between groups (P > 0.05).
Table 1. PO$_2$mv parameters of the spinotrapezius muscle at rest and following the onset of contractions under control and NaNO$_2$ conditions

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>NO$_2^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO$<em>2$mv$</em>{BL}$, mmHg</td>
<td>28.4 ± 1.1</td>
<td>31.6 ± 1.2*</td>
</tr>
<tr>
<td>$\Delta_1$PO$_2$mv, mmHg</td>
<td>10.5 ± 0.9</td>
<td>12.7 ± 0.8*</td>
</tr>
<tr>
<td>PO$<em>2$mv$</em>{\text{(steady-state)}}$, mmHg</td>
<td>18.0 ± 0.9</td>
<td>18.9 ± 1.2</td>
</tr>
<tr>
<td>TD$_1$, s</td>
<td>7.0 ± 1.2</td>
<td>6.0 ± 1.6</td>
</tr>
<tr>
<td>$\tau_1$, s</td>
<td>12.3 ± 1.2</td>
<td>19.7 ± 2.2*</td>
</tr>
<tr>
<td>MRT$_1$, s</td>
<td>19.3 ± 1.9</td>
<td>25.6 ± 3.3*</td>
</tr>
<tr>
<td>$\Delta_2$PO$_2$mv, mmHg</td>
<td>3.4 ± 0.5</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>TD$_2$, s</td>
<td>78.5 ± 22.3</td>
<td>68.9 ± 9.5</td>
</tr>
<tr>
<td>$\tau_2$, s</td>
<td>67.9 ± 7.7</td>
<td>77.1 ± 14.2</td>
</tr>
<tr>
<td>MRT$_2$, s</td>
<td>146.4 ± 25.7</td>
<td>97.3 ± 48.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. PO$_2$mv$_{BL}$, resting PO$_2$mv; $\Delta_1$PO$_2$mv and $\Delta_2$PO$_2$mv amplitude of the first and second components; PO$_2$mv$_{\text{(steady-state)}}$, contracting steady-state PO$_2$mv; TD$_1$ and TD$_2$, time delay of the first and second component; $\tau_1$, and $\tau_2$, time constant of the first and second component; MRT$_1$ and MRT$_2$, mean response time of the first and second component. Primary components were calculated for all rats (n=12). Secondary components were present in 3 CON and 2 NO$_2^-$ rats. *P ≤ 0.05 vs. CON.
Figure 1. NaNO\textsubscript{2} Superfusion vs. Intra-arterial Infusion on PO\textsubscript{2}mv

Averaged PO\textsubscript{2}mv from preliminary results (n=3) during 180 s of NaNO\textsubscript{2} superfusion (30 mM) and a bolus intra-arterial (IA) infusion of NaNO\textsubscript{2} (7 mg kg\textsuperscript{-1}). Inset black box denotes the superfusion period. Measurements were recorded for an additional 600 s to monitor the effects of direct vs. systemic administration in the absence of muscle contraction. Following a plateau in PO\textsubscript{2}mv, there was a decline in PO\textsubscript{2}mv at the rate of -0.012 mmHg s\textsuperscript{-1} in the superfusion condition.
Figure 2. NaNO₂ Superfusion vs. Intra-arterial Infusion on MAP

Preliminary results (n=3) showed that mean arterial pressure (MAP) during 180 s of NaNO₂ superfusion (30 mM) remained constant whereas a bolus intra-arterial (IA) infusion of NaNO₂ (7 mg kg⁻¹) produced a sustained reduction in MAP by as much as ∼14-15 mmHg. Data are presented as means ± SE.

Figure 3. Microvascular oxygen pressure (PO₂mv) during NaNO₂ superfusion

Microvascular oxygen pressure (PO₂mv) of the spinotrapezius muscle for the experimental group of rats (n=12) was measured every 2 s during the 180 s superfusion of 30 mM NaNO₂ in Krebs-Henseleit solution. Inset black box denotes the superfusion period.
Figure 4. PO$_2$mv and MAP during contractions, and PO$_2$mv difference between NaNO$_2$ and Control

A: Average spinotrapezius PO$_2$mv kinetics for the experimental group of rats (n=12) during 180 s electrically stimulated contractions in the control condition (closed circles) and following NaNO$_2$ superfusion (open circles). Mean arterial pressure (closed squares) was recorded at the beginning and end of the contraction protocol. Data are presented as means ± SE. B: PO$_2$mv differences for each 2 s measurement for the experimental group of rats (n=12) between NaNO$_2$ and CON conditions during spinotrapezius muscle contractions. Note that prior to (i.e. left of) the dashed line, NaNO$_2$ is significantly different from CON (P < 0.05).
Discussion

The principal original findings of the present investigation demonstrate that NaNO₂ superfusion enhances the muscle PO₂mv at rest (i.e. elevated PO₂mvBL) and during the transition from rest-to-contractions, in part, by slowing the PO₂mv fall (i.e. increased τ and MRT). Importantly, these effects occurred concomitant with stable MAP. The ability of NO₂⁻ to enhance the driving pressure of O₂ before and for the 40 s after the onset of muscle contractions (Figures 4A and 4B) would be expected to potentially decrease reliance on immediate, nonoxidative energy pathways and delay the production of fatigue-inducing metabolic products (29, 50). In disease conditions attended by cardiovascular complications, the inability to deliver O₂ may induce ischemic damage, impair recovery and, for active skeletal muscle, constrain exercise performance and perhaps daily activities essential to life quality. The delivery of exogenous NO₂⁻ is known to improve the central and systemic derangements prevalent in CHF (5, 37, 42) and we now demonstrate that it rapidly enhances peripheral control directly by circumventing the NOS pathway.

Microvascular Oxygen Pressure (PO₂mv) Dynamics

In skeletal muscle at rest, NaNO₂ has the potential to elevate the driving pressure of O₂ from blood to myocyte (PO₂mvBL) under normoxic conditions. This NOS-independent effect coheres with previous investigations that have administered NO precursors (20, 22, 25). With respect to the PO₂mv elevations, these may be the consequence of increased ŴO₂, decreased ŴO₂, or a combination of both. ŴO₂ increases with increasing [NO₂⁻] because the one step reduction to NO occurs in the low PO₂ environment of resistance vessels in healthy individuals (18, 19). The advantage of the NO₂⁻-NO reduction is that NOS reliance is avoided, rather NO₂⁻ is
reduced to NO in environments of low O₂/pH via interactions with deoxyhemoglobin, tissue and vascular myoglobin, xanthine oxidoreductase, and aldehyde dehydrogenase. Furthermore, by experimental blockade of endogenous production of NO from eNOS and nNOS pharmacologically (L-NAME and SMTC) blood flow is reduced independent of any increases in sympathetic activity/vasoconstriction (10, 27)(9, 10, 27, 28). The present results clearly demonstrate that NaNO₂ increases PO₂mvBL and that the NO₂⁻-NO reduction compliments any NO emanating from the intact NOS system to augment PO₂mv. The direct measurement of PO₂mv herein extends previous observations of NO₂⁻-induced vasorelaxation and increased Q̇O₂ in healthy subjects (11, 12, 38).

Potentially, the increased PO₂mv could also be explained by mitochondrial inhibition (↓VO₂) where NO competitively binds to complexes I and IV of the respiratory chain (6, 7). However, if the primary effect of NO₂⁻ on PO₂mv is mitochondrial inhibition, then a continuous increase in steady-state PO₂mv during muscle contractions would be expected but clearly this is not found (Figure 4A). It is pertinent that using a similar protocol with SNP, Hirai and colleagues (25) demonstrated the integrity of mitochondrial respiration (and thus VO₂) concomitant with elevated NO₂⁻ and NO. Thus, the elevation in baseline PO₂mv seen herein is most likely the consequence of enhanced blood flow and QO₂ rather than decreased VO₂.

The heightened vascular response and increased QO₂ with NaNO₂ superfusion (11, 28, 46) persists into the onset of contractions (slowed PO₂mv kinetics) as demonstrated in Figures 4A and 4B. In Figure 4B the rate at which PO₂mv is falling is slowed by disproportionate changes in QO₂ and VO₂ across the initial 40 seconds. Enhancing the QO₂-to-VO₂ relationship at this crucial transition would potentially improve energy production via oxidative metabolism,
decrease the acidic sequelae of glycolytic pathways, and delay the onset of fatigue processes (29, 50).

**Potential Therapeutic Significance**

NO\textsubscript{2\textsuperscript{-}} precursors such as NO\textsubscript{3\textsuperscript{-}} containing beetroot juice effectively increase plasma [NO\textsubscript{2\textsuperscript{-}}] and can improve exercise performance in healthy individuals (1, 32, 53) and patient populations where cardiovascular function is impaired such as CHF (54) and peripheral artery disease (PAD) (31). Specifically, CHF patients have an increased cardiac output reserve secondary to the reduction of vascular resistance (54). Likewise, PAD patients exhibit improved exercise/walking performance and delayed onset of claudication following beetroot juice supplementation (31). However, elevating circulating [NO\textsubscript{2\textsuperscript{-}}] via dietary means takes 2-3 hours which may constitute too great a delay for an effective therapeutic outcome. A potential alternative is oral administration of NaNO\textsubscript{2} which elevates circulating [NO\textsubscript{2\textsuperscript{-}}] within 30 minutes in older adults (14) and diabetic patients (24). More direct routes of NaNO\textsubscript{2} (i.e. intravascular infusion or oral administration) can bypass the need for bacterial breakdown of NO\textsubscript{3\textsuperscript{-}} and absorption in the gut and increase vascular [NO\textsubscript{2\textsuperscript{-}}] rapidly. Thus, i.v. NO\textsubscript{2\textsuperscript{-}} infusions have improved cardiac function and exercise performance in CHF patients (5). Additionally, NO\textsubscript{2\textsuperscript{-}} infusion during L-NAME induced NOS blockade or CHF (18, 23) reverses the consequences of absent endogenous NO bioavailability and provides an avenue for enhanced ÊO\textsubscript{2\textsuperscript{-}}-ÊV\textsubscript{O\textsuperscript{2}} matching.

Administration of NaNO\textsubscript{2} protects against hypoxic damage to liver, cerebral, and myocardial tissue via the reduction of NO\textsubscript{2\textsuperscript{-}} to NO (17, 30, 47, 49), but possibly not the kidney (3, 48). Therefore topical administration of NaNO\textsubscript{2}, potentially via implantable osmotic micro-pump, may provide tissue protection during acute surgical or other medical conditions by
elevating $\dot{Q}_O$ and $PO_{2mv}$ within 3 minutes of application (see Figure 3) without confounding peripheral vascular effects (i.e. reductions in tissue $PO_{2mv}$ [Figure 1] that are coincident with reductions in MAP [Figure 2]). Local administration may open up the avenue for long-term administration at a desired location enhancing $\dot{Q}_O$ and $PO_{2mv}$ for extended durations. The progressive decay of NO$_2^-$'s effects on $PO_{2mv}$ (Figures 1 and 4B) indicates that setting the required [NaNO$_2$] and timing of application would be crucial.

**Experimental Considerations**

Although $PO_{2mv}$ was significantly different at rest and the beginning of muscle contractions, the effect of NO$_2^-$ is largely diminished by the time the steady-state $PO_{2mv}$ is reached (Figure 4A and 4B). It remains unclear whether this is a direct effect of NO$_2^-$ wearing off irrespective of muscle contractions, or whether contractions and the attendant hypoxia are elevating NO$_2^-$ utilization. Figure 1 demonstrates that in the absence of muscle contractions, $PO_{2mv}$ plateaus and then declines at a rate of $\sim$0.7 mmHg min$^{-1}$ after superfusion has ceased. This would indicate, in part, why NaNO$_2$ superfusion does not enhance steady-state $PO_{2mv}$ relative to the control condition. Importantly, the linear decay in $PO_{2mv}$ sheds light on the utilization of NO$_2^-$ in the absence of systemic circulation which continually delivers NO$_2^-$ stores. Future investigations into various delivery methods of NaNO$_2$ (i.e. injectable pellets or cutaneous patches) may be warranted considering that the administration of NO$_2^-$ augments $PO_{2mv}$ at rest and during the rest-exercise transition.

**Conclusion**

NaNO$_2$ serves therapeutically as a hypoxic vasodilator with efficacy for improving exercise performance in patients with cardiovascular disease (1, 5, 36, 41, 42). The present
investigation demonstrates the ability of NaNO₂ to locally elevate skeletal muscle PO₂ mv at rest and following the onset of contractions in the healthy rat without altering MAP. Enhancing the muscle vascular O₂ driving pressure via NaNO₂ would provide a fast-acting modality to potentially improve metabolic control and thus delay fatigue and/or hypoxic damage. Improving PO₂ mv dynamics alongside what remains of the endogenous NOS system under these conditions, NaNO₂ may ameliorate perturbations in the ŌO₂-to-VO₂ ratio commonly found in disease states such as CHF and PAD. Fast-acting improvements in both resting and exercise PO₂ mv dynamics may add to the current standard of care in populations with limited exercise tolerance along with individuals that may be suffering from focal tissue hypoxia and/or ischemia (i.e. frostbite, PAD, stroke).
References


39. McDonagh STJ, Wylie LJ, Winyard PG, Vanhatalo A, Jones AM. The Effects of


