

Physiologic responses and plasma endothelin-1 concentrations associated with abrupt cessation of nitric oxide inhalation in isoflurane-anesthetized horses

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Objective—To assess physiologic responses and plasma endothelin (ET)-1 concentrations associated with abrupt cessation of nitric oxide (NO) inhalation in isoflurane-anesthetized horses.

Animals—6 healthy adult Standardbreds.

Procedures—Horses were anesthetized with isoflurane in oxygen and placed in dorsal recumbency. Nitric oxide was pulsed into the respiratory tract for 2.5 hours, and then administration was abruptly discontinued. Just prior to commencement and at cessation of NO administration, and at intervals during a 30-minute period following cessation of NO inhalation, several variables including P_{aO_2} , mean pulmonary artery pressure, venous admixture or pulmonary shunt fraction (Q_s/Q_t), and plasma ET-1 concentration were recorded or calculated.

Results—After cessation of NO inhalation, P_{aO_2} decreased slowly but significantly (172.7 ± 29.8 mm Hg to 84.6 ± 10.9 mm Hg) and Q_s/Q_t increased slowly but significantly ($25 \pm 2\%$ to $40 \pm 3\%$) over a 30-minute period. Mean pulmonary artery pressure increased slightly (14.0 ± 1.3 mm Hg to 16.8 ± 1 mm Hg) over the same time period. No change in serum ET-1 concentration was detected, and other variables did not change or underwent minor changes.

Conclusions and Clinical Relevance—The improvement in arterial oxygenation during pulsed inhalation of NO to healthy isoflurane-anesthetized horses decreased only gradually during a 30-minute period following cessation of NO inhalation, and serum ET-1 concentration was not affected. Because a rapid rebound response did not develop, inhalation of NO might be clinically useful in the treatment of hypoxemia in healthy isoflurane-anesthetized horses. (*Am J Vet Res* 2008;69:423–430)

Anesthetized, recumbent horses commonly develop hypoxemia secondary to \dot{V}/\dot{Q} mismatch in which the dependent portions of the lung are perfused but not well ventilated and the nondependent portions of the lung are well ventilated but poorly perfused.^{1,2} Ventilation-perfusion ratio mismatch and hypoxemia are also

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ABBREVIATIONS

\dot{V}/\dot{Q}	Ventilation-perfusion ratio
NO	Nitric oxide
ET	Endothelin
MPAP	Mean pulmonary arterial blood pressure
F_{iO_2}	Fraction of inspired oxygen
TV	Tidal volume
pHa	pH of arterial blood
pHv	pH of central (mixed) venous blood
$P\bar{V}O_2$	Partial pressure of oxygen, mixed venous
$P\bar{V}CO_2$	Partial pressure of carbon dioxide, mixed venous
Q_s/Q_t	Venous admixture or pulmonary shunt fraction
P_{AO_2}	Alveolar oxygen tension
$(A-\bar{a})DO_2$	Alveolar-arterial oxygen difference
$(a-v)DO_2$	Arterial-venous oxygen difference
NO_2	Nitrogen dioxide
$S\bar{v}O_2$	Oxygen saturation, mixed venous
$\dot{V}E$	Expired minute ventilation
HPV	Hypoxic pulmonary vasoconstriction

a problem in some anesthetized humans and humans with various forms of acute lung disease, especially diseases that cause pulmonary hypertension.³⁻⁵ Systemic

administration of vasodilators has been used to improve pulmonary blood flow in well-ventilated but poorly perfused portions of the lungs; however, these agents can cause hypotension and may not be the ideal choice in anesthetized patients.⁵ Nitric oxide, a potent vasodilator, can be inhaled directly into the lungs, thereby allowing selective dilatation of pulmonary blood vessels in ventilated areas, reduction of pulmonary vascular resistance in the area exposed to NO, and subsequent improvement of arterial oxygenation as blood flow is redistributed to ventilated areas of the lung.⁶ Inhalation of NO is known to improve oxygenation secondary to \dot{V}/\dot{Q} mismatch in sheep,⁷ pigs,⁸ and humans.^{5,6,9} In anesthetized horses, continuous inhalation of NO is not associated with improvement in P_{aO_2} ,¹⁰ but delivery of NO as pulses in the early phase of inhalation does improve oxygenation.^{11,12}

In some species, abrupt cessation of inhalation of NO, such as that which might occur at the end of an anesthetic period, causes a rapid and potentially drastic decrease of arterial oxygen tension and an increase in pulmonary arterial pressure,^{6,7,13} and these effects may be related to a compensatory increase in the circulating concentration of the potent endogenous vasoconstrictor ET-1.¹³⁻¹⁹ If this phenomenon, termed the rebound effect, occurs in horses, it could limit the usefulness of NO inhalation in this species. The purpose of the study reported here was to assess physiologic responses and plasma ET-1 concentrations associated with abrupt cessation of NO inhalation in isoflurane-anesthetized horses.

Materials and Methods

Horses—Six healthy Standardbreds (2 mares and 4 geldings) were used for this study; mean weight of the horses was 488 kg (range, 450 to 510 kg), and mean age was 5 years (range, 4 to 6 years). The study was approved by the local ethics committee for animal experiments, Uppsala, Sweden.

Anesthesia—Food but not water was withheld for 12 hours prior to anesthesia. On the day of the procedure, acepromazine^a (0.03 mg/kg) was administered IM to each horse approximately 30 minutes before IV administration of detomidine^b (0.005 mg/kg). Ten minutes after the detomidine injection, 7.5% guaifenesin^c (approx 100 mg/kg) was infused IV to effect or until the horse developed ataxia. Anesthesia was then induced via IV administration of a bolus of thiopentone sodium^d (4 to 5 mg/kg). The horse was intubated with a 30-mm cuffed endotracheal tube^e and placed in dorsal recumbency on a padded table. The endotracheal tube was connected to a semiclosed circle rebreathing system that was attached to a large-animal anesthesia machine. Anesthesia was maintained with spontaneous breathing of oxygen (> 90%) and isoflurane^f delivered through an out-of-circle, agent-specific, precision vaporizer.^g Throughout the study, end-tidal isoflurane concentrations of 1.5% to 1.7% (approx 1.2X to 1.25X the minimum alveolar concentration of isoflurane in horses) were maintained. The gas monitor was calibrated before each research period by use of a commercially prepared calibration gas.^h Following the last data collection, each

horse was allowed to recover from anesthesia without assistance in a padded stall.

Instrumentation—For each horse, an ECG was placed for lead II analysis and measurement of heart rate. On 1 side of the head, the facial artery region was clipped free of hair and aseptically prepared; a 20-gauge, 5-cm catheterⁱ was introduced percutaneously for measurement of arterial blood pressure and for collection of arterial blood samples for blood gas analysis. An area over the right jugular vein was clipped free of hair and aseptically prepared; an introducer kit^j was used to place a 7-F thermodilution catheter^k through the jugular vein and into the pulmonary artery for measurement of pulmonary arterial blood pressure and for collection of mixed venous blood samples for blood gas analysis. A pig-tail, multihole catheter^l was introduced by the same technique into the same jugular vein, advanced to the right ventricle, and retracted into the right atrium for injection of saline (0.9% NaCl) solution for cardiac output (ie, Q_t) determination. Catheters were positioned by use of pressure-tracing guidance and simultaneous ECG monitoring and were locked in position via a Luer-lock adapter. Systolic, diastolic, and mean arterial blood pressure values and MPAP were measured by use of pressure transducers positioned at the level of the scapulohumeral joint, which was considered to correspond to the level of the right atrium. Cardiac output was determined by use of a thermodilution technique in which a bolus of 20 mL of cold (0°C) saline solution was injected rapidly by hand through the pig-tailed catheter. A minimum of 3 injections was performed, and those data were averaged at each time period. Cardiac output, arterial blood pressures, heart rate, F_{IO_2} , respiratory rate, TV, end-tidal carbon dioxide fraction, and end-tidal isoflurane fraction were recorded from a standard anesthesia monitor.^m To our knowledge, the measurement of TV in horses by use of this monitor has not been validated but is a standard measurement used in our laboratory.^{11,12}

Arterial and central (mixed) venous blood samples were obtained for assessment of pHa, pH \bar{v} , P_{aO_2} , $P\bar{v}O_2$, P_{aCO_2} , and $P\bar{v}CO_2$ by use of a standard electrode technique.ⁿ Arterial and mixed venous oxygen saturation and blood hemoglobin concentration were measured spectrophotometrically.^o Blood gas analysis was corrected for atmospheric pressure.

Calculated data—Venous admixture was calculated by use of the Berggren shunt equation²⁰ as follows:

$$Q_s/Q_t = (Cc'O_2 - CaO_2)/(Cc'O_2 - C\bar{v}O_2)$$

where $Cc'O_2$, CaO_2 , and $C\bar{v}O_2$ are oxygen content of capillary, arterial, and mixed venous blood, respectively.

Expired minute ventilation was calculated as TV multiplied by respiratory rate. Alveolar oxygen tension was calculated as follows:

$$PAO_2 = F_{IO_2} - (P_{ACO_2}/R)$$

where P_{ACO_2} is used as the alveolar carbon dioxide tension (P_{ACO_2}) and R is the respiratory quotient (0.8).

Alveolar-arterial oxygen difference ($[A-a]DO_2$) was calculated as PAO_2 minus PaO_2 , and arterial-venous oxygen difference ($[a-v]DO_2$) was calculated as PaO_2 minus $P\bar{v}O_2$.

Delivery and measurement of NO—Following data collection at 45 minutes after the start of isoflurane anesthesia (anesthesia baseline; time [T]–AB), NO was pulsed into the circle system during the early phase of each inspiration for 2.5 hours and then was abruptly discontinued. The NO was administered by use of a device that had been developed at the Datex-Ohmeda Research Unit, Helsinki, Finland,^{21,p} specifically for pulsed delivery of NO. The device delivered a mean volumetric dose of NO into the endotracheal tube during the first $31 \pm 3\%$ of the inspiration, which resulted in a dose of approximately $39 \pm 5 \text{ g/m}^3$ ($39 \pm 5 \text{ ppm}$) of $NO \cdot \text{breath}^{-1}$. A pressure sensor was used to trigger the gas delivery and was fitted onto the endotracheal tube to detect breathing. The delivery device was connected to a cylinder supply of $2,000 \text{ g/m}^3$ ($2,000 \text{ ppm}$) of NO in N_2 .^q The amount (in g/m^3 [ppm]) of NO exhaled with each breath was measured by use of a chemiluminescence monitor.^r An electrochemical NO_2 analyzer^s was connected to the expiratory limb of the breathing circuit to measure the NO_2 fraction.

ET measurements—Venous blood was collected in chilled tubes containing EDTA (final concentration, 10mM) and centrifuged at 4°C for 10 minutes to separate the plasma. Acid ethanol was added to precipitate the protein. The precipitate was analyzed for ET-1–like immune reactivity by use of a radioimmunoassay involving E1 antiserum raised against ET-1 in rabbits. The detection limit of the assay was 1.91 pmol. The cross-reactivity of the E1 antiserum was as follows: ET-1, 100%; ET-2, 27%; ET-3, 8%; and big ET-1, 0.14%. The plasma concentration of ET-1 is expressed as picomole per milliliter of plasma.

Data collection—All data including heart rate; mean, systolic, and diastolic arterial blood pressures; MPAP; systolic and diastolic pulmonary arterial blood pressures; Qt; FIO_2 ; respiratory rate; TV; end-tidal carbon dioxide fraction; end-tidal isoflurane fraction; pHa and $pH\bar{v}$; PaO_2 ; $P\bar{v}O_2$; SaO_2 ; $S\bar{v}O_2$; Qs/Qt; $\dot{V}E$; PAO_2 ; $(A-a)DO_2$;

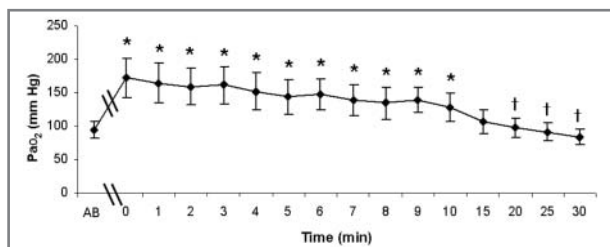


Figure 1—Mean \pm SEM PaO_2 values in 6 healthy isoflurane-anesthetized horses that inhaled pulses of NO delivered during the early phase of each inspiration for 2.5 hours until NO inhalation was abruptly discontinued. Values were determined following 45 minutes of equilibration after commencement of isoflurane anesthesia prior to NO administration (anesthesia baseline; time [T]–AB) and immediately following the last NO pulse (T–0 minutes). Data were then collected at 1-minute intervals for 10 minutes followed by collection once every 5 minutes for 20 minutes. *Value significantly ($P < 0.05$) different from that at T–AB. †Value significantly ($P < 0.05$) different from that at T–0 minutes.

and $(a-v)DO_2$ were measured or calculated following 45 minutes of equilibration after commencement of isoflurane anesthesia prior to NO administration (anesthesia baseline; T–AB) and immediately following the last NO pulse (T–0 minutes). Data were then collected at 1-minute intervals for 10 minutes followed by collection once every 5 minutes for 20 minutes. All data were collected or calculated at T–AB and T–0, T–1, T–5, T–10, T–25, and T–30 minutes. At all other times, all data were measured or calculated except for $pH\bar{v}$, $P\bar{v}O_2$, $S\bar{v}O_2$, Qt, $(A-a)DO_2$, and Qs/Qt. Venous blood samples for ET-1 analysis were collected at T–AB and T–0, T–10, and T–14 minutes.

Data analysis—Repeated-measures ANOVA was used to compare data at various time points within the group. The Tukey honest significant difference test was used for post hoc comparisons, and probability values were calculated. For all statistical calculations, a software package^t was used and a value of $P < 0.05$ was considered significant. Data are presented as mean \pm SEM.

Results

The horses in the present study received an FIO_2 of 0.95. From T–0 to T–30 minutes, PaO_2 and SaO_2 decreased gradually but significantly ($172.7 \pm 29.8 \text{ mm Hg}$ to $84.6 \pm 10.9 \text{ mm Hg}$ and $98.7 \pm 0.6\%$ to $93.0 \pm 2.1\%$, respectively; Figure 1); however, Qs/Qt increased gradually but significantly ($25 \pm 2\%$ to $40 \pm 3\%$) during that interval (Figure 2). Other variables that increased gradually but significantly from T–0 to T–30 minutes included heart rate ($33 \pm 1.0 \text{ beats/min}$ to $36 \pm 1.5 \text{ beats/min}$), MPAP ($14.0 \pm 1.3 \text{ mm Hg}$ to $16.8 \pm 1.0 \text{ mm Hg}$), $(A-a)DO_2$ ($54.65 \pm 4.0 \text{ mm Hg}$ to 68.98 ± 2.50

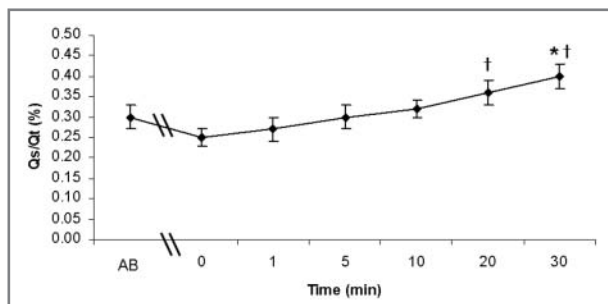


Figure 2—Mean \pm SEM percentage Qs/Qt in the 6 healthy isoflurane-anesthetized horses in Figure 1 before and after abrupt cessation of NO inhalation. See Figure 1 for key.

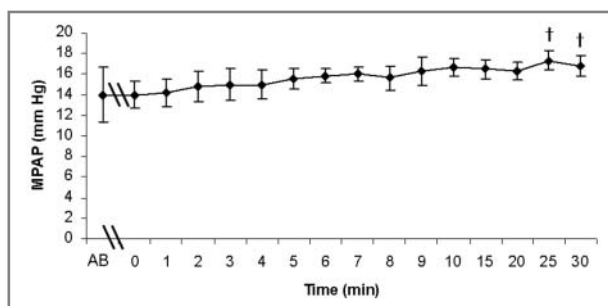


Figure 3—Mean \pm SEM MPAP values in the 6 healthy isoflurane-anesthetized horses in Figure 1 before and after abrupt cessation of NO inhalation. See Figure 1 for key.

Table 1—Mean \pm SEM values of heart rate, respiratory rate, P_{aCO_2} , $\dot{V}E$, and $(A-a)Do_2$ in 6 isoflurane anesthetized horses that inhaled pulses of NO delivered during the early phase of each inspiration for 2.5 hours until NO inhalation was abruptly discontinued. Values were determined following 45 minutes of equilibration after commencement of isoflurane anesthesia prior to NO administration (anesthesia baseline; time [T]–AB) and immediately following the last NO pulse (T–0 minutes). Data were then collected at 1-minute intervals for 10 minutes followed by collection once every 5 minutes for 20 minutes.

Time (min)	Heart rate (beats/min)	Respiratory rate (breaths/min)	P_{aCO_2} (mm Hg)	$\dot{V}E$ (L/min)	$(A-a)Do_2$ (mm Hg)
AB	33 \pm 1	5 \pm 1	54.0 \pm 3.8	33.47 \pm 4.95	67.85 \pm 2.09
0	33 \pm 1	4 \pm 1	65.4 \pm 4.6*	23.68 \pm 2.96*	54.65 \pm 4.00*
1	34 \pm 1	4 \pm 1	67.4 \pm 5.1*	22.91 \pm 2.93*	55.52 \pm 3.83*
2	33 \pm 1	4 \pm 1	67.1 \pm 4.2*	24.62 \pm 2.85*	56.52 \pm 3.56*
3	34 \pm 1	4 \pm 1	65.2 \pm 5.2*	25.10 \pm 3.38*	56.46 \pm 3.70*
4	34 \pm 1	4 \pm 1	65.6 \pm 4.8*	23.96 \pm 4.57*	58.00 \pm 3.78*
5	34 \pm 1	4 \pm 1	66.1 \pm 5.3*	25.08 \pm 4.49*	59.22 \pm 3.90*
6	35 \pm 1	4 \pm 1	67.1 \pm 5.6*	23.59 \pm 3.99*	58.44 \pm 3.40*
7	35 \pm 1	4 \pm 1	66.9 \pm 5.5*	22.56 \pm 3.00*	59.68 \pm 3.42*
8	35 \pm 1	4 \pm 1	67.4 \pm 5.9*	26.29 \pm 2.96	60.28 \pm 3.55*
9	35 \pm 1	4 \pm 1	64.9 \pm 5.9*	26.70 \pm 3.12	60.14 \pm 2.62*
10	34 \pm 1	4 \pm 1	65.0 \pm 6.0*	28.41 \pm 4.36†	61.72 \pm 3.52
15	33 \pm 2	5 \pm 1	61.8 \pm 6.8*	29.33 \pm 3.63†	65.46 \pm 3.12†
20	34 \pm 2	5 \pm 1	61.8 \pm 6.8*	28.98 \pm 3.63†	66.64 \pm 2.79†
25	36 \pm 1*†	5 \pm 1	64.0 \pm 7.2*	30.91 \pm 4.62	67.22 \pm 2.54†
30	36 \pm 1*†	5 \pm 1	60.5 \pm 7.2*	31.47 \pm 5.01	68.98 \pm 2.50†

*Value significantly ($P < 0.05$) different from that at T–AB. †Value significantly ($P < 0.05$) different from that at T–0 minutes.

mm Hg), and $\dot{V}E$ (26.7 to 31.5 L/min; Table 1; Figure 3). The $(a-v)Do_2$ decreased gradually but significantly (38.46 ± 1.48 mm Hg to 33.64 ± 1.80 mm Hg) during the same time period. The $\dot{V}E$ value increased slightly as a result of a slight but significant increase in TV. Throughout the study, end-tidal carbon dioxide fraction was greater than the value at T–AB but did not change significantly over time. Other variables (mean, systolic, and diastolic arterial blood pressures; systolic and diastolic pulmonary arterial blood pressures; Qt ; F_{IO_2} ; respiratory rate; end-tidal isoflurane fraction; pHa and pH \bar{v} ; $P\bar{v}O_2$; $S\bar{v}O_2$; and PAO_2) did not change significantly. At T–0 minutes, the total exhaled NO concentration was 1.5 g/m³ (1.5 ppm). This concentration decreased to 0.2 g/m³ (0.2 ppm) in 4.5 minutes and was below measurable concentrations at all other time points. The toxin NO_x was not detected in the system at any time. Plasma ET-1 concentrations did not change significantly at any time during the study. At T–AB, mean plasma ET-1 concentration was 4.68 ± 0.40 pmol/mL; at T–0 minutes, the value was 4.40 ± 0.46 pmol/mL. At T–10 minutes, mean plasma ET-1 concentration was 4.62 ± 0.42 pmol/mL; at T–30 minutes, the value was 5.04 ± 0.50 pmol/mL.

Discussion

In the present study in isoflurane-anesthetized horses, cessation of pulse-delivered inhalation of NO resulted in a gradual but not abrupt decrease in arterial oxygen concentration and an increase in pulmonary arterial pressure with no change in plasma concentrations of ET-1. These results indicated that, following the withdrawal of pulsed NO inhalation, an ET-induced rebound effect does not develop in healthy isoflurane-anesthetized horses. Other variables that increased gradually but significantly following termination of NO

inhalation included heart rate, TV, and $\dot{V}E$, whereas $(a-v)Do_2$ decreased gradually but significantly during the same time period. These changes were most likely a response to decreasing P_{aO_2} .

Endogenous NO is critical for maintenance of basal dilator tone in the pulmonary (and systemic) vasculature of many species.²² Exogenous NO delivered via inhalation provides selective pulmonary vasodilation,^{5-7,9} and this method of delivery is used therapeutically in humans to reduce pulmonary hypertension and subsequent development of hypoxemia.^{5,6} However, discontinuation of NO inhalation can cause a precipitous decrease in oxygen saturation secondary to development of pulmonary hypertension with worsening of \dot{V}/\dot{Q} mismatch; this worsening of pulmonary function, or rebound effect, following cessation of NO inhalation has been detected in humans,⁶ pigs,¹³ sheep,¹⁶ and rats.²³ Although the mechanisms responsible for the rebound effect are not fully understood, an increase in the circulating concentration of the potent endogenous vasoconstrictor, ET-1, has been implicated.¹³⁻¹⁹ Inhalation of NO by human adult and pediatric patients with pulmonary hypertension following cardiopulmonary bypass resulted in increases in plasma ET-1 concentrations to 127% and 147% of baseline values, respectively, after 12 hours.¹⁹ Control patients in that study did not receive NO, and plasma ET-1 concentrations in those individuals actually decreased following the bypass procedure. In neonatal lambs with surgically induced aortopulmonary shunts, inhalation and subsequent withdrawal of NO were associated with a 34.8% increase in plasma ET-1 concentration and a 45% increase in pulmonary vascular resistance, compared with baseline values.¹⁶ In healthy neonatal lambs, inhalation of NO was associated with a $119.5 \pm 42.2\%$ increase in plasma ET-1 concentrations, with an increase in pulmonary vascular resistance of 77.8% following NO withdrawal.¹⁵ In

that same study, pulmonary vascular resistance did not increase in lambs that were pretreated with an ET receptor antagonist. Increased MPAP and decreased P_{aO_2} were also associated with increased plasma ET-1 concentrations in endotoxemic pigs.¹³ Endothelin-1 is also a potent vasoconstrictor in the pulmonary vessels in horses^{24,25} and could potentially contribute to hypoxia in anesthetized horses. However, plasma ET-1 concentrations did not change at any time during the study of this report, which could explain the lack of a rebound effect.

The present study is not the first in which a lack of a rebound effect following NO withdrawal in healthy adult isoflurane-anesthetized horses was detected. The gradual decrease of P_{aO_2} and increase in MPAP and Q_s/Q_t to, but not beyond, baseline values following the cessation of pulsed NO inhalation have been previously reported by Heinonen et al.^{11,12} There are many components of those 2 studies that might explain the lack of a rebound effect, including the species studied, anesthetic agents used, NO delivery method, and health of study animals.

The response of the pulmonary vasculature to hypoxia is highly variable among species²⁶⁻²⁸; for example, horses develop moderate HPV yet pigs develop profound HPV.²⁷ This profound response could predispose pigs to a more precipitous decrease in arterial oxygen concentrations secondary to more profound \dot{V}/\dot{Q} mismatch. To our knowledge, the HPV response in humans has not been directly compared with that of horses or pigs, but results of research in altitude-induced HPV suggest that there is great variability among individuals,^{29,30} which could contribute to the fact that some humans respond to NO inhalation and some do not. This might also explain why some humans develop a rebound effect following cessation of NO inhalation, whereas some develop a gradual decrease in P_{aO_2} and an increase in Q_s/Q_t similar to those detected in the horses of the present study. Furthermore, the amount of endogenous NO in the respiratory tract is highly variable among mammalian species,³¹ and this could explain the different responses to the treatment with and withdrawal of exogenous inhaled NO.

The sedatives and general anesthetic agents used in our study could also have affected the results and account for the discrepancies between our findings and those of other researchers. First, detomidine (a potent α_2 -adrenoceptor agonist) was administered to the horses as a premedication. α_2 -Adrenoceptor agonists cause pulmonary vascular constriction in some species³² and could potentially limit the likelihood of a rebound effect if this vasoconstriction persisted into the period of recovery from anesthesia. In their study of NO administration in pigs, Chen et al¹³ administered xylazine, another α_2 -adrenoceptor agonist; xylazine has a shorter duration of action than detomidine,³³ and the effects of xylazine in the pigs could have dissipated before data collection occurred. However, among other studies^{6,19,34-37} in humans, α_2 -adrenoceptor agonists were not administered to any human in any of the clinical or research trials in which a rebound effect occurred. Thus, the use of detomidine cannot be the sole contributing factor to the lack of a rebound effect in our study.

Following the administration of detomidine, horses of the present study underwent isoflurane anesthesia; isoflurane could also have influenced our findings because inhalant anesthetic agents are known to affect HPV. Inhalant anesthetic agents generally inhibit the HPV response,^{38,39} whereas injectable anesthetic agents may inhibit, maintain, or even potentiate the HPV response.^{34,39} However, a rebound response has been detected in conscious humans,^{19,34-36} anesthetized humans,^{19,37} anesthetized pigs,^{13,40} conscious sheep,⁷ and anesthetized sheep.^{15,16,41,42} Thus, because of the wide variety of anesthetics used and the inclusion of conscious subjects in those studies, it is unlikely that the rebound effect or lack of a rebound effect could be wholly attributed to a difference in anesthetic protocol. Nevertheless, it could be argued that the inhalant anesthetic agent used in the present study blunted the HPV response, thereby decreasing the degree of pulmonary vasoconstriction and minimizing the effect of NO inhalation among the study horses. This would indeed decrease the likelihood of a rapid rebound response following cessation of NO inhalation but would also have limited the initial response to NO. Although the horses in our study responded to inhalation of NO, whether the response would have been greater if isoflurane had not been used cannot be determined. Finally, the variety of effects caused by different anesthetic agents could explain the facts that halothane-anesthetized horses failed to respond to NO inhalation¹⁰ and yet isoflurane-anesthetized horses in our study and other investigations^{11,12} did respond to NO inhalation.

To compound matters, not only were the horses anesthetized with isoflurane in the present study, but also the depth of anesthesia was fairly deep and anesthetic depth is directly related to respiratory depression. An end-tidal isoflurane concentration of 1.25 \times the minimum alveolar concentration of isoflurane in horses was chosen for our study to maintain a steady plane of anesthesia and prevent any movement by the horses, a practice that is standard for our laboratory.^{11,12} Although this depth of anesthesia exceeds that which would be clinically necessary, maintenance of a stable plane of anesthesia throughout the entire investigative period eliminates the effects of changing anesthetic depth on the data collected. However, the horses were hypercarbic throughout the study, most likely as a result of respiratory depression caused by depth of anesthesia. Hypercarbia causes increases in MPAP and pulmonary vascular resistance⁴³ and could have contributed to the increase in MPAP in the horses of the present study. Interestingly, inhalation of NO has been used to reverse the effects of hypercarbia in humans⁴³ and would likely have counteracted the effects of hypercarbia in the study horses. In fact, regardless of the causes of increased MPAP in general, NO inhalation resulted in a decrease in MPAP, with subsequent improvement in P_{aO_2} in the horses of the present study. A gradual but significant increase in MPAP occurred after NO inhalation was discontinued. Also, because P_{aCO_2} is an integral part of the formula used to calculate $P_{Aa}O_2$, hypercarbia would have affected this value and that of $(A-a)DO_2$. However, P_{aCO_2} was increased but stable throughout the study, and any changes in $P_{Aa}O_2$ and $(A-a)DO_2$ would not have been at-

tributable to P_{aCO_2} . Furthermore, $(A-a)Do_2$ increased gradually but significantly during the 30-minute period following cessation of NO inhalation, and this was not accompanied by a concurrent change in P_{aCO_2} .

Second, the mode of ventilation or F_{IO_2} during anesthesia could also have contributed to the differences between results of our study and studies performed previously. The horses in the present study and the sheep in the study by Frostell et al⁷ were allowed to breathe spontaneously, whereas many of the humans,^{34,37} most of the pigs,^{13,44} and many of the sheep^{15,16,41,45} in other investigations were mechanically ventilated; however, a rebound effect was detected in all of those other published studies. The rebound effect has been detected in conscious patients receiving no mechanical ventilation and in patients that were completely mechanically ventilated and maintained on positive end-expiratory pressure; thus, the mode of ventilation is unlikely to be the only explanation for the presence or absence of a detectable rebound effect. With regard to F_{IO_2} , the horses in the present study received an F_{IO_2} of 0.95; in other studies, pigs received an F_{IO_2} of 0.3² to 0.5¹³ and conditions for humans varied from breathing room air (F_{IO_2} , 0.21)^{19,34-36} to breathing high concentrations of oxygen (F_{IO_2} , 0.95) during anesthesia.^{19,37} Therefore, it is unlikely that F_{IO_2} is the sole cause of the rebound effect. Furthermore, high F_{IO_2} (0.95) causes greater compromise of pulmonary function than low F_{IO_2} (0.21) in anesthetized horses.^{46,47} Thus, if differences in response to NO inhalation were based solely on F_{IO_2} , we would have predicted either a lack of response to NO or a precipitous rebound effect as the inhalation of NO was discontinued in the horses of the present study. Neither of these events occurred in our study.

Third, NO was delivered as an inhalational pulse during the initial phase of each inspiration, which maximized improvement in oxygenation by allowing NO to be delivered only to well-ventilated alveoli and by minimizing the accumulation of NO in the breathing circuit. Because alveoli that are already distended are easier to fill, gases entering the airways during the initial phase of inhalation move easily into the well-ventilated alveoli. Once these alveoli are filled, inhaled gases began to move into alveoli that are in the so-called transitional zone (the zone between well-ventilated and nonventilated alveoli). Transitional zone alveoli have a fairly high closing pressure and do not distend with every breath; thus, they do not consistently participate in gas exchange. Therefore, continuous delivery of NO allows distribution to all alveoli that can be distended, including those that may be poorly ventilated, whereas delivery of NO during the early phase of inhalation allows selective NO distribution to the well-ventilated alveoli only. The vasodilation and increased blood flow following distribution of inhaled NO to areas of poor ventilation may contribute to the failure of NO to improve oxygenation in some humans.²¹ This may also be the reason that inhalation of NO (continuous delivery) failed to increase oxygenation in halothane-anesthetized horses.¹⁰ Studies by Heinonen et al^{11,12} revealed that compared with continuous delivery of NO, pulses

of NO administered during the initial phase of inhalation were more effective in improving P_{aO_2} in anesthetized horses. Also, via administration of NO pulses, we were able to decrease the total dose of NO that each horse received in the present study, which decreased the chance of NO accumulation in the rebreathing system of the anesthesia machine. In fact, NO was not detected in the circuit within 5 minutes after the discontinuation of administration, which indicates that the lack of a rebound effect was not the result of a weaning effect from retained NO in the circuit. Also, excess NO is often converted to the toxin NO_2 ; however, NO_2 was not detected in the circuit at any time in the present study.

Another important difference (and potential limitation) of our study, compared with other investigations, is the fact that we included only healthy horses, whereas the health status of most clinical patients and research animals used in other studies has been compromised. A rebound effect in humans with pulmonary hypertension, acute respiratory distress syndrome, hypoxic respiratory failure, or other forms of pulmonary disease has been reported.^{6,19,34,39} The rebound effect has also been reported in human adults and infants with pulmonary hypertension during or following cardiothoracic surgery or lung transplantation.^{6,19,35} Pigs with endotoxemia^{13,36} and neonatal sheep with cardiopulmonary compromise^{15,16,36,37} can also develop the rebound effect. Because ET synthesis is increased in disease states,⁴⁸ an ET-induced rebound effect may be more likely to develop in compromised patients than in healthy patients.

Although study limitations include the use of an inhalant anesthetic agent and a population of only healthy horses, we propose that the anesthetic protocol chosen for our study was typical of anesthetic protocols used in clinical settings and that not all anesthetized patients are compromised. Thus, the results of our study have suggested a possible use for NO inhalation in healthy horses. However, a final limitation of the present study is that the horses were not in a typical situation during recovery from anesthesia; horses were still under the influence of inhalant anesthetic and supported via inhalation of oxygen. Thus, although the effects of discontinuation of NO inhalation in this type of recovery situation can be predicted from our findings, the effects of discontinuation of NO inhalation during a recovery phase in which horses are not influenced by inhalant anesthetic and the F_{IO_2} is comparatively low cannot be predicted.

The findings of our study indicated that the improvement in P_{aO_2} induced by pulsed inhalation of NO during the early phase of inspiration in healthy isoflurane-anesthetized horses slowly but significantly declined following termination of NO inhalation. Healthy adult isoflurane-anesthetized horses do not appear to develop a rapid rebound effect immediately after cessation of NO inhalation.

- a. Plegicil, Pharmacia & Upjohn Animal Health, Helsingborg, Sweden.
- b. Domosedan, Orion Animal Health, Stockholm, Sweden.
- c. Myolaxin, Chassot & Cie AG, Berne, Switzerland.
- d. Pentothal Natrium, Abbott, Kista, Sweden.
- e. Bivona, Smiths Medical, London, England.
- f. Forene, Abbott, Kista, Sweden.

- g. Vapor 19.1, North American Drager, Telford, Pa.
- h. Anesthesia agent/CO₂ calibration gas, BCI International, Waukesha, Wis.
- i. Insyte-W, 18GA, Becton-Dickson, Ohmeda, Helsingborg, Sweden.
- j. Arrow International Inc, Reading, Pa.
- k. Swan-Ganz thermodilution catheter, Columbus Instruments, Columbus, Ohio.
- l. Cook Europe A/S, Søborg, Denmark.
- m. AS/3-AM, Datex-Ohmeda, Helsinki, Finland.
- n. ABL 5, Radiometer, Copenhagen, Denmark.
- o. OSM 5, Radiometer, Copenhagen, Denmark.
- p. NOrse, Datex-Ohmeda Research Unit, Helsinki, Finland.
- q. AGA AB, Lidingö, Sweden.
- r. Sievers NOA 280, Sievers Instruments, Boulder, Colo.
- s. Nomius, Dan Sjö Medical, Stockholm, Sweden.
- t. Statistica/w 6.0, StatSoft Inc, Tulsa, Okla.

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Correction: In “In vitro efficacy of an ophthalmic drug combination against corneal pathogens of horses”, published January 2008 (*Am J Vet Res* 2008;69:101–107), the last sentence in the results section of the abstract should read as follows:

Antiproteinase activity of serum was a concentration-dependent event, which enabled serum to achieve significantly greater activity than the drug combination after 3.5 and 4 hours of incubation for the gelatin and collagen I assays, respectively.