Effect of L-NAME on oxygen uptake kinetics during heavy-intensity exercise in the horse

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Received 8 December 2000; accepted in final form 15 March 2001

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Kindig, Casey A., Paul McDonough, Howard H. Erickson, and David C. Poole. Effect of L-NAME on oxygen uptake kinetics during heavy-intensity exercise in the horse. J Appl Physiol 91: 891–896, 2001.—There is evidence that oxidative enzyme inertia plays a major role in limiting/setting the O_2 uptake (V_O_2) response at the transition to higher metabolic rates and also that nitric oxide (NO) competitively inhibits V_O_2 within the electron transport chain. To investigate whether NO is important in setting the dynamic response of V_O_2 at the onset of high-intensity (heavy-domain) running in horses, five geldings were run on a treadmill across speed transitions from 3 m/s to speeds corresponding to 80% of peak V_O_2 with and without nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor (20 mg/kg; order randomized). L-NAME did not alter (both P > 0.05) baseline (3 m/s, 15.4 ± 0.3 and 16.2 ± 0.5 l/min for control and L-NAME, respectively) or end-exercise V_O_2 (56.9 ± 5.1 and 55.2 ± 5.8 l/min for control and L-NAME, respectively). However, in the L-NAME trial, the primary on-kinetic response was significantly (P < 0.05) faster (i.e., reduced time constant, 27.0 ± 2.7 and 18.7 ± 3.0 s for control and L-NAME, respectively), despite no change in the gain of V_O_2 (P > 0.05). The faster on-kinetic response was confirmed independent of modeling by reduced time to 50, 63, and 75% of overall V_O_2 response (all P < 0.05). In addition, onset of the V_O_2 slow component occurred earlier (124.6 ± 11.2 and 65.0 ± 6.6 s for control and L-NAME, respectively), and the magnitude of the O_2 deficit was attenuated (both P < 0.05) in the L-NAME compared with the control trial. Acceleration of the V_O_2 kinetics by L-NAME suggests that NO inhibition of mitochondrial V_O_2 may contribute, in part, to the intrinsic metabolic inertia evidenced at the transition to higher metabolic rates in the horse.

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Attain maximal levels of V_O_2 nearing 200 ml·kg⁻¹·min⁻¹ places a heavy burden on the equine cardiovascular system to match adequately O_2 delivery to metabolic demand, and thus the horse offers an exciting and relevant model in which to study the V_O_2 kinetic response to exercise.

Two mechanisms are thought to limit V_O_2 on-kinetics: 1) the rapidity with which the mitochondria adjust oxidative ATP supply to demand (oxidative enzyme inertia hypothesis) (7, 13, 14, 19, 43) or, alternatively, 2) the rate of O_2 delivery to working muscle (O_2 delivery hypothesis) (12, 15, 28; for review see Ref. 42). Although O_2 delivery is generally not considered to be a limiting factor in response to aerobic work performed below the lactate threshold (i.e., limiting factors reside primarily within the inertia of the oxidative enzymes), there is clear evidence that perturbations expected to alter O_2 delivery during the transition to heavy-domain exercise may result in similarly altered V_O_2 on-kinetic responses (12, 15, 28).

Nitric oxide (NO), synthesized from L-arginine and O_2 in a reaction catalyzed by NO synthase (NOS), has been implicated in a myriad of biological functions (6, 10, 21, 38); however, its ability to reduce vascular smooth muscle tone has been most widely documented (for review see Ref. 21). Although not unequivocal (35, 40), NOS inhibition by L-arginine analogs has been shown to reduce skeletal muscle blood flow in response to exercise in humans (9), rats (18), and dogs (38). Thus, if nitro-L-arginine methyl ester (L-NAME) reduces O_2 delivery significantly at the trot-to-gallop transition, the rapid V_O_2 on-kinetic profile characteristic of the horse may be slowed.

In contrast to the role of NO in facilitating O_2 transport to skeletal muscle, recent studies have demonstrated that NO may regulate mitochondrial function through competitive inhibition of V_O_2 in the electron transport chain, specifically at cytochrome c oxidase (6, 37). Thus NO may serve as part of a feedback mechanism to reduce the reliance on O_2 extraction to meet tissue O_2 needs, and this would serve to maintain higher intramyocyte P_O_2 levels during exercise (38). Indeed, concomitant with a reduced cardiac output,
NOS blockade resulted in an elevated fractional O₂ extraction at peak exercise compared with control conditions in the horse (23). In this scenario, NOS blockade acted to reduce whole body O₂ delivery while simultaneously relieving the NO inhibition of mitochondrial function. Both of these mechanisms will have contributed to the increased fractional O₂ extraction observed. From the evidence presented above, it is feasible that NO inhibition of mitochondrial V˙O₂ may contribute to an intrinsic oxidative enzyme inertia at exercise onset.

The purpose of the present investigation was to determine the V˙O₂ kinetic profile in the horse during heavy exercise under control and NOS inhibition (by l-NAME) conditions. We hypothesized that if inhibition of mitochondrial function by NO across the on-transient plays a deterministic role in setting the V˙O₂ on-kinetic response, alleviation of this inhibition by l-NAME will speed V˙O₂ on-kinetics. Alternatively, l-NAME may reduce muscle blood flow to such a degree that V˙O₂ on-kinetics are slowed because of the decreased O₂ delivery. In this instance, any improvement in mitochondrial function would be masked.

METHODS

Five geldings (4 Thoroughbreds and 1 quarter horse, 547 ± 21 kg, 10 ± 2 yr) acclimatized to running on a treadmill (conditioned twice weekly) were housed in a dry lot (loafing shed with paddock) and given unrestricted access to water and fed twice daily. Food was withheld ≥4 h before each experimental session. All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Initially, peak V˙O₂ (V˙O₂ peak) was assessed 2 wk before this investigation [V˙O₂ and CO₂ output (V˙CO₂), obtained by open-flow system as described below]. The protocol consisted of an 800-m trot at 3 m/s followed by a 1 m/s per 1-min interval incremental ramp (starting at 7 m/s) performed on a level equine treadmill (SATO, Uppsala, Sweden) to volitional fatigue.

Experimental Protocol

Each horse performed one control and one l-NAME exercise trial (order randomized; 2 wk between runs). Before both runs, the temperature of the equine laboratory was lowered to ~11°C (relative humidity ~55%), and two overhead fans were used to counteract the horse’s inability to thermoregulate after NOS inhibition (30). After each horse was led onto the treadmill, l-NAME (20 mg/kg in 180 ml of sterile saline) or sterile saline (180 ml) was infused intravenously over a 4-min period. At 3 min after l-NAME (or saline) infusion, the exercise protocol was initiated. The protocol, which began and ended with an 800-m trot at 3 m/s, consisted of two constant-speed (i.e., increase to the required speed in <10 s) exercise bouts separated by a 4-min recovery trot at 3 m/s. These exercise bouts consisted of an initial 4-min run at 8–10 m/s (warm-up at ~50% V˙O₂ peak) followed by a 6-min run at the speed corresponding to 80% of control V˙O₂ peak (experimental run).

Respiratory Gas Measurement

Measurements of V˙O₂ and V˙CO₂ (STPD corrected) were obtained as described previously (26) using an open-flow system. Briefly, a loose-fitting mask was placed over the horse’s muzzle while industrial fans drew air past the mask at a flow rate >7,500 l/min, such that no end-expired air escaped. Flow through the system was calculated by measurement of pressure differences (differential pressure transducer, model CD1-3-871, Validyne, Northridge, CA) across a flow nozzle (standard 2 in ASME). Concentrations of O₂ and CO₂ (mass spectrometer, model 1100, Perkin-Elmer, Pomona, CA) as well as temperature and relative humidity (model HS-ZCHDT-2R, Thunder Scientific, Albuquerque, NM) were measured distal to the flow nozzle and recorded (100-Hz sample rate) by means of a computer-based data acquisition system (DATAQ, Akron, OH).

Kinetic Modeling/Analysis

The open-flow system utilized does not permit measurement of tidal volumes or assessment of changes in lung gas stores; however, evidence to date (17) suggests that functional residual capacity is not altered in ponies from rest to exercise. Values for V˙O₂ were smoothed using a 4-s rolling average. Data were then analyzed by means of Kaleidograph data analysis software (Synergy Software, Reading, PA), using a one- and two-exponential model of the following forms

\[ \dot{V}O_2(t) = \dot{V}O_2(b) + A_1 \cdot [1 - e^{-t/\tau_{D1}}] \]

and

\[ \dot{V}O_2(t) = \dot{V}O_2(b) + A_1 \cdot [1 - e^{-t/\tau_{D1}}] + A_2 \cdot [1 - e^{-t/\tau_{D2}}] \]

where \( t \) is time, \( b \) is baseline (trotting at 3 m/s), \( A_1 \) and \( A_2 \) are the response amplitudes, \( \tau_1 \) and \( \tau_2 \) are the independent time delays, and \( \tau_1 \) and \( \tau_2 \) are the time constants. In all cases, a significantly improved fit to the data was found for the two-component model. In addition, time to 50, 63, 75, and 95% of the overall amplitude was determined independent of modeling procedures.

O₂ deficit was calculated in two ways. First, it was calculated in the traditional manner as the area above the V˙O₂ curve and below a horizontal line drawn from the end-exercise asymptotic value. The second method was derived from that presented by Bearden and Moffatt (5) and calculated O₂ deficit in the traditional manner less the area represented by \( A_2 \cdot \tau_2 \).

Statistical Analysis

Values are means ± SE. Differences between the l-NAME and control trials were tested by Student’s paired \( t \)-test. Statistical significance was accepted at \( P \leq 0.05 \).

RESULTS

V˙O₂ peak (initial incremental protocol) averaged 62.2 ± 5.0 l/min. The speed that corresponded to 80% V˙O₂ peak was 11.9 ± 0.5 m/s and yielded end-exercise (control) V˙O₂ values of 55.9 ± 5.1 l/min (89.5 ± 3.3% V˙O₂ peak measured on the initial incremental protocol). The V˙O₂ on-kinetic parameters for the control and l-NAME runs are shown in Table 1 and represented graphically in Fig. 1. The mean response for all five horses (2-s average) is shown in Fig. 1, top. Figure 1, bottom, depicts a common logarithmic plot of the V˙O₂ difference at time \( t \) (4-s average) from its asymptotic (or end-exercise) value. Although l-NAME did not affect V˙O₂ during the warm-up at 3 m/s or at end exercise, the primary on-kinetic response was significantly

\[ J \text{ Appl Physiol} \bullet \text{ VOL 91} \bullet \text{ AUGUST 2001} \bullet \text{ www.jap.org} \]
faster (i.e., reduced $\tau_1; P < 0.05$), with no change in the amplitude of the response ($A_1$). The reduced $\tau_1$ was confirmed, independent of time delay and monoeponential curve modeling, by a significant reduction ($P < 0.05$) in time to 50, 63, and 75% of the overall $V_\dot{O}_2$ amplitude (however, time to 95% was not statistically different; Fig. 2). The onset of the slow component (i.e., $TD_2$) occurred significantly sooner ($P < 0.05$) in the L-NAME than in the control trial. Furthermore, L-NAME significantly ($P < 0.05$) attenuated the magnitude of the $O_2$ deficit irrespective of the model used (see METHODS) for its calculation (Fig. 3). In Fig. 1 (bottom) the L-NAME-induced reduction in $O_2$ deficit is represented by the area between the control and L-NAME points [i.e., ($control - L$-NAME)] as a function of time.

The effect of L-NAME on the $VCO_2$ on-kinetic response was qualitatively similar to the effect of L-NAME on the $V_\dot{O}_2$ response (Table 2).

Table 1. $V_\dot{O}_2$ response and related kinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>L-NAME</th>
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</thead>
<tbody>
<tr>
<td>$V_\dot{O}_2$, l/min</td>
<td>15.4 ± 0.3</td>
<td>16.2 ± 0.5</td>
</tr>
<tr>
<td>Baseline (3 m/s)</td>
<td>56.9 ± 5.1</td>
<td>55.2 ± 5.8</td>
</tr>
<tr>
<td>End exercise</td>
<td>37.1 ± 5.4</td>
<td>33.7 ± 3.9</td>
</tr>
<tr>
<td>$A_2$, l/min</td>
<td>4.5 ± 1.9</td>
<td>5.3 ± 1.8</td>
</tr>
<tr>
<td>$\tau_1$, s</td>
<td>27.0 ± 2.7</td>
<td>18.7 ± 3.0</td>
</tr>
<tr>
<td>$\tau_2$, s</td>
<td>52.6 ± 8.4</td>
<td>60.7 ± 11.3</td>
</tr>
<tr>
<td>$TD_1$, s</td>
<td>6.7 ± 3.4</td>
<td>9.4 ± 1.9</td>
</tr>
<tr>
<td>$TD_2$, s</td>
<td>124.6 ± 11.2</td>
<td>65.0 ± 6.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 5$. $V_\dot{O}_2$, $O_2$ uptake; $A_1$ and $A_2$, response amplitudes; $TD_1$ and $TD_2$, time delays; $\tau_1$ and $\tau_2$, time constants; L-NAME, N-methyl-L-arginine methyl ester. *Significantly different ($P < 0.05$) from control.

Fig. 1. Top: mean response (2-s mean; SE bars omitted for clarity) for all 5 horses. Solid lines fit the mean primary on-kinetic parameters as presented in Table 1. Bottom: common logarithmic plot of $O_2$ uptake ($V_\dot{O}_2$) difference at time $t$ (4-s average) from its asymptotic value (44). ss, Steady state. Solid lines are consistent with those at top and thus demonstrate the significant acceleration of the primary fast on-kinetic response in the nitro-L-arginine methyl ester (L-NAME) compared with the control trial.

Fig. 2. Time from baseline $V_\dot{O}_2$ (at 3 m/s) to 50, 63, 75, and 95% of the overall $V_\dot{O}_2$ amplitude as determined independent of modeling procedures. Time to 50% ($T_{50}$), 63% ($T_{63}$), and 75% ($T_{75}$) was significantly reduced (*$P < 0.05$) for L-NAME compared with control, confirming the findings of the 2-component model (i.e., reduced $\tau_1$). There was no significant difference (NS) in time to 95% ($T_{95}$).

Fig. 3. $O_2$ deficit was significantly less (*$P < 0.05$) in the L-NAME than in the control run whether calculated in the traditional manner as the area above the curve and below the end-exercise asymptotic value or by a second method (5), which calculates $O_2$ deficit in the traditional manner less the area represented by ($A_2 \times TD_2$, where $A_2$ is slow component response amplitude and $TD_2$ is time delay for the slow component).
Mechanism for Altered $\dot{V}_{\text{O}_2}$ Kinetic Profile

$O_2$ delivery. It is generally accepted (although not unequivocally so) that $NO$ plays a modest role in skeletal muscle exercise hyperemia (21). Thus, although some investigators have reported that $NO$ inhibition does not reduce blood flow in exercising human muscle (35, 40), other studies in humans (9) and also in rats (18) and dogs (38) demonstrated significant reductions in the exercise hyperemic response when subjects/animals were challenged with $NO$ inhibition. In the present investigation, skeletal muscle blood flow was not measured. However, $\dot{V}_{\text{O}_2}$ does significantly reduce cardiac output (274 ± 23 and 242 ± 20 l/min for control and $\dot{V}_{\text{O}_2}$, respectively, $P < 0.05$) (23) and concomitantly increases fractional $O_2$ extraction during heavy-domain exercise in the horse, suggesting that skeletal muscle blood flow may be reduced by $\dot{V}_{\text{O}_2}$.

At the onset of exercise or increased work rates in humans, there is strong evidence that increases in muscle blood flow precede increases in $\dot{V}_{\text{O}_2}$ (45) and that muscle $O_2$ fractional extraction falls, such that venous effluent $O_2$ content rises (albeit transiently (10–15 s)) (16). During work consistent with moderate-intensity exercise in the dog gastrocnemius muscle, neither elevated $O_2$ delivery (13) nor a rightward-shifted $O_2$ dissociation curve (designed to enhance hemoglobin $O_2$ off-loading) (14) speeds $\dot{V}_{\text{O}_2}$ kinetics. However, in a recent investigation, Grassi and colleagues (15) determined that increasing blood flow to the isolated canine gastrocnemius muscle before electrically stimulated contractions at near-maximal values resulted in faster muscle $\dot{V}_{\text{O}_2}$-on kinetics. This study, in combination with the work of others (12, 25), suggests that if $O_2$ delivery was reduced by $\dot{V}_{\text{O}_2}$, then a resultant slowing of $\dot{V}_{\text{O}_2}$ dynamics may occur during heavy-domain exercise. However, if this effect was present, it must have been overcome by the inhibition of oxidative enzyme function (inertia), and the result was a net acceleration of the $\dot{V}_{\text{O}_2}$ on-kinetic response (see below).

Oxidative enzyme inertia. Many experiments have supported the notion that the site of control of $\dot{V}_{\text{O}_2}$ kinetics is located within the muscle (7, 13, 14, 19, 43), and this has been corroborated recently using a frog myocyte preparation (19). Specifically, Hogan (19) demonstrated that intramyocyte $O_2$ did not fall immediately at the onset of contractions. Rather, there was a delay of ~13 s followed by a monoexponential decline to the steady state, suggesting that a delay in $\dot{V}_{\text{O}_2}$ at the mitochondrial level is responsible, in part, for the delayed $\dot{V}_{\text{O}_2}$ kinetics. Given that there is strong evidence implicating intramuscular factors in the control (or limitation) of $\dot{V}_{\text{O}_2}$ kinetics, numerous mechanisms could achieve this control. Potential mechanisms include phosphorylation state, redox potential, pyruvate dehydrogenase (PDH) activation, mitochondrial Ca$^{2+}$ transients, and mitochondrial $O_2$. Although there is some suggestive evidence that dichloroacetate activation of PDH may speed $\dot{V}_{\text{O}_2}$ kinetics at exercise onset in humans (20, 41), rigorous dissection of the relative importance of each of the potential controllers listed previously must await future investigation. Notwithstanding the factors described above, the $\dot{V}_{\text{O}_2}$ condition did elevate core blood temperature ~0.6 ± 0.1°C above that found in the control condition (23). Elevated temperature can speed the rate of chemical reactions. However, there is solid evidence that hyperthermia does not increase the speed of $\dot{V}_{\text{O}_2}$ kinetics in humans performing exercise that is moderate (25) or severe (32) in intensity.

There are at least three possible mechanisms by which $NO$ inhibition may reduce the intrinsic metabolic inertia at the on-transient to higher metabolic rates. First, it has been reported that $NO$ inhibits creatine kinase (22). Thus the removal of this inhibition may speed phosphocreatine hydrolysis and ATP...
turnover and, in turn, increase $\dot{V}O_2$ more rapidly. Second, Howlett et al. (20) demonstrated an increased contribution of oxidative phosphorylation to ATP demand at exercise onset by means of PDH activation by dichloroacetate. Given that NO-mediated damage of PDH has been described in heart mitochondria during postischemia reperfusion (1), the possibility exists that NO may inhibit PDH activation at exercise onset. Finally, as described in the introduction, it is well documented that NO inhibits $\dot{V}O_2$ by competitive inhibition of cytochrome c oxidase in the electron transport chain (6, 37, 38). Removal of this inhibition may serve to accelerate mitochondrial $O_2$ flux and thus speed the $\dot{V}O_2$ on-kinetic response.

**Alleviation of NO Inhibition on Net $\dot{V}O_2$**

In the present investigation, whole body $O_2$ cost did not differ between L-NAME and control trials (Table 1, Fig. 1). This finding is in agreement with other studies in which NOS inhibition did not alter whole body $\dot{V}O_2$ in exercising horses (31) and humans (35) and dogs at rest (8). However, some investigations have reported an elevated $\dot{V}O_2$ in canine skeletal muscle at rest (24) and during exercise (38) after NOS inhibition. In the former study, whole body $\dot{V}O_2$ remained unchanged (24). If alterations in net muscle $\dot{V}O_2$ occurred in this investigation, this would have been manifested in the pulmonary $\dot{V}O_2$ values, given that, during near-maximal exercise, skeletal muscle receives the bulk of cardiac output (29). Furthermore, unless NO reduces mechanical or oxidative enzyme efficiency, increases muscle fiber recruitment, and/or decreases anaerobic contributions to aerobic steady-state exercise (which are thought to be minimal at best), no readily available explanation exists for an increased steady-state $\dot{V}O_2$ under L-NAME conditions.

**Mechanisms for Early Onset of $\dot{V}O_2$ Slow Component With L-NAME**

It is not known what precise mechanism(s) results in the “excess” $O_2$ cost or $\dot{V}O_2$ slow component associated with sustained work performed above the lactate threshold, although the origin appears to be primarily within the working muscle (11, 34). In contrast to the magnitude of the slow component, relatively little attention has been afforded to the temporal displacement of the slow component (i.e., TD4) from the on-transition to higher metabolic rates (4, 27, 33).

To our knowledge, there is no evidence to suggest that the muscle mass recruited to perform a given workload is altered by NOS inhibition. Nevertheless, the likelihood does exist that a population of muscle fibers may fatigue more rapidly if their $O_2$ delivery is reduced. Neither the primary ($A_1$) nor the slow component ($A_2$) $\dot{V}O_2$ amplitudes were significantly different between conditions. This suggests that L-NAME does not cause substantial changes in muscle recruitment. Moreover, if less-economical type IIb fibers were recruited earlier during the L-NAME condition, a reduction in $A_1$ and an increase in $A_2$ with no change in the time of onset (i.e., TD2) of the $\dot{V}O_2$ slow component might be expected (3). Furthermore, recent analysis of electromyogram frequency and integrated electromyogram in concert with $\dot{V}O_2$ determination during heavy-domain cycle ergometry demonstrated no correlation between the slow component and altered muscle recruitment patterns (36), in contrast to previous work (39).

As discussed above, evidence presented previously (9, 18, 23, 38) suggests that muscle blood flow was likely to be reduced during exercise in the L-NAME condition; however, this variable was not measured in the present investigation. Also it is not known to what degree the matching of $O_2$ delivery to $\dot{V}O_2$ requirement may have been altered. In this regard, there was a trend toward elevated venous plasma lactate with L-NAME, but this was not statistically different between trials (11.7 ± 4.1 and 13.7 ± 3.4 mM for control and L-NAME, respectively) (23). Irrespective of the precise mechanism responsible, we believe that the present investigation is the first to demonstrate an experimental condition that results in an acceleration of the onset of the $\dot{V}O_2$ slow component and suggests that some component of NO control of mitochondrial function may be deterministic in setting the onset of the slow component.

To our knowledge, this is the first investigation to quantify the $\dot{V}O_2$ on-kinetic response in the exercising horse under control and NOS-inhibited (by L-NAME) conditions. Although L-NAME did not affect the primary component or slow component amplitude, the primary $\dot{V}O_2$ response increased at a greater rate and the $\dot{V}O_2$ slow component onset occurred earlier in horses performing exercise in the heavy domain. Regardless of the effect of L-NAME on muscle blood flow, these data suggest that NO may be responsible, in part, for the initial intrinsic metabolic inertia seen at exercise onset and that this effect may be mediated by inhibition of mitochondrial $\dot{V}O_2$.

The authors thank Mark Brentano and Holly Brown for help with data acquisition. This investigation was supported in part by grants from the American Quarter Horse Association and the Kansas Racing Commission and National Heart, Lung, and Blood Institute Grant HL-50306.

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