Nitric oxide synthase inhibition does not affect the exercise-induced arterial hypoxemia in Thoroughbred horses

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Manohar, Murli, Thomas E. Goetz, and Aslam S. Hassan. Nitric oxide synthase inhibition does not affect the exercise-induced arterial hypoxemia in Thoroughbred horses. J Appl Physiol 91: 1105–1112, 2001.—Because sensitivity of equine pulmonary vasculature to endogenous as well as exogenous nitric oxide (NO) has been demonstrated, we examined whether endogenous NO production plays a role in exercise-induced arterial hypoxemia. We hypothesized that inhibition of NO synthase may alter the distribution of ventilation-perfusion mismatching, which may affect the exercise-induced arterial hypoxemia. Arterial blood-gas variables were examined in seven healthy, sound Thoroughbred horses at rest and during incremental exercise protocol leading to galloping at maximal heart rate without (control; placebo = saline) and with Nω-nitro-L-arginine methyl ester (L-NAME) administration (20 mg/kg iv). The experiments were conducted in random order, 7 days apart. At rest, L-NAME administration caused systemic hypertension, pulmonary hypertension, and bradycardia. During 120 s of galloping at maximal heart rate, significant arterial hypoxemia, desaturation of hemoglobin, hypercapnia, hyperthermia, and acidosis occurred in the control as well as in NO synthase inhibition experiments. However, statistically significant differences between the treatments were not found. In both treatments, exercise caused a significant rise in hemoglobin concentration, but the increment was significantly attenuated in the NO synthase inhibition experiments, and, therefore, arterial O2 content (CaO2) increased to significantly lower values. These data suggest that, whereas L-NAME administration does not affect pulmonary gas exchange in exercising horses, it may affect splenic contraction, which via an attenuation of the rise in hemoglobin concentration and CaO2 may limit performance at higher workloads.

arterial blood-gas tensions in exercise; Nω-nitro-L-arginine methyl ester; exertion

THE OCCURRENCE OF ARTERIAL hypoxemia in strenuously exercising horses has been well documented (1, 2, 4, 30, 32), and it is reported that the occurrence of arterial hypoxemia poses a limit to exercise performance (9, 31). Although “relative” alveolar hypoventilation, as evidenced by the increasing arterial CO2 tension (PaCO2) during strenuous exercise, contributes to the observed reduction in arterial O2 tension (PaO2; 2), this mechanism usually does not account for the entire decrease in PaO2 observed in exercising horses (12). Thus it is often suggested that diffusion limitation probably related to the dramatic shortening of the pulmonary capillary transit time as well as ventilation-perfusion inhomogeneity play a role in the exercise-induced arterial hypoxemia in horses (1, 2, 4, 9, 12, 30–32). Exercising horses exhibit significant pulmonary arterial, capillary, and venous hypertension (13–17), and the ensuing high transmural pulmonary capillary pressures contribute to the stress failure of pulmonary capillaries, resulting in exercise-induced pulmonary hemorrhage (EIPH; 33). Although the role of exercise-induced structural changes in the blood-gas barrier (8, 25, 33) in causing exercise-induced arterial hypoxemia continues to be a contentious issue, it has been reported in healthy human subjects (3, 27) and Thoroughbred horses (18) that a successive bout of high-intensity exercise, performed soon after the first high-intensity exercise bout, did not accentuate the arterial hypoxemia. Inference from these reports (3, 7, 18, 27) is that the exercise-induced structural changes in the blood-gas barrier (8, 25, 33) may not contribute to the exercise-induced arterial hypoxemia. Thus, despite considerable interest, uncertainty exists as to the precise mechanism(s) responsible for the exercise-induced arterial hypoxemia in the horse.

Nitric oxide (NO) is an endogenous vasodilator substance that is reported to play a significant role in modulating the pulmonary vascular tone in resting healthy animals and humans (5). Although the sensitivity of equine pulmonary vasculature to endogenous as well as exogenous NO has been demonstrated at rest (15, 16), the role of NO in modulating the equine pulmonary vascular tone during exercise is uncertain. However, it has been reported that NO synthase inhibition with Nω-nitro-L-arginine methyl ester (L-NAME) caused an intensification of the exercise-induced pulmonary arterial hypertension in horses (22) and that exogenous NO inhalation caused a significant reduction in the severity of exercise-induced pulmonary hypertension in horses (21). Despite the possibility that NO may modulate the equine pulmonary vascular tone
at rest (15, 16) and during exercise (21, 22), only recently has attention been directed at examining the effects of NO on arterial blood-gas tensions during exercise (6, 10, 26). In horses, L-NAME administration to cause NO synthase inhibition significantly decreased PaCO2 during exercise at 50 and 80% maximal O2 consumption (V02 max), whereas PaO2 remained unchanged (10), indicating that endogenous NO production plays a role in pulmonary gas exchange of exercising horses. Recently, it was also reported that increased endogenous NO production (through administration of L-arginine) enhances aerobic exercise capacity (20). In view of the above reports (10, 20), our objective in the present study was to ascertain whether endogenous NO production plays a role in exercise-induced arterial hypoxemia in horses. We hypothesized that inhibition of NO synthase with L-NAME may alter the distribution of ventilation-perfusion mismatching within the lungs, which may affect the exercise-induced arterial hypoxemia. Toward this goal, we examined changes in arterial blood-gas tensions in healthy horses performing an incremental exercise protocol leading to galloping at maximal heart rate without (control) and with L-NAME administration. In the present study, L-NAME was infused intravenously at 20 mg/kg, a dose known to cause significant inhibition of NO synthase in healthy horses (10, 15, 22, 23).

**MATERIALS AND METHODS**

**Horses**

Experiments were carried out on seven healthy, sound Thoroughbred horses (2 fillies, 5 geldings), 2.5–5 yr old and weighing 466 ± 14 kg. The horses were housed in an air-conditioned building and were accustomed to being handled by people. They were fed a diet of alfalfa hay and oats, and free access to water was provided. The horses were dewormed periodically and were inoculated with tetanus toxoid and strangles vaccine. Our protocols and procedures were randomized for all horses, and 7 days were allowed before experiments. All experiments were carried out in an air-conditioned laboratory, where ambient temperature was maintained at 20°C.

**Exercise Training**

All horses were exercise trained for a period of 7 wk before undertaking the blood-gas studies. Our exercise training regimen has been described in detail previously (14–18).

**Work Intensity Eliciting Maximal Heart Rate**

Trials to ascertain work intensity needed to elicit maximal heart rate were undertaken on completion of exercise training (see above). It was observed that galloping at 14 m/s on a 3.5% uphill grade not only elicited maximal heart rate (217 ± 2 beats/min) but also induced EIPH in all horses, as demonstrated by the presence of fresh blood in the trachea on airway endoscopic examination (11, 29). These trials also revealed that our horses could not sustain galloping at 14 m/s on a 3.5% uphill grade for >120 s despite vigorous humane encouragement. Thus, for the present study, this workload, i.e., 14 m/s on a 3.5% uphill grade, was selected because it represented a strenuous effort capable of eliciting maximal heart rate and inducing EIPH consistently.

**Experimental Procedures**

Our procedures for hemodynamic and pulmonary gas-exchange studies have been described in detail previously (13–19, 24); therefore, only a brief description is given here. On the day of the study, after local infiltration of 2% lidocaine HCl in the 17th intercostal space, the abdominal aorta was percutaneously catheterized as described previously (19, 24). Thereafter, using local infiltration of 2% lidocaine HCl, cardiac catheters (8F) equipped with tip manometer (Millar Instruments, Houston, TX), fluid-filled lumen, and a thermistor (Edward Laboratories, Santa Clara, CA) were advanced into the pulmonary artery via introducers inserted into the left jugular vein. The location of various catheters was confirmed by monitoring the characteristic phasic blood pressure waveforms on an oscillographic recorder (E for M, Lanexa, KS), and all pressure signals were referenced at the point of the left shoulder. These catheters permitted sampling of the aortic blood and continuous monitoring of the pulmonary arterial blood (core) temperature during the experiments. After catheter placement, horses stood quietly on the treadmill for ∼45–50 min before blood-gas studies were undertaken.

Blood-gas tensions, arterial pH (pHa), hemoglobin concentration, arterial hemoglobin O2 saturation (SaO2), and O2 content (CaO2) were determined using a carefully calibrated blood-gas analyzer/CO-oximeter (ABL520 system, Radiometer, Copenhagen, Denmark), and all blood-gas tensions/pH data were corrected to the simultaneously measured pulmonary artery blood temperature. The calibration of our blood-gas/pH analyzer/CO-oximeter was checked frequently (at 30-min intervals) and was verified using tonometered solutions of known blood-gas tensions, pH, hemoglobin concentration, and O2 saturation.

**Experimental Design and Protocol**

All horses were studied in the control (placebo) as well as the L-NAME experiments. The sequence of these treatments was randomized for all horses, and 7 days were allowed between experiments. All experimentation was carried out in an air-conditioned laboratory, where ambient temperature was maintained at 20°C.

**Control (placebo) study.** Measurements were first made on quietly standing horses when heart rate and the aortic and pulmonary arterial blood pressures had been stable for ∼10 min (rest 1). Thereafter, physiological saline (250 ml of 0.9% NaCl) was infused intravenously over a period of 5 min via the side port of the introducer used for advancing the pulmonary arterial blood temperature. The calibration of our blood-gas/pH analyzer/CO-oximeter was checked frequently (at 30-min intervals) and was verified using tonometered solutions of known blood-gas tensions, pH, hemoglobin concentration, and O2 saturation.

**Experimental (L-NAME) study.** After the horses had trotted for 60 s at 6 m/s, the belt speed was raised in increments of 1 m/s every 60 s until the speed was 6 m/s. After the horses had trotted for 60 s at 6 m/s, belt speed was raised to 8 m/s for 60 s and then to 14 m/s. On completion of 120 s of galloping at 14 m/s on a 3.5% uphill grade, the belt speed was decreased to 5 m/s (trot) for 60 s and then to 2 m/s (walk) for 5 min before the treadmill was stopped.

In this exercise protocol, along with continuous core temperature measurement, aortic blood samples were obtained for determining PaCO2, PaO2, pH, hemoglobin concentration, SaO2, and CaO2 at 55 s of trotting at 6 m/s; at 55 s of exercise at 8 m/s; at 30, 60, 90, and 120 s of galloping at 14 m/s on a 3.5% uphill grade; and at 2 min of walk at 2 m/s (recovery).
L-NAME study. In these experiments, measurements were first made on quietly standing horses (without any drugs) when heart rate and the aortic and pulmonary arterial blood pressures had been stable for ~10 min (rest 1). Thereafter, a freshly prepared solution of L-NAME (20 mg/kg; Sigma Chemical, St. Louis, MO) in 250 ml of physiological saline was infused intravenously over 5 min in exactly the same manner as described above for the saline injection in the control study. Several reports have documented the efficacy of this dosage in inhibiting NO synthase in horses (10, 15, 22, 23). Postinfusion resting measurements (rest 2) were then made in quietly standing horses during the 9th and 10th min after administration of L-NAME. Exercise was initiated in the 11th min after L-NAME administration and was performed on the high-speed treadmill set at a 3.5% uphill grade exactly as described above for the control study. Along with continuous measurement of core temperature, PaO_2, PaCO_2, pH, hemoglobin concentration, SaO_2, and CaO_2 were determined at exactly the same intervals as described above for the control experiments.

Immediately after the treadmill was stopped, L-arginine (Sigma Chemical) was administered intravenously at 200 mg/kg to reverse the effects of L-NAME (15, 22, 23). For each experiment, this dose of L-arginine was dissolved in 500 ml of physiological saline.

Postexercise Airway Endoscopic Examination
In all experiments, using a flexible fiber-optic endoscope (Pentax Fiberscopes, Orangeburg, NY), careful endoscopic examination of the nasopharynx, larynx, and trachea (up to the carina) was undertaken 45–50 min postexercise, and the presence of fresh blood in the airway(s) was regarded as indicative of the occurrence of EIPH (11, 29).

Data Analysis
The data were first subjected to repeated measures, split-plot design analysis of variance (28) using the SAS statistical software package (SAS version 6.12, SAS Institute, Cary, NC), and the treatment comparisons were made using the least squares significant difference method (28). Data for the control as well as L-NAME experiments were also subjected to Newman-Keuls multiple-range test (28) to determine the significant effects of work intensity/duration within each treatment. For all statistical analyses, the level of significance was set at P < 0.05 and the data are presented as means ± SE.

RESULTS
General Observations
In keeping with our previous work (15), the cardiovascular effects of L-NAME were very rapid in onset. Within 60 s of completion of the L-NAME injection, heart rate had decreased significantly (P < 0.0001) to 27 ± 2 beats/min (from 36 ± 1 beats/min pre-L-NAME administration), and consistent with the effects of NO synthase inhibition, mean aortic (128 ± 7 mmHg) and pulmonary artery (37.2 ± 1.0 mmHg) blood pressures of standing horses had increased significantly (P < 0.01) from pre-L-NAME resting values of 97 ± 2 and 30.1 ± 1.2 mmHg, respectively. Further significant changes in these variables did not occur during the 10 min after completion of L-NAME injection. The bradycardia after L-NAME injection was due to the development and/or exaggeration of the second-degree atrioventricular block. Also, in agreement with our previous observations (15), it was readily apparent that the horses had a much calmer and more submissive demeanor after L-NAME administration.

Similar to our previous work (15), after L-NAME administration, it was a consistent observation that, unlike in the control study, the horses were not aggressive in charging at the front bar of the treadmill during submaximal exercise, and the heart rates recorded during submaximal exercise at 6 as well as 8 m/s on a 3.5% uphill grade (142 ± 4 and 170 ± 5 beats/min, respectively) were significantly (P < 0.001) less than corresponding values in the control study (168 ± 5 and 192 ± 4 beats/min, respectively). However, in the L-NAME experiments, during galloping at 14 m/s on a 3.5% uphill grade, heart rate increased to its maximal value (217 ± 2 beats/min) as observed in the control study. These changes in heart rate are consistent with our previous observations with L-NAME administration to galloping Thoroughbreds (15).

Changes in PaO_2 and SaO_2
Preinfusion resting values of PaO_2 and SaO_2 were similar in the control and the L-NAME experiments, and the administration of L-NAME to standing horses did not cause significant changes (Fig. 1). During submaximal exercise at 6 and 8 m/s, PaO_2 and SaO_2 were well maintained in both treatments. In both experiments, during galloping at 14 m/s on a 3.5% uphill grade, a statistically significant (P < 0.0001) decrease in PaO_2 was evident at 30 s, but further significant changes in PaO_2 did not occur as exercise duration increased to 120 s. The PaO_2 values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments were 72.9 ± 1.6 and 76.4 ± 1.6 Torr, respectively. Statistically significant differences between the treatments were not discerned at any point during the exercise protocol.

In both treatments, galloping at 14 m/s on a 3.5% uphill grade also caused statistically significant desaturation of hemoglobin in the arterial blood at 30 s. As exercise duration increased to 120 s, the desaturation of hemoglobin intensified in both treatments, but statistically significant differences were not found between the control and the L-NAME experiments. The
increasing desaturation of arterial hemoglobin observed in going from 30 to 120 s of galloping at 14 m/s on a 3.5% uphill grade probably resulted from the rightward shift of the hemoglobin-O₂ dissociation curve as hypercapnia (Fig. 2), acidosis (Fig. 3), and hyperthermia (from core temperature of 39.2 ± 0.2 and 39.1 ± 0.2°C at 30 s in the control and L-NAME experiments, respectively, to 40.6 ± 0.2 and 40.7 ± 0.2°C at 120 s in the control and L-NAME experiments, respectively) intensified with increasing exercise duration. SaO₂ values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments were 85.0 ± 1.7 and 87.5 ± 1.9%, respectively, and statistically significant differences between the treatments were not discerned at any point during the exercise protocol.

Changes in PaCO₂

In standing horses, PaCO₂ values were unaffected by inhibition of NO synthase with L-NAME (Fig. 2). Whereas submaximal exercise at 6 and 8 m/s in both experiments was attended by hyperventilation, during galloping at 14 m/s on a 3.5% uphill grade a significant hypercapnia developed. The extent of exercise-induced hypercapnia in galloping Thoroughbreds was similar between the control and the L-NAME experiments. PaCO₂ values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments were 50.8 ± 1.8 and 49.3 ± 2.1 Torr, respectively.

Changes in pHₐ

Preinfusion and postinfusion values of pHₐ were similar between the control and the L-NAME experiments (Fig. 3). Exercise at 6 and 8 m/s was not attended by significant changes in pHₐ in either treatment.
ment. During galloping at 14 m/s on a 3.5% uphill grade, a progressive, significant acidosis of a similar magnitude developed in both treatments. The $pH_a$ values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments approached 7.125 ± 0.031 and 7.143 ± 0.036, respectively.

**Changes in Arterial Blood Hemoglobin Concentration**

Preinfusion values of arterial hemoglobin concentration in standing horses were similar in the control and the L-NAME experiments, and L-NAME administration did not cause significant changes (Fig. 4). In both treatments, exercise caused significant ($P < 0.0001$) increments in arterial hemoglobin concentration, but the increment in hemoglobin concentration in the L-NAME experiments was found to be significantly ($P < 0.01$) attenuated at all work intensities. Arterial hemoglobin concentration in the control study at 6 and 8 m/s and at 60 s of galloping at 14 m/s on 3.5% uphill grade was $19.5 ± 0.4$, $20.7 ± 0.3$, and $21.5 ± 0.2$ g/dl, respectively. Corresponding values in the L-NAME study were significantly less, being $16.3 ± 0.3$, $17.6 ± 0.5$, and $19.5 ± 0.2$ g/dl, respectively.

**Changes in $CaO_2$**

The $CaO_2$ of standing horses was unaffected by L-NAME administration (Fig. 5). In both experiments, a large significant ($P < 0.0001$) increment in $CaO_2$ was observed during exercise as hemoglobin concentration increased significantly (Fig. 4). However, for all work intensities, the values of $CaO_2$ in the L-NAME experiments remained significantly less ($P < 0.05$) than in the control experiments. $CaO_2$ values in the control study at 6 and 8 m/s and at 60 s of galloping at 14 m/s
on a 3.5% uphill grade were 26.5 ± 0.5, 27.6 ± 0.4, and 26.6 ± 0.4 ml O2/dl blood, respectively. Corresponding values in the L-NAME study were significantly (P < 0.05) lower, being 22.3 ± 0.4, 23.8 ± 0.7, and 24.9 ± 0.6 ml O2/dl blood, respectively.

**Airway Endoscopy**

It was observed that all horses had experienced EIPH in the control as well as the L-NAME experiments, as demonstrated by the presence of fresh blood in the trachea (11, 29).

**DISCUSSION**

Our findings regarding arterial hypoxemia, desaturation of hemoglobin, hypercapnia, acidosis, increased hemoglobin concentration, and CaO2, as well as significant hyperthermia during high-intensity short-term exercise in the control study (Figs. 1–5), are similar to those reported previously (1, 2, 4, 9, 18, 30–32). Also, our observations regarding the effects of L-NAME administration in resting and exercising horses on heart rate, peak core temperature, and sweating mirror those reported previously (10, 15). Regarding the primary objective of the present study, i.e., to examine the effects of NO synthase inhibition on exercise-induced arterial hypoxemia, the new findings in the present study were as follows. 1) L-NAME administration to horses did not significantly affect the significant reductions in PaO2 and SaO2 (Fig. 1) or the rise in PaCO2 (Fig. 2) observed during high-intensity short-term exercise. Thus our data indicate that NO synthase inhibition did not significantly affect pulmonary gas exchange in exercising horses. 2) L-NAME administration to horses caused a significant reduction in hemoglobin concentration at all work intensities (Fig. 4), and as a consequence, CaO2 values remained significantly less than corresponding values in the control study (Fig. 5). The significant attenuation of the increment in arterial hemoglobin concentration throughout the exercise protocol in the L-NAME experiments suggests that the drug may have deleterious effects on equine splenic contraction. In view of the significant reduction in CaO2 in horses exercising after L-NAME administration (Fig. 5), it is appropriate to ask, “How were the metabolic needs being met during strenuous exercise?” Because the extent of metabolic acidosis in our L-NAME experiments was not significantly different from that in the control study (Fig. 3), it is likely that NO synthase inhibition did not cause a further increase in anaerobic metabolism in exercising horses. This is in agreement with the report by Kindig et al. (10), wherein lactate production did not increase in horses performing peak exercise after L-NAME administration, although an increase in O2 extraction was observed (10). Because mixed venous blood samples were not obtained in the present study, we cannot provide direct evidence for increased O2 extraction in our NO synthase inhibition experiments.

In the present study, in both treatments, arterial hypoxemia occurred rapidly, being readily evident at 30 s of galloping at 14 m/s on a 3.5 uphill grade (Fig. 1). The extremely rapid development of arterial hypoxemia with onset of high-intensity exercise and the fact that its magnitude was unaffected by the intensifying hypercapnia (Fig. 2) suggest that the exercise-induced arterial hypoxemia in horses may not have a structural basis related to the changes in the thickness/integrity of the blood-gas barrier (25, 33) brought about via stress failure of pulmonary capillaries resulting from the high transmural pulmonary capillary pressures (13–17). This is because, whereas the structural changes in the blood-gas barrier would be expected to intensify with increasing exercise duration and should, therefore, cause an intensification of the arterial hypoxemia in exercising horses, this was not the case in the present study (Fig. 1; Ref. 18). Thus our data are more consistent with the thesis that the exercise-induced arterial hypoxemia in strenuously exercising horses has a functional basis, probably related to the significant shortening of the transit time for blood in the pulmonary capillaries as cardiac output increases dramatically (18). A similar conclusion was reached by St. Croix et al. (27) in exercising human subjects.

As shown in Figs. 1 and 2, inhibition of endogenous NO production with L-NAME did not significantly affect pulmonary gas exchange in resting or exercising horses. In this context, in contrast with the findings of Mills et al. (21, 22), we have demonstrated that neither L-NAME administration to inhibit endogenous NO production (15) nor inundation of the pulmonary circulation with exogenous NO (with intravenous nitroglycerin infusion at 20 μg·kg⁻¹·min⁻¹, a dose fourfold of that required to cause maximal pulmonary vasodilation in healthy resting horses; 16) were effective in modifying the pulmonary arterial, capillary, or venous blood pressures in horses performing strenuous exercise. The inability of exogenous NO to modify pulmonary vascular tone in exercising horses (16) was attributed to the fact that pulmonary vascular resistance of exercising horses reaches its minimal value (achieved at maximal pulmonary vasodilation) during moderate exercise (performed at 8 m/s) and becomes a fixed quantity, such that further reductions in pulmonary vascular resistance do not occur as workload is increased to maximal exercise (17).

Our observations that PaO2, SaO2, and PaCO2 of horses were not significantly affected by L-NAME administration at rest or at any of the work intensities examined (Figs. 1 and 2) are, however, in conflict with those of Kindig et al. (10). These investigators (10) reported that, after L-NAME administration, PaCO2 of horses decreased significantly during exercise performed at 50 and 80% V̇O2 max but that PaO2 remained unchanged. Although the reasons for divergent findings are difficult to discern, it is important to note that in the experiments of Kindig et al. (10), even during exercise performed at V̇O2 max, horses did not exhibit the usual exercise-induced arterial hypoxemia and/or hypercapnia. This is not only in contrast with the data in the present study (Figs. 1 and 2), but also with several previous reports (1, 2, 4, 9, 12, 18, 30–33).
Furthermore, the findings of Kindig et al. regarding O\textsubscript{2} consumption and lactate production after L-NAME administration also conflict with other data in the literature (23). For example, whereas Mills et al. (23) reported that after L-NAME administration, O\textsubscript{2} consumption of exercising horses remains unaffected but their anaerobic metabolism (as indicated by lactate production) increases significantly, Kindig et al. (10) reported V\textsubscript{O\textsubscript{2}}\textsubscript{max} and lactate production to decrease significantly after L-NAME administration.

An alternate strategy to elucidate the role of NO in pulmonary gas exchange has been to study the effects of exogenous NO added to the inhaled gas (6, 26). However, these studies did not yield unequivocal results. Whereas NO inhalation at 15 parts/million was reported to cause a significant reduction in Pa\textsubscript{O\textsubscript{2}} of highly trained athletes at rest and during exercise performed at 50 and 80% V\textsubscript{O\textsubscript{2}}\textsubscript{max}, these findings were not confirmed in a recent study wherein NO inhalation at 20 parts/million failed to significantly affect the pulmonary gas exchange of highly trained cyclists at rest as well as during exercise performed during normoxia and hypoxia (26).

Consistent with previous reports that splenic contraction dramatically augments the O\textsubscript{2}-carrying capacity of blood in exercising horses (1, 2, 4, 9, 12, 18, 30–32), in the present study, arterial hemoglobin concentration of blood in exercising horses (1, 2, 4, 9, 12, 18, 19, 24, 27) significantly in both treatments with exertion (Fig. 4). These observations lead us to suggest that L-NAME administration may have direct or indirect effects on the splenic contraction.

In summary, our data demonstrated that L-NAME administration to horses did not significantly affect pulmonary gas exchange at rest or during exercise. Thus endogenous NO production is unlikely to play a role in mediating the exercise-induced arterial hypoxemia and hypercapnia in horses. However, because L-NAME administration caused a significant attenuation of the exercise-induced rise in arterial hemoglobin concentration, the Ca\textsubscript{O\textsubscript{2}} of exercising horses decreased significantly; the latter may diminish exercise performance at high workloads.

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