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Effects of pulsed inhaled nitric oxide delivery on the distribution of pulmonary perfusion in spontaneously breathing and mechanically ventilated anesthetized ponies

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OBJECTIVE
To measure changes in pulmonary perfusion during pulsed inhaled nitric oxide (PiNO) delivery in anesthetized, spontaneously breathing and mechanically ventilated ponies positioned in dorsal recumbency.

ANIMALS
6 adult ponies.

PROCEDURES
Ponies were anesthetized, positioned in dorsal recumbency in a CT gantry, and allowed to breathe spontaneously. Pulmonary artery, right atrial, and facial artery catheters were placed. Analysis time points were baseline, after 30 minutes of PiNO, and 30 minutes after discontinuation of PiNO. At each time point, iodinated contrast medium was injected, and CT angiography was used to measure pulmonary perfusion. Thermodilution was used to measure cardiac output, and arterial and mixed venous blood samples were collected simultaneously and analyzed. Analyses were repeated while ponies were mechanically ventilated.

RESULTS
During PiNO delivery, perfusion to aerated lung regions increased, perfusion to atelectatic lung regions decreased, arterial partial pressure of oxygen increased, and venous admixture and the alveolar-arterial difference in partial pressure of oxygen decreased. Changes in regional perfusion during PiNO delivery were more pronounced when ponies were spontaneously breathing than when they were mechanically ventilated.

CLINICAL RELEVANCE
In anesthetized, dorsally recumbent ponies, PiNO delivery resulted in redistribution of pulmonary perfusion from dependent, atelectatic lung regions to nondependent aerated lung regions, leading to improvements in oxygenation. PiNO may offer a treatment option for impaired oxygenation induced by recumbency.

Perioperative mortality rates for healthy horses are around 0.9%,[1–4] with much higher rates reported for horses with systemic disease.[3,5] Factors that may contribute to these high mortality rates include hypotension, hypoxemia, and acid-base derangements during general anesthesia.[3] Matching of alveolar ventilation to pulmonary perfusion is critical for effective gas exchange and tissue oxygenation. However, in anesthetized horses, large areas of ventilation-perfusion inequality or mismatch develop, resulting in a low arterial partial pressure of oxygen (Pa_{O_2}) and an elevated alveolar-arterial difference in the partial pressure of oxygen.[6,7] This ventilation-perfusion mismatching occurs predominantly as a consequence of compression atelectasis of dependent portions of the lung, leading to development of a large intrapulmonary shunt, and is of particular concern when animals are positioned in dorsal recumbency.[6,8] Treatments that target ventilation (eg, the open lung concept)[9] or pulmonary perfusion (eg, inhalation of nitric oxide or β_2-adrenoceptor agonists) have been shown to improve arterial oxygenation in anesthetized horses.[7,10–13] However, the increased intrathoracic pressure generated during mechanical ventilation can lead to unpredictable effects. For example, mechanical ventilation can decrease cardiac output by preventing efficient venous return[14]; increased lung volume impedes cardiac filling through cardiac

chamber and pericardial compression effects[15,16]; and the positive pressure may drive pulmonary blood flow downwards toward atelectatic lung regions, worsening intrapulmonary shunting.[17]

Inhalation of nitric oxide results in selective pulmonary vasodilation,[7,18] and when nitric oxide is given as a pulse (pulsed inhaled nitric oxide [PiNO]) early in inspiration to anesthetized horses that are spontaneously breathing or being mechanically ventilated, Pa_{O_2} and venous admixture improve.[11,12] These improvements occur because of movement of blood against gravity from dependent, presumably atelectatic lung regions to nondependent, aerated lung regions.[19] Pulsed delivery given at the start of inspiration ensures that well-ventilated alveoli are targeted.[11,12]

Recently, CT angiography and the maximum slope model have been described to measure regional pulmonary perfusion in anesthetized ponies.[20] The safety of repeated injections of iodinated contrast medium means that serial measurements can be made following various treatments to assess their effects on pulmonary perfusion.

The objective of the study reported here was to measure changes in pulmonary perfusion during PiNO delivery in anesthetized, spontaneously breathing and mechanically ventilated ponies positioned in dorsal recumbency. Our hypothesis was that blood would be redistributed from dependent atelectatic lung regions to nondependent aerated lung regions during PiNO administration. This redistribution would be observed while ponies were spontaneously breathing and when they were mechanically ventilated and would be associated with improvements in arterial oxygenation.

Materials and Methods

The study protocol was approved by the local Ethical Committee on Animal Experiments in Uppsala, Sweden. Six healthy ponies (5 Shetland ponies and 1 crossbreed pony) with a mean weight of 190 kg (range, 150 to 241 kg) and mean age of 12 years (range, 4 to 18 years) were used in the study. The ponies comprised 1 mare, 3 geldings, and 2 stallions. All ponies were confirmed to be healthy prior to the study by means of a complete physical examination, CBC, and serum biochemical testing.

Anesthetic protocol

Food was withheld for 12 hours prior to induction of general anesthesia; water was provided ad libitum until the time of venous catheterization. Ponies were premedicated with acepromazine (0.03 mg/kg, IM) approximately 45 minutes prior to induction to general anesthesia. A 14-gauge, 9-cm catheter was placed in the left jugular vein, and two 8.5F sheath introducers were inserted in the right jugular vein following SC infiltration with lidocaine. Ponies were further premedicated with xylazine (1.1 mg/kg, IV) and butorphanol (0.025 mg/kg, IV), and general anesthesia was induced with ketamine (2.2 mg/kg) and diazepam (0.05 mg/kg) mixed in the same syringe and given IV. Once ponies were unconscious, the trachea was intubated with a 20-mm endotracheal tube, and the ponies were hoisted onto an appropriately padded CT table with their head toward the CT gantry. The forelimbs and hind limbs were allowed to fall into natural positions, with padding inserted between the lower portion of the antebrachium and the metacarpal bones. The endotracheal tube was connected to a large animal anesthetic circuit and mechanical ventilator. A specially designed arrangement of pitot tubes for measuring lung volumes and flow and a simple ball-valve tap to facilitate a breath hold during acquisition of CT images was inserted between the breathing system and the endotracheal tube. General anesthesia was maintained with isoflurane in oxygen with an inspired oxygen fraction (F_{IO_2}) of approximately 0.9. The mean arterial blood pressure was maintained at a pressure > 70 mm Hg with a variable-rate infusion of dobutamine (0.5 to 5.0 µg/kg/min). Ringer's acetate solution was administered throughout the anesthetic period (5 mL/kg/h). Ponies were initially allowed to breathe spontaneously and then were mechanically ventilated. At the end of the experiment, anesthesia was discontinued, and ponies were hoisted into a padded stall and allowed to recover. Analgesia was provided at this time with flunixin meglumine (1.1 mg/kg, IV) and morphine (0.1 mg/kg, IM).

Instrumentation and hemodynamic analysis

The facial artery was catheterized with an 18-gauge catheter for collection of arterial blood samples and measurement of systemic blood pressure. A 7F Swan-Ganz catheter was advanced into the pulmonary artery through one of the preplaced introducers in the right jugular vein. This catheter was used to collect mixed venous blood samples and measure mean pulmonary arterial pressure, pulmonary capillary wedge pressure, and cardiac output. A pigtail, multi-hole catheter was similarly inserted through the other preplaced introducer, advanced into the right ventricle, and then retracted into the right atrium. This catheter was used for injection of contrast medium for determination of pulmonary perfusion and administration of ice-cold saline (0.9% NaCl) solution for measurement of cardiac output by thermodilution. Catheters were positioned with pressure waveform guidance and simultaneous ECG monitoring. Pressures were measured by connecting catheters to calibrated transducers and saline solution–filled columns. The transducers were positioned at the level of the shoulder joint and zeroed to atmospheric pressure.

Each pony was connected to a multiparameter monitor for ECG monitoring and measurement of heart rate, oxygen saturation of hemoglobin (by pulse oximetry), end-tidal partial pressure of carbon dioxide (P_{ETCO_2}), F_{IO_2}, end-tidal isoflurane concentration, respiratory rate, tidal volume, peak inspiratory pressure, mean pulmonary arterial blood pressure, pulmonary capillary wedge pressure, car-

diac output, and systolic, mean, and diastolic arterial blood pressures.

Thermodilution for determination of cardiac output was performed by injecting a 20-mL bolus of ice-cold saline solution through the pigtail catheter by hand during the expiratory phase of respiration. A minimum of 3 measurements were made at each data collection time point, and the mean value was recorded.

Blood gas measurements

Arterial and mixed venous blood samples were collected simultaneously at each data collection time point from the facial artery and pulmonary artery catheters, respectively, into heparinized syringes and analyzed immediately with a standard electrode technique. Arterial pH, Pa_{O_2}, arterial partial pressure of carbon dioxide (Pa_{CO_2}), arterial oxygen saturation of hemoglobin, hemoglobin concentration, mixed venous partial pressure of oxygen, mixed venous oxygen saturation of hemoglobin, and lactate concentration were measured. Blood gas partial pressures were corrected for atmospheric pressure.

Ventilation and nitric oxide

PiNO was delivered using an airway pressure–triggered device (Norse; Datex-Ohmeda Research Unit) that intermittently delivered a volumetric dose of nitric oxide from the beginning of inspiration for 30% to 45% of total inspiratory time. The device was connected to a cylinder with 2,000 ppm of nitric oxide in nitrogen gas. The approximate dose of nitric oxide delivered during each breath was 2.5 to 5.0μM.

Ponies were initially allowed to breathe spontaneously. After instrumentation was completed, baseline measurements were obtained. PiNO was continuously delivered for 30 minutes, and measurements were repeated. PiNO was then discontinued, and measurements were recorded again after 30 minutes. Mechanical ventilation was then initiated, with tidal volume and rate adjusted to maintain Pa_{CO_2} between 45 and 68 mm Hg while maintaining a peak inspiratory pressure < 30 cm H_2O. Approximately 30 minutes after mechanical ventilation was started, another set of baseline measurements was obtained. PiNO was continuously delivered for 30 minutes, and measurements were repeated. PiNO administration was then discontinued, and measurements were recorded again after 30 minutes.

Measurement of pulmonary perfusion

Pulmonary perfusion was measured by means of CT angiography as described elsewhere.[20] In brief, dynamic axial scans were acquired at each data collection time point with a 64-slice multidetector CT scanner (Somatom Definition AS; Siemens Medical Systems) at exposures of 70 mAs and 120 kV, slice thickness of 15.0 mm, and rotation time of 0.33 seconds. Iohexol (Omnipaque; GE Healthcare; 300mg/mL) was injected directly into the right atrium through the multihole pigtail catheter with an automated injector (Medrad Stellant dual-syringe CT injection system; Bayer AG). For measurements performed while the ponies were spontaneously breathing, CT scans were acquired at end expiration. For measurements performed while ponies were mechanically ventilated, CT scans were acquired at end expiration and peak inspiration. Each scan sequence lasted 50 seconds. DICOM files were exported at a rate of 3 images/s and viewed with imaging software (OsiriX 64-bit version 5.8.5; Pixmeo SARL). The CT images were analyzed with standard software (ImageJ version 1.49; National Institutes of Health). Lung regions were delineated by means of threshold windows (atelectatic lung, +100 to –100 HU; poorly aerated lung, –100 to –350 HU; and aerated lung, –350 to –1,000 HU), and perfusion was calculated as described.[20] As pulmonary perfusion is directly related to cardiac output and cardiac output differed among animals, calculations were corrected for each individual pony.

Calculated data

Oxygen content of pulmonary end-capillary blood (Cc'_{O_2}), oxygen content of arterial blood (Ca_{O_2}), and oxygen content of mixed venous blood ($C\bar{v}_{O_2}$) were calculated as $Cx_{O_2} = (1.36 \bullet [Hb] \bullet Sx_{O_2}) + (0.003 \bullet Px_{O_2})$, where x was c′, a, or $\bar{v}$ and [Hb] was hemoglobin concentration. The alveolar partial pressure of oxygen (P_{AO_2}) was used to calculate Cc'_{O_2}, with P_{AO_2} calculated as (F_{IO_2} • [barometric pressure – water vapor pressure]) – (Pa_{CO_2}/RQ), where Pa_{CO_2} was substituted for alveolar partial pressure of carbon dioxide (Pa_{CO_2}) and the respiratory quotient (RQ) was assumed to be 0.8.[21] Oxygen delivery was calculated as Ca_{O_2} times cardiac output. Venous admixture was calculated with the Berggren shunt formula[22] as $(Cc'_{O_2} - Ca_{O_2})/(Cc'_{O_2} - C\bar{v}_{O_2})$. Cardiac index was calculated as cardiac output divided by body weight (in kg). Minute ventilation was calculated as the respiratory rate times the tidal volume. Alveolar dead space was estimated as $(Pa_{CO_2} - P_{ETCO_2})/Pa_{CO_2}$. Oxygen extraction ratio was calculated as $(Ca_{O_2} - C\bar{v}_{O_2})/Ca_{O_2}$. Pulmonary vascular resistance was calculated as (mean pulmonary arterial blood pressure – pulmonary capillary wedge pressure)/(cardiac output′ • ′80). Cardiac output–corrected perfusion at each time point for each individual pony while spontaneously breathing was calculated as perfusion divided by cardiac output. The percentage change in cardiac output–corrected perfusion for each individual pony from baseline to the time when PiNO had been delivered for 30 minutes was calculated as cardiac output–corrected perfusion at baseline/(cardiac output–corrected perfusion after 30 minutes of PiNO – 1). The percentage change in cardiac output–corrected perfusion for each individual pony after 30 minutes of PiNO to 30 minutes after PiNO was discontinued was calculated as cardiac output–corrected perfusion after 30 minutes of PiNO/(cardiac output–corrected perfusion 30 minutes after PiNO was discontinued – 1). Similar calculations were made for measurements obtained while ponies were being mechanically ventilated.

Statistical analysis

A mixed procedure performed with standard software (SAS version 9.4; SAS Institute Inc) was

Table 1—Mean value ± SD pulmonary perfusion (mL/min/g of lung tissue) at baseline, after 30 minutes of pulsed inhaled nitric oxide (PiNO) delivery, and 30 minutes after PiNO was discontinued in aerated and atelectatic lung regions in 6 anesthetized ponies positioned in dorsal recumbency.

	Pulmonary perfusion (mL/min/g of lung tissue)		
Variable	**Baseline**	**After 30 minutes of PiNO**	**30 minutes after PiNO discontinued**
Aerated lung region			
SB end expiration	4.0 ± 1.9	7.2 ± 3.0[a]	4.9 ± 1.6[b]
MV end expiration	4.6 ± 1.2	7.5 ± 3.1[a]	5.2 ± 1.7[b]
MV peak inspiration	4.1 ± 0.5	5.2 ± 0.6[a]	4.5 ± 0.9[b]
Atelectatic lung region			
SB end expiration	5.0 ± 1.2	3.6 ± 1.3[c]	4.0 ± 0.5
MV end expiration	2.7 ± 0.7[d]	2.2 ± 0.9[c,d]	2.3 ± 1.1[d]
MV peak inspiration	2.7 ± 0.6[d]	2.3 ± 1.2[c,d]	2.5 ± 1.0[d]

Measurements were obtained at end expiration while ponies were spontaneously breathing (SB) and at end expiration and peak inspiration while ponies were mechanically ventilated (MV).

[a]Significantly (P < 0.01) different from baseline perfusion. [b]Significantly (P < 0.05) different from perfusion after 30 minutes of PiNO. [c]Significantly (P < 0.05) different from baseline perfusion. [d]Significantly (P < 0.01) different from perfusion measured during spontaneous breathing.

used for statistical analyses. Assumptions underlying the models were checked with diagnostic plots. Post hoc comparisons were adjusted for multiplicity with the Tukey method. Because several observations were performed on each pony, data were analyzed by means of mixed linear models.[23] For each response variable, data collection time point (baseline, 30 minutes of PiNO, and 30 minutes after discontinuation of PiNO), ventilation mode (spontaneous breathing and mechanical ventilation), and the interaction between these 2 factors were included as fixed factors. Pony was included as a random factor. Values of P < 0.05 were considered significant. Data are presented as mean ± SD.

Results

Distribution of perfusion

Perfusion to poorly aerated lung regions (–100 to –350 HU) did not differ significantly among data collection time points (baseline vs after 30 minutes of PiNO vs 30 minutes after discontinuation of PiNO); therefore, data for these regions were not included in further analyses and are not reported.

Pulmonary perfusion values obtained for aerated and atelectatic lung regions during a 50-second breath hold while ponies were spontaneously breathing (end expiration) and were being mechanically ventilated (end expiration and peak inspiration) were summarized **(Table 1)**. Mixed linear modeling showed that pulmonary perfusion was affected by both data collection time point and ventilation mode. For both ventilation modes, perfusion was significantly increased in aerated lung regions after 30 minutes of PiNO administration, compared with baseline values, and was significantly decreased 30 minutes after dis-

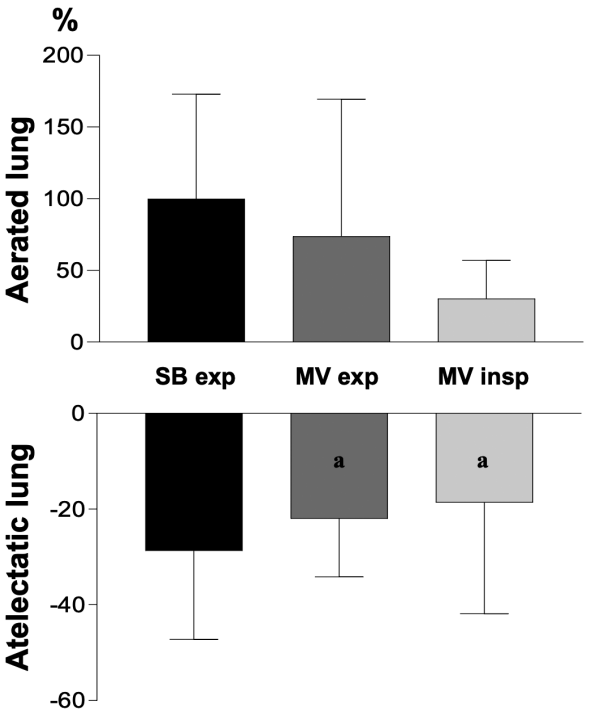

Figure 1—Mean ± SD percentage change in cardiac output–corrected pulmonary perfusion in aerated and atelectatic lung regions between baseline perfusion and perfusion after 30 minutes of pulsed inhalation of nitric oxide in 6 anesthetized ponies positioned in dorsal recumbency. Measurements were obtained at end expiration while ponies were spontaneously breathing (SB exp) and at end expiration (MV exp) and peak inspiration (MV insp) while ponies were mechanically ventilated. For all 3 conditions, the percentage change was significantly (P < 0.001) different from 0. [a]Value was significantly (P < 0.001) different from value obtained when ponies were spontaneously breathing.

continuation of PiNO, compared with values obtained after 30 minutes of PiNO. Similarly, for both ventilation modes, perfusion was significantly decreased in atelectatic lung regions after 30 minutes of PiNO, compared with baseline values. When perfusion was corrected for cardiac output, perfusion was again significantly increased in aerated lung regions and significantly decreased in atelectatic lung regions between baseline measurements and measurements obtained after 30 minutes of PiNO **(Figure 1)**. However, the decrease in perfusion in atelectatic lung regions was significantly greater during spontaneous breathing than during mechanical ventilation.

Cardiovascular changes

During both spontaneous breathing and mechanical ventilation, mean pulmonary arterial blood pressure and pulmonary vascular resistance were significantly decreased after 30 minutes of PiNO, compared with baseline values **(Table 2)**. Venous admixture was significantly lower during mechanical ventilation than during spontaneous breathing at all data collection time points, and for both ventilation modes, venous admixture was significantly lower after 30 minutes of PiNO than at baseline. There were no significant differences in mean arterial blood pressure, cardiac index, lactate concentration, or dobutamine dose among data collection time points. At all data collection time points, heart rate and hemoglobin concentration were significantly higher during mechanical ventilation than during spontaneous breathing.

Ventilation and indices of oxygenation

End-tidal isoflurane concentrations did not differ significantly among data collection time points or between ventilation modes. Minute ventilation, respiratory rate, and pH were all significantly higher during mechanical ventilation than during spontaneous breathing **(Table 3)**. The $Paco_2$ was significantly lower during mechanical ventilation than during spontaneous breathing and differed significantly among data collection time points. Estimated alveolar dead space was significantly lower after 30 minutes of PiNO than at baseline. The alveolar-arterial difference in partial pressure of oxygen was lower and Pao_2 was higher after 30 minutes of PiNO than at baseline during both spontaneous breathing and mechanical ventilation, even though the inspired oxygen fraction was significantly lower after 30 minutes of PiNO. The mixed venous partial pressure of oxygen was significantly higher after 30 minutes of PiNO than at baseline but was significantly lower during mechanical ventilation than dur-

Table 2—Mean ± SD cardiovascular measurements for the six ponies in Table 1.

Variable	Baseline	After 30 minutes of PiNO	30 minutes after PiNO discontinued
Heart rate (beats/min)			
SB	56 ± 18	53 ± 18	56 ± 18
MV	58 ± 16[a]	59 ± 16[a]	65 ± 20[a]
Cardiac index (mL/kg/min)			
SB	9.9 ± 4.2	9.7 ± 4.1	10.3 ± 4.4
MV	8.5 ± 1.9	8.9 ± 2.4	9.0 ± 2.1
Mean arterial blood pressure (mm Hg)			
SB	80 ± 13	76 ± 13	74 ± 10
MV	73 ± 13	73 ± 11	73 ± 15
Mean pulmonary arterial blood pressure (mm Hg)[b]			
SB	20 ± 6	16 ± 4	18 ± 7
MV	18 ± 5	16 ± 4	20 ± 8
Pulmonary vascular resistance (dynes/s/cm^{-5})[b]			
SB	19.7 ± 5.9	14.9 ± 4.0	17.6 ± 6.8
MV	17.1 ± 4.9	15.1 ± 4.1	16.6 ± 3.6
Venous admixture (%)[c]			
SB	49 ± 9	37 ± 10	43 ± 8
MV	34 ± 7[d]	29 ± 9[d]	35 ± 6[d]
Hemoglobin (g/dL)			
SB	89 ± 15	95 ± 13	100 ± 15
MV	105 ± 19[e]	106 ± 23[e]	108 ± 25[e]

[a]Significantly ($P < 0.05$) different from value obtained when ponies were spontaneously breathing. [b]Values differed significantly ($P < 0.05$) among data collection time points. [c]Values differed significantly ($P < 0.001$) among data collection time points. [d]Significantly ($P < 0.001$) different from value obtained when ponies were spontaneously breathing. [e]Significantly ($P < 0.01$) different from value obtained when ponies were spontaneously breathing.

Table 3—Mean ± SD ventilation and oxygenation measurements obtained for the ponies in Table 1.

Variable	Baseline	After 30 minutes of PiNO	30 minutes after PiNO discontinued
Minute ventilation (L/min)			
SB	15.9 ± 4.3	13.2 ± 3.1	14.2 ± 5.9
MV	24.2 ± 4.5[d]	21.7 ± 1.8[d]	26.6 ± 7.2[d]
Alveolar dead space (%)[b]			
SB	28 ± 11	20 ± 7	24 ± 7
MV	26 ± 5	23 ± 5	27 ± 5
Respiratory rate (breaths/min)			
SB	6.3 ± 2.7	5.7 ± 2.4	5.3 ± 2.5
MV	7.3 ± 1.2[a]	6.3 ± 0.5[a]	6.8 ± 1.2[a]
Peak inspiratory pressure (cm H_2O)			
SB	0	0	0
MV	25.2 ± 5.0[d]	26.2 ± 5.3[d]	25.2 ± 6.5[d]
Arterial pH			
SB	7.24 ± 0.05	7.24 ± 0.08	7.23 ± 0.07
MV	7.38 ± 0.06[d]	7.40 ± 0.04[d]	7.37 ± 0.08[d]
Pa_{CO_2} (mm Hg)[b]			
SB	76.5 ± 11.3	84.8 ± 15.0	89.3 ± 15.0
MV	57.8 ± 8.3[a]	51.2 ± 7.5[a]	52.5 ± 9.0[a]
F_{IO_2}[b]			
SB	0.85 ± 0.06	0.83 ± 0.04	0.85 ± 0.05
MV	0.84 ± 0.06	0.83 ± 0.05	0.84 ± 0.06
$P_{AO_2} - Pa_{O_2}$ (mm Hg)[c]			
SB	396 ± 56	274 ± 113	326 ± 80
MV	274 ± 90[d]	192 ± 82[d]	256 ± 40[d]
Pa_{O_2} (mm Hg)[c]			
SB	128 ± 48	230 ± 114	186 ± 80
MV	266 ± 94[d]	345 ± 86[d]	287 ± 55[d]
$P\bar{v}_{O_2}$ (mm Hg)[b]			
SB	52.5 ± 7.5	60.0 ± 8.3	59.3 ± 7.5
MV	51.8 ± 6.0[a]	54.8 ± 9.0[a]	53.3 ± 7.5[a]
Sa_{O_2} (%)			
SB	93.8 ± 2.9	95.9 ± 1.1	95.3 ± 1.5
MV	96.4 ± 0.6[d]	96.8 ± 0.3[d]	96.7 ± 0.1[d]
Ca_{O_2} (mL/L)			
SB	117 ± 16	131 ± 14	135 ± 18
MV	146 ± 25[d]	150 ± 30[d]	150 ± 33[d]
$Ca_{O_2} - C\bar{v}_{O_2}$ (mL/L)			
SB	21.2 ± 8.4	21.7 ± 6.2	20.8 ± 5.3
MV	25.3 ± 5.4[a]	25.2 ± 9.3[a]	23.6 ± 5.4[a]
Oxygen delivery (L/min)			
SB	2.0 ± 0.4	2.2 ± 0.6	2.5 ± 0.9
MV	2.3 ± 0.6	2.5 ± 0.8	2.6 ± 1.2
Oxygen extraction ratio (%)			
SB	18 ± 7	17 ± 5	15 ± 4
MV	18 ± 6	17 ± 8	16 ± 6

Ca_{O_2} = Oxygen content of arterial blood. $Ca_{O_2} - C\bar{v}_{O_2}$ = Arterial–mixed venous difference in oxygen content. F_{IO_2} = Inspired oxygen fraction. PaCO2 = Partial pressure of carbon dioxide. $P_{AO_2} - Pa_{O_2}$ = Alveolar-arterial difference in partial pressure of oxygen. $P\bar{v}_{O_2}$ = Mixed venous partial pressure of oxygen. Sa_{O_2} = Arterial oxygen saturation of hemoglobin.

See Tables 1 and 2 for remainder of key.

ing spontaneous breathing. The arterial–mixed venous difference in oxygen content was significantly higher during mechanical ventilation than during spontaneous breathing. Oxygen delivery and oxygen extraction ratio did not differ significantly among data collection time points or between ventilation modes.

Discussion

Results of the study reported here showed that in anesthetized, spontaneously breathing and mechanically ventilated anesthetized ponies positioned in dorsal recumbency, PiNO administration increased

perfusion in nondependent, aerated lung regions and decreased perfusion in dependent, atelectatic lung regions. This effect was more pronounced when ponies were spontaneously breathing than when they were being mechanically ventilated. This favorable redistribution of blood flow in the lung improved arterial oxygenation by reducing venous admixture and alveolar dead space.

Ever since derangements in gas exchange were first recognized in anesthetized horses, numerous methods to improve arterial oxygenation and oxygen delivery have been attempted.[24] Although many of these interventions have been successful to some extent, many of them result in unpredictable adverse effects on cardiac output or have little impact on oxygen delivery and tissue oxygenation.[10,25,26] Additionally, most techniques necessitate mechanical ventilation, which, in itself, can have cardiopulmonary consequences.[14,15] Without attempts to reinflate atelectatic lung regions through recruitment maneuvers or the use of positive end-expiratory pressure, arterial oxygenation can only improve by redistribution of pulmonary blood flow to alveoli that are being ventilated. In the present study, we have found that PiNO favorably redistributed pulmonary perfusion, leading to significant improvements in arterial oxygenation, irrespective of ventilation mode.

Redistribution of blood flow against gravity in the large, vertical thorax of horses has been proposed as a possible mechanism of action for PiNO. Using a scintigraphic method in anesthetized horses, Grubb et al[19] demonstrated that administration of PiNO leads to movement of blood from atelectatic regions of lung to aerated regions. However, that study was limited by the small number of animals and the poor spatial and temporal resolution of the images. Additionally, quantification of pulmonary perfusion was not possible. It also differed from the present study in that horses breathed spontaneously and were not mechanically ventilated. By developing a novel CT angiographic method for measuring pulmonary perfusion,[20] we were able to better quantify regional pulmonary perfusion in anesthetized ponies and document the effect of PiNO on pulmonary perfusion during different ventilation modes.

Beneficial effects of PiNO on arterial oxygenation and oxygen delivery in anesthetized horses in the absence of any deleterious effects has been reported in several previous studies.[7,11,27] However, the precise, quantitative effect on pulmonary perfusion has been elusive, largely owing to difficulties in imaging the equine lung. In the present study, we demonstrated the effects of PiNO as a selective pulmonary vasodilator during different modes of ventilation. We did not document any adverse effects of PiNO, providing further evidence of the safety and efficacy of this technique.

The effect of PiNO on pulmonary vascular resistance in anesthetized horses has not been established. The present study demonstrated that with PiNO, pulmonary vascular resistance decreases, but the magnitude of that decrease was clinically unimportant, suggesting that pulmonary vasodilation was selective. There was no effect on arterial blood pressure, further supporting the presumption of a selective action in the pulmonary circulation. This is worthwhile to document because hypotension commonly occurs in anesthetized equids through a variety of mechanisms and should not be compounded by administration of a drug that further reduces blood pressure. Dobutamine was being administered concurrently to support cardiac output and blood pressure in the present study, and this may have masked the effect of PiNO on the systemic vasculature. However, there was no increased requirement for dobutamine during PiNO administration.

Mechanical ventilation is frequently necessary in anesthetized horses to manage hypoventilation associated with general anesthesia and recumbency. Therefore, it was important that the effect of PiNO on pulmonary perfusion during periods of mechanical ventilation was investigated. In the present study, arterial oxygenation was superior during mechanical ventilation, compared with spontaneous breathing, even prior to PiNO, supporting previous findings.[28] This may be explained by an improvement in alveolar ventilation during mechanical ventilation.[29] However, regardless of the Pa_{O_2} starting point, the administration of PiNO resulted in a similar redistribution of blood flow in the lung during both mechanical ventilation and spontaneous breathing. During mechanical ventilation, well-ventilated regions of lung may become overventilated and pulmonary blood flow may be pushed into dependent regions of lung. This may worsen oxygenation owing to increased intrapulmonary shunting as more blood is driven into nonventilated regions of the lung.[17] We could not demonstrate that shunting was worse during periods of mechanical ventilation in the present study, and venous admixture was actually lower during mechanical ventilation. Nevertheless, during PiNO delivery, there was an immediate and significant reduction in venous admixture and significant improvement in arterial oxygenation, demonstrating that even during periods of mechanical ventilation, gas exchange was improved. This was verified by increased perfusion of aerated lung regions and reduced perfusion of atelectatic lung regions, as occurred during spontaneous breathing. Thus, it seems possible that PiNO may be able to counteract the detrimental effect on pulmonary blood flow when mechanical ventilation is used. Although these ponies were not hypoxemic prior to PiNO, the mechanism of action was important to document, because successful treatment of hypoxemia in anesthetized and mechanically ventilated horses with PiNO has been described elsewhere.[13]

The Pa_{CO_2} was higher when ponies were spontaneously breathing than when they were mechanically ventilated in the present study, likely because of decreased alveolar ventilation, and this may have affected our results. The independent effect of carbon dioxide concentration on the pulmonary vasculature in anesthetized horses has not been described, and there are contradictory experimental reports in other species. In anesthetized hypoxemic dogs, pulmonary vascular resistance increased significantly

in the presence of high $Paco_2$, suggestive of a vasoconstrictive effect.[30] Conversely, in isolated rat lung preparations, hypercapnia resulted in vasodilatation in the presence of preexisting high pulmonary arterial blood pressures.[31] Furthermore, this latter study also showed that endogenous nitric oxide did not augment the hypercapnia-induced vasodilatation in the pulmonary vasculature. It is unclear whether exogenously administered nitric oxide in our study would have had effects similar to those of endogenous nitric oxide. We did not observe differences in pulmonary vascular resistance between modes of ventilation, suggesting that $Paco_2$ differences did not influence our results.

Ventilation-perfusion relationships are directly affected by changes in cardiac output.[32] For this reason, when we compared pulmonary perfusion among data collection time points, the change in cardiac output in individual ponies at the time of measurements was also considered. From our cardiac output–corrected perfusion calculations, it was clear that PiNO was more effective during spontaneous breathing versus mechanical ventilation. Thus, although oxygenation improves, mechanical ventilation may have a negative impact on perfusion to aerated regions of lung.[17] The favorable respiratory mechanics of spontaneous breathing and the superior effect of PiNO during spontaneous breathing in anesthetized horses has been described in earlier work.[33] During mechanical ventilation, Pao_2 values were significantly greater at baseline, compared with values measured during spontaneous breathing, owing to improved ventilation and a lower alveolar-arterial difference in partial pressure of oxygen. Consequently, the effect of PiNO during mechanical ventilation may appear somewhat blunted as a result.

There were some limitations to the present study. Limitations associated with the CT angiographic method of measuring pulmonary perfusion have been reported previously.[20] Owing to the size of the CT gantry, it was only possible to perform this experiment with ponies. Although the cardiopulmonary physiology will be similar in larger breeds of horse, the magnitude of the effects may be different. The ponies in this study did not become hypoxemic (Pao_2 < 60 mmHg) at any time during the experiment. Once again, this may have been due to the fact that the horses we used, because of size limitation of the CT gantry, were small, and the development of gas exchange derangements and hypoxemia is known to be related to body mass and thoracic dimensions.[34] Importantly, however, the focus of the present study was to document the change in distribution of pulmonary perfusion in response to PiNO during spontaneous breathing and mechanical ventilation regardless of the baseline Pao_2. We did not randomize the ventilation mode (ie, measurements were always obtained first while ponies were spontaneously breathing and then while they were being mechanically ventilated). This was based on our previous experience and pilot studies demonstrating that it is problematic to switch from prolonged mechanical ventilation to spontaneous breathing in a single experimental period in anesthetized, dorsally recumbent ponies. Furthermore, because spontaneous breathing is physiologically normal, we did not want to disrupt pulmonary perfusion characteristics with an extended period of mechanical ventilation before examining the effects of PiNO in spontaneously breathing animals. Therefore, we opted to allow ponies to first breathe spontaneously prior to initiating mechanical ventilation.

In conclusion, PiNO in dorsally recumbent, anesthetized ponies favorably redistributed pulmonary blood to nondependent ventilated lung regions so that arterial oxygenation improves, regardless of ventilation mode. However, the effect of PiNO on perfusion was more pronounced during spontaneous breathing. This selective pulmonary vasodilatory effect did not appear to have deleterious effects in the systemic circulation. Our findings suggest that PiNO may be a useful treatment option for hypoxemic anesthetized horses and could now be developed for clinical use.

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