



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Veterinary Medicine
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Department of Animal Environment and Health

Pulsed inhaled Nitric Oxide - a possible way to treat hypoxemia in colic horses during abdominal surgery

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Pulsed inhaled Nitric Oxide - a possible way to treat hypoxemia in colic horses undergoing abdominal surgery

Pulsad inhalerad NO (kvävemonoxid) - en möjlig metod för att behandla hypoxemi hos kolikhästar vid bukkirurgi

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SUMMARY

The aim of the present study was to evaluate if pulsed inhaled nitric oxide (PiNO) could be used as a treatment for hypoxemia during general anaesthesia in colic horses. Previous studies have shown a positive response in horses receiving PiNO in comparison to controls, but these studies have only included healthy individuals. A total of 30 horses were included in this present study, they all underwent abdominal surgery because of acute colic that could not be medically treated. Fifteen of the horses received PiNO and 15 horses served as a control group. Arterial oxygenation was improved in all horses receiving PiNO. PaO_2 , SaO_2 , CaO_2 and $P(A-a)O_2$ increased as a result of a decrease in right to left vascular-shunt (Q_s/Q_t) due to the inhalation of NO. The conclusion is that PiNO is an effective way to treat hypoxemia in colic horses undergoing abdominal surgery.

SAMMANFATTNING

Syftet med detta arbete var att utvärdera effekterna av pulsad inhalerad kväveoxid (PiNO) och se om det kan användas för att behandla hypoxemi under allmän narkos på kolikhästar. Tidigare studier har visat positiva effekter hos hästar som fått PiNO i jämförelse med kontroller, dessa studier har dock bara inkluderat friska individer. Totalt ingick 30 hästar i denna studie och alla dessa buköppnades på grund av akut kolik som inte gick att behandla medicinskt. Femton hästar fick PiNO under narkos och 15 hästar ingick i kontrollgruppen. Alla hästar som fick PiNO fick en förbättrad syresättning; de fick ökat syretryck (P_{aO_2}), ökad syremättnad (S_{aO_2}), ökat syrenehåll i arteriellt blod (C_{aO_2}) och minskad alveolär-arteriell syredifferens ($P(A-a)O_2$) vilket berodde på en minskad shunt (Q_s/Q_t) som var ett resultat av den inhaleerade kväveoxiden. Slutsatsen är att PiNO är en effektiv metod för att behandla hypoxemi hos kolikhästar vid bukkirurgi.

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ABBREVIATIONS

CaO ₂	Oxygen content in arterial blood
CcO ₂	Oxygen content in pulmonary capillaries
CvO ₂	Oxygen content in mixed venous (pulmonary arterial) blood
ET-1	Endothelin-1
FiO ₂	Fraction of inspired oxygen
Hb	Hemoglobin
iNO	Inhaled nitric oxide
IPPV	Intermittent positive pressure ventilation
N ₂	Nitrogen gas
NO	Nitric oxide
NO ₂	Nitrogen dioxide
P(A-a)O ₂	Partial pressure difference of alveolar-arterial O ₂
PaCO ₂	Partial pressure of CO ₂ in arterial blood
PAO ₂	Partial pressure of oxygen in the alveolar gas
PaO ₂	Partial pressure of oxygen in arterial blood
PAP	Pulmonary arterial pressure
PEEP	Positive end-expiratory pressure
PiNO	Pulsed inhaled nitric oxide
Q _s /Q _t	Ratio of shunted blood (Q _s) to total blood flow
Q _t	Cardiac output
SaO ₂	Oxygen/hemoglobin saturation of arterial blood
V _A /Q	Ventilation/perfusion ratio

INTRODUCTION

During general anaesthesia many horses become hypoxemic. This is due to a ventilation/perfusion mismatch, which is worst when the horse is positioned in dorsal recumbency (Dobson *et al.*, 1985; Nyman & Hedenstierna, 1989). Hypoxemia in horses is associated with many side effects including lactic acidemia, increased lactate concentrations, postanaesthetic cerebral necrosis and decreased oxygenation in skeletal muscles (Taylor, 1999; McKay *et al.*, 2002; Portier *et al.*, 2009).

Recent studies (Grubb *et al.*, 2008; Grubb *et al.*, 2012; Nyman *et al.*, 2012; Grubb *et al.*, 2013) have shown that pulsed inhaled nitric oxide (PiNO) during the first part of inspiration effectively redistributes blood flow from dependent atelectatic lung regions to non-dependent ventilated areas. The effect and security of the treatment with PiNO has so far been demonstrated in healthy horses. This study is a follow-up of previous studies and a pilot study where PiNO was given to clinic cases. If the results in this pilot study are in agreement with previous results, PiNO can become a general treatment to improve oxygenation in horses during general anaesthesia.

Based on previous results, the hypothesis is that arterial oxygen tension, arterial oxygen saturation, arterial oxygen content, alveolar-arterial gradient and degree of shunt is improved in horses receiving PiNO compared to control horses. Further, another hypothesis is that a low arterial oxygen tension at the start of anaesthesia results in a larger relative increase in arterial oxygen tension within the group of horses receiving PiNO.

The aim of the present study was to evaluate the effect of PiNO on arterial oxygenation in colic horses during abdominal surgery.

LITERATURE REVIEW

The mortality rate is much higher in colic horses undergoing abdominal surgery compared to non-colic surgery cases. A prospective observational study showed a mortality rate of 0.9% for non-colic horses and a mortality rate of 8.0% for colic horses (Johnston *et al.*, 2002). Colic horses are in a negative oxygen balance even before they are anaesthetized and during anaesthesia the muscle oxygenation is insufficient compared to healthy horses (Edner *et al.*, 2007). In a retrospective study from 1983, 13.1% of horses that underwent colic surgery between September 1974 and February 1980 were hypoxemic during anaesthesia (Pascoe *et al.*, 1983). In another study, 17% of the horses showed hypoxemia during surgery, and the horses that did not survive had a higher prevalence of complication during anaesthesia, hypoxemia being one of them (Trim *et al.*, 1989). Proudman *et al.* (2006) suggest in a study that hypoxemia during anaesthesia influence the post-operative prognosis negatively for colic horses. It is also suggested that hypoxemia contributes to an increased risk of developing pulmonary edema (Borer, 2005). Once a horse have developed hypoxemia during general anaesthesia it will remain throughout the whole duration of anaesthesia and also during the recovery period (Trim & Wan, 1990). In the same study they concluded that nothing in the evaluation of the horses before anaesthesia could help to predict which horses were at risk to develop hypoxemia as a complication during surgery.

When a horse is positioned in dorsal recumbency during general anaesthesia, atelectasis develops in the lowest part of the lung (Nyman *et al.*, 1990). The best ventilated area is in the upper cranioventral part of the lungs but perfusion is mainly distributed to the lower caudodorsal part (Dobson *et al.*, 1985). Thus, most of the circulated blood will not come in contact with well-ventilated areas of lungs; the blood is shunted through the dependent lungs without gas exchange. There are two hypothetical ways to improve the matching of ventilation and perfusion, either the ventilation can be directed to lung regions that are well perfused or the perfusion could be redistributed to the well-ventilated lung areas.

Nyman & Hedenstierna (1989) and Edner *et al.* (2005) showed in different studies that neither conventional mechanical ventilation (intermittent positive pressure ventilation (IPPV)) nor positive end-expiratory pressure (PEEP) had a positive effect on PaO₂ or the ventilation/perfusion ratio. In other words it did not decrease the degree of the shunt. However, selective mechanical ventilation of dependent lung regions in horses in dorsal recumbency does increase PaO₂ during general anaesthesia (Nyman *et al.*, 1987). Selective mechanical ventilation is a recruitment maneuver where collapsed alveoli get reinflated and remains opened with PEEP (Nyman *et al.*, 1987; Lapinsky & Mehta, 2005). One disadvantage of this method is that it requires tracheotomy.

Nitric oxide (NO) is a potent vasodilator that is short lived and released from arteries and veins and causes smooth muscle relaxation (Ignarro *et al.*, 1987). Inhaled nitric oxide (iNO) works as a selective pulmonary vasodilator and does not cause systemic effects in the rest of the body (Frostell *et al.*, 1991). One problem with iNO is that in higher concentration it can form nitrogen dioxide (NO₂) which is a toxic metabolite (Warren & Higenbottam, 1996). These earlier studies have been done in humans. One study that looked at the effects of continuous iNO in horses, showed that it had no positive effect (Young *et al.*, 1999). Instead of giving iNO continuously it can be administered in the beginning of each breath, which reduces the total amount of given NO and therefore reduces the risk of toxic effects (Katayama *et al.*, 1998). In horses pulsed inhaled nitric oxide (PiNO) turned out to be a possible way to counteract hypoxemia during general anaesthesia (Heinonen *et al.*, 2001). The best effect to achieve a reduced shunt was received when NO was given during the first 30 or 60% of the inspiration (Heinonen *et al.*, 2002). In the same study no side effects concerning cardiac output (Qt) or pulmonary arterial pressure (PAP) occurred. The improvement in arterial oxygenation during pulsed delivery of iNO can be sustained throughout 2.5 hours of anaesthesia. A pulse length between 30-45% was optimal in most treated cases. However, all previous studies with PiNO in horses have so far only included healthy horses. One study focused on what occurred after NO was discontinued since rebound has occurred in other species (Chen *et al.*, 2001). In horses, PaO₂ tend to decrease gradually but no rebound effects have been observed (Grubb *et al.*, 2008). A recent study showed that horses receiving PiNO both improved the ventilation/perfusion ratio (V_A/Q) and reduced the shunt (Q_s/Qt) thus leading to improved arterial oxygenation (Grubb *et al.*, 2013).

MATERIALS AND METHODS

The criteria for horses to become part of the study were that they showed signs of colic and underwent acute abdominal surgery at the equine clinic of UDS, SLU, Uppsala, Sweden between May 2012 and December 2013. Fifteen horses received PiNO and fifteen served as controls. Horses in the group that received PiNO aged from 1 year to 21 years with an average of 10 years, the controls were between 1 year and 20 years with an average of 11 years. The average weight in horses receiving PiNO was 543 kg (337-688 kg) and in controls 508 kg (250-616 kg). Both mares, geldings and stallions as well as different breeds were included in the study. The most common breed in both groups was Swedish warmblood. The same protocol was followed each time but since colic surgery in horses varies a lot in time the number of samples that was collected from each horse also varies. Some horses were euthanized on the operation table due to prognosis and the owner's decision.

Nitric oxide (NO) was given through a special device that has been designed for spontaneous breathing (Datex-Ohmeda Research Unit, Helsinki, Finland). The device triggers when the horse inhales due to the negative pressure that precede the start of delivery of the gas. The device makes it possible to change how long the inhaled pulse, in percent of the inspiration time, will be. Results from previous studies showed that at least 30% of the inspiration time is needed to get a positive effect by the NO in the horse (Nyman *et al.*, 2012). Since all horses are different and do not respond in the same way, blood gases were analyzed during surgery so the delivery time of iNO could be adapted for each case. The delivery device was connected to an adapter located at the proximal end of the endotracheal tube. The NO was supplied in a cylinder of 2,000 ppm medical grade NO in N₂ (AGA AB, Sweden).

Anaesthesia

The horses were anaesthetized following the standard protocol used in the Uppsala University equine clinic. Premedication included 1.1 mg kg⁻¹ flunixin meglumine (Flunixin N-vet; N-vet AB, Sweden), 0.03 mg kg⁻¹ acepromazine (Plegicil; Pharmaxim, Sweden), 0.1 mg kg⁻¹ romifidine (Sedivet; Boehringer Ingelheim Vetmedica, Sweden) and 0.025 mg kg⁻¹ butorphanol (Butador; Vetoquinol, Sweden) and induction with 0.03 mg kg⁻¹ diazepam (Diazepam-ratiopharm, Ratiopharm, Germany) and 2.2 mg kg⁻¹ ketamine (Ketaminol; Intervet, Sweden) was used. The horses were intubated and anaesthesia was maintained with isoflurane (IsoFlo; Orion Pharma Animal Health, Sweden) in oxygen. Most of the horses received a lidocaine infusion during surgery, 2 mg kg⁻¹ for bolus given over 15 to 20 minutes and thereafter a CRI with 2 mg kg⁻¹ h⁻¹. Low blood pressure was treated with dobutamine (Dobutamin Carino[®] 250 mg/50 ml) as required. Since the device for delivery of NO was triggered by a negative inspiratory pressure during spontaneous breathing, none of the horses in the study were on a ventilator; but some horses were given extra breaths manually if the respiratory rate was low for a longer period of time.

Instrumentation

All horses were instrumented with ECG electrodes placed for lead II analysis and measurement of heart rate. The skin over the facial artery was clipped free of hair and

aseptically prepared and a catheter was introduced for measurement of arterial blood pressure and for collection of arterial blood samples for blood gas analysis.

Collection of data

Once completely instrumented, data were then collected and used as anaesthesia baseline data. After baseline data collection, PiNO delivery commenced. Pulse rate was counted by manual palpation of the pulse on the facial artery and respiratory rate was counted by watching thoracic movements. Fraction of inspired and end-tidal oxygen (FiO_2 , EtO_2), carbon dioxide ($FiCO_2$, $EtCO_2$) as well as isoflurane was measured with side-stream capnography collecting gas from the endotracheal tube (Mindray, BeneView T5).

Blood gas analysis

Arterial and central or jugular venous blood samples were obtained for assessment of arterial and central venous oxygen tension, PaO_2 , PvO_2 , carbon dioxide tension, $PaCO_2$, $PvCO_2$, pH, lactate and glucose. Devices from Radiometer, ABL 500 and ABL 90 flex, was used for the blood gas analyzes. Venous blood samples were also collected in EDTA tubes for analyzes of hemoglobin (Hb) and endothelin-1 (ET-1). All blood samples were stored on ice. Hemoglobin was analyzed at the Hematology and chemistry laboratory that is located at the Swedish university of agricultural science, SLU, in Uppsala, where they use the Advia 2120 device for analysis of hemoglobin.

Calculated data

Shunt fraction (Q_s/Q_t) was calculated using:

$$Q_s/Q_t = (C_{cO_2} - C_{aO_2}) / (C_{cO_2} - C_{vO_2})$$

C_{cO_2} , C_{aO_2} , and C_{vO_2} are oxygen content in $ml L^{-1}$ of capillary, arterial, and central venous blood, respectively. These values were calculated as: $C_xO_2 = (Hb \times 1.36 \times S_xO_2) + (0.227 \times P_xO_2)$.

Alveolar-arterial oxygen difference $P(A-a)O_2$ was calculated using:

$$P(A-a)O_2 = PAO_2 - PaO_2$$

PAO_2 is the partial pressure of oxygen in the alveoli and PaO_2 is the partial pressure of oxygen in the arterial blood.

Partial pressure of alveolar oxygen (PAO_2) was calculated using:

$$PAO_2 = FiO_2 \times ((\text{atmospheric pressure} - 47) / 7.5) - (PaCO_2 / R)$$

R is the respiratory quotient (0.8). FiO_2 is the fraction of inspired oxygen. 47 is the water vapour pressure in alveoli in mmHg. To convert mmHg to kPa the pressure was divided by 7.5.

Statistics

All raw data collected was entered in Microsoft Excel 2010 and then processed. For the statistics the GraphPad Prism 5 program was used to calculate the data. Two-way ANOVA, linear regression, one-tailed and two-tailed unpaired t test was used to compare horses receiving PiNO and controls. For the statistics on SaO₂, changes had to be done to fulfill the conditions for doing a linear regression analysis. The formula used was $-\ln(100-\text{SaO}_2)$ and these data was then used to compare the two groups, horses receiving PiNO and controls. In the figures and tables the data is presented as mean \pm SEM. The difference was considered significant when $P < 0.05$ with a confidence interval of 95%.

All conditions for a two-way ANOVA is not fulfilled in this study since repeated measures was done on each individual, however this only gives a less significance in comparison to if a more advanced parametric test had been used.

RESULTS

Partial pressure of oxygen in arterial blood, PaO₂

PaO₂ increased compared to the baseline value in all of the horses receiving PiNO. Five of these horses had at the start of anaesthesia a PaO₂ below 8 kPa, all these horses increased their PaO₂ to above 10 kPa after receiving PiNO. Calculating the difference between highest measured PaO₂ during PiNO with the individual baseline value in each horse, there was a significant (P=0.0001) difference between horses receiving PiNO compared to controls (Table 1). Horses receiving PiNO had an 8.73 kPa ± 1.46 (mean ± SEM) increase in PaO₂ during anaesthesia (N=15). In the control group the same measurement showed a change of 0.24 kPa ± 1.22 (N=15). In Figure 1 the change in each horse is shown. Figure 2 shows the difference between the baseline value and the last measured value. Figure 3 shows the baseline value of each individual horse and the last measured value during anaesthesia. All horses receiving PiNO presented a higher PaO₂ in the end compared to baseline value. All but one had at the last measured PaO₂ a value above 10 kPa. Most controls (N=12) presented a lower PaO₂ at the end of anaesthesia in comparison to baseline value. Nine controls had a PaO₂ below 10 kPa at the last measured value and seven of these horses had a PaO₂ below 8 kPa.

Table 1. *Difference between the highest measured PaO₂ and baseline PaO₂ in kPa*

	PiNO	Control
Mean ± SEM	8.73 ± 1.46	0.24 ± 1.22
Minimum	2.55	-6.38
Maximum	22.08	14.26
Number of values	15	15

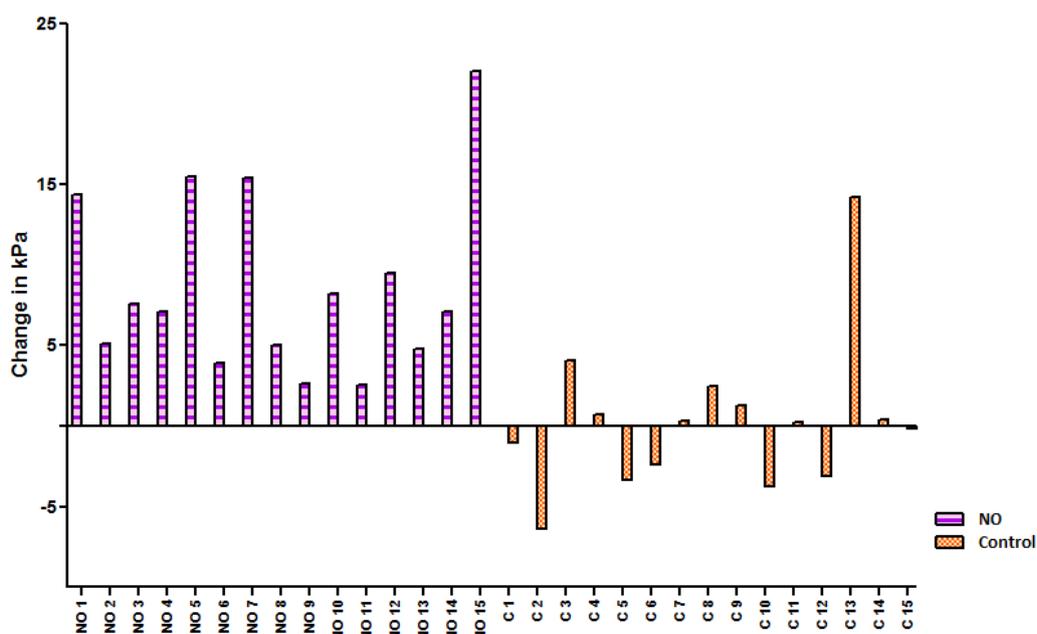


Figure 1. *Difference in PaO₂ in kPa when comparing the highest measured PaO₂ during anaesthesia with the baseline value in individual horses.*

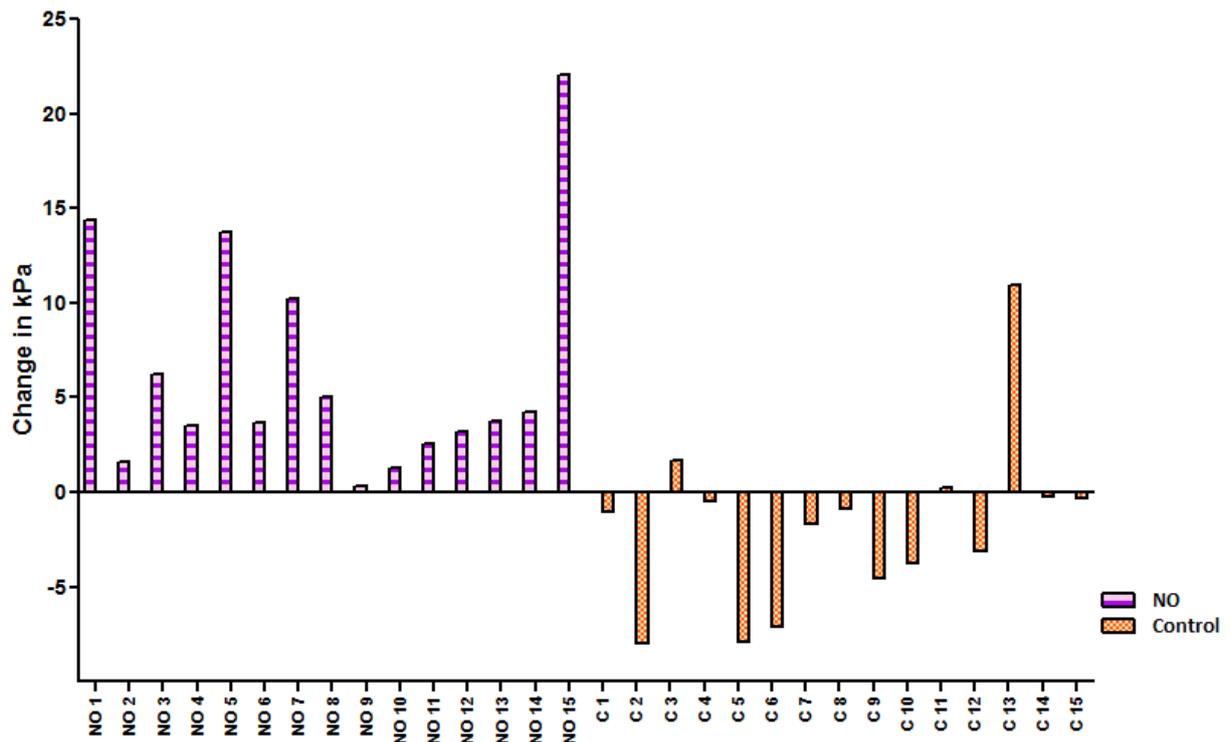


Figure 2. Difference in PaO₂ in kPa when comparing the last measured PaO₂ during anaesthesia with the baseline value in individual horses.

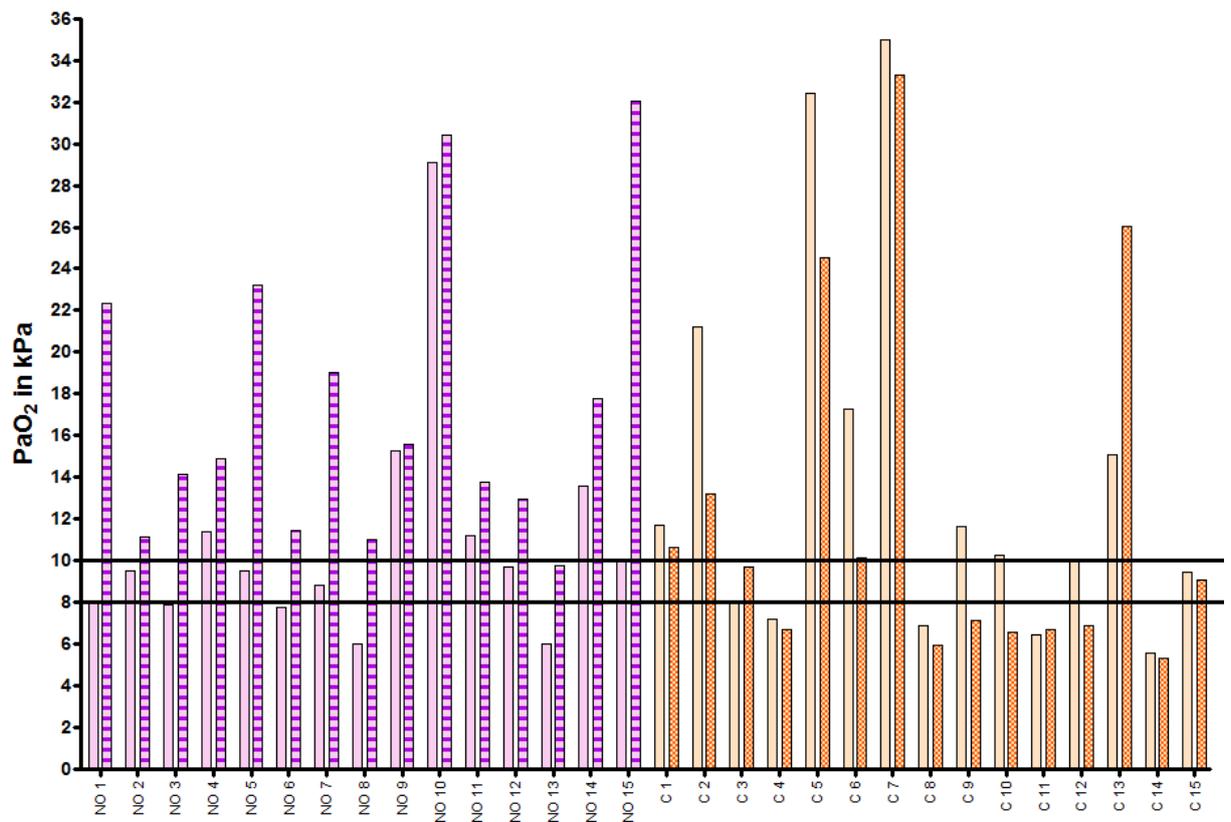


Figure 3. Illustration of the change in PaO₂ in individual horses, 15 receiving PiNO and 15 controls. For every horse the bar to the left is the baseline PaO₂ and the bar to the right is the last measured PaO₂ during anaesthesia.

Looking at change in PaO₂ over time with a two-way ANOVA the effect of PiNO was considered highly significant (P<0.0001). The difference between horses receiving PiNO and controls was significant at all times during anaesthesia. This is illustrated in Figure 4 and Table 2, where the change in percent in relation to the baseline value is displayed at different times during the anaesthesia.

Table 2. Mean change in % in relation to the baseline value at different times during anaesthesia. Comparison of horses receiving PiNO and controls, the P value shows how significant the difference between the two groups is.

Time	PiNO	Control	P value
0.5 hour	62.6	-2.3	< 0.001
1.0 hour	61.5	3.2	< 0.01
1.5 hour	61.1	-3.9	< 0.01
2.0 hours	70.5	-6.0	< 0.01
2.5 hours	101.3	-24.4	< 0.001
3.0 hours	82.0	-29.3	< 0.01
3.5 hours	88.0	-38.5	< 0.05
Overall mean	75.4	-14.5	

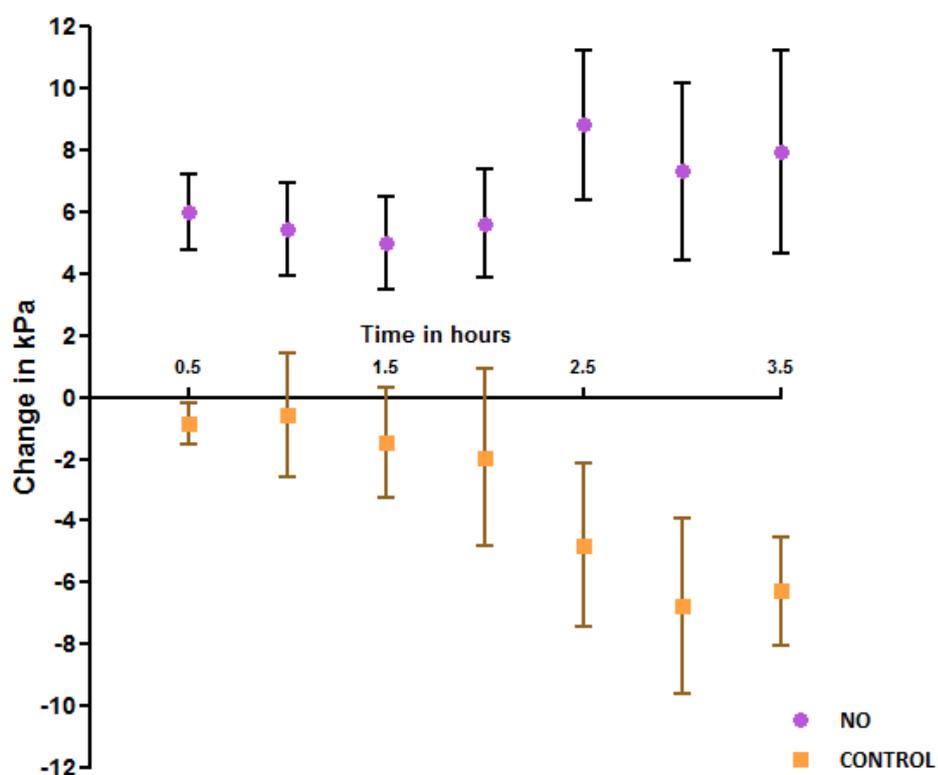


Figure 4. Change in PaO₂ (mean ± SEM) over time, a comparison between horses receiving PiNO and controls.

In Table 3 and 4 the individual change in PaO₂ for horses receiving PiNO is shown. Most of these horses (N=10) had a PaO₂ below 10 kPa at the beginning of anaesthesia while the rest (N=5) had a PaO₂ above 10 kPa. The horses with the lower baseline PaO₂ had a higher percentage change in comparison to the horses with a baseline value above 10 kPa. The change in PaO₂ was analyzed using a one-tailed unpaired t test and the difference between these two groups was significant (P<0.006).

Table 3. *The individual change in PaO₂ in % after receiving PiNO*

Horse	Baseline value	Change in %
NO 1	7.92	182
NO 2	9.48	54
NO 3	7.85	97
NO 4	11.37	62
NO 5	9.46	164
NO 6	7.75	50
NO 7	8.81	175
NO 8	5.98	84
NO 9	15.26	17
NO 10	29.11	28
NO 11	11.18	23
NO 12	9.69	98
NO 13	6.00	73
NO 14	13.57	53
NO 15	9.96	222

Table 4. *Mean change in % for horses with lower and higher PaO₂ baseline value*

	Number of values	Mean change in %
Baseline < 10 kPa	10	120
Baseline > 10 kPa	5	37

Oxygen saturation of hemoglobin in arterial blood, SaO₂

When looking at each individual horse and comparing the baseline value of SaO₂ with the last SaO₂ during anaesthesia, all the horses receiving PiNO (N=15) showed an increase in SaO₂ (Figure 5). Five of the horses had a SaO₂ of 90% or less at start and in four of these SaO₂ improved to above 95%. In the fifth of these SaO₂ was measured to 94.5% at end of anaesthesia compared to its baseline value of 81%. Another five horses had a SaO₂ between 91% and 95% at the start of anaesthesia and saturation improved to above 96% in all of them.

In the control group (N=15), the SaO₂ improved during anaesthesia in three horses. One of them started with a SaO₂ of 84% and in the end of anaesthesia it was 85%. Another horse had a baseline value of 91% and although the SaO₂ increased it was not above 95% at the end of anaesthesia. Three horses had a baseline value of SaO₂ around 95% (95-97%), in the last

sample collected during anaesthesia the SaO₂ had decreased to below 90% (84-87%). Two control horses had a SaO₂ below 90% at both the baseline value and the last sample.

A one-tailed unpaired t test on the change between the last and the baseline value of SaO₂ for each individual showed a highly significant (P<0.0001) difference between horses receiving PiNO and controls.

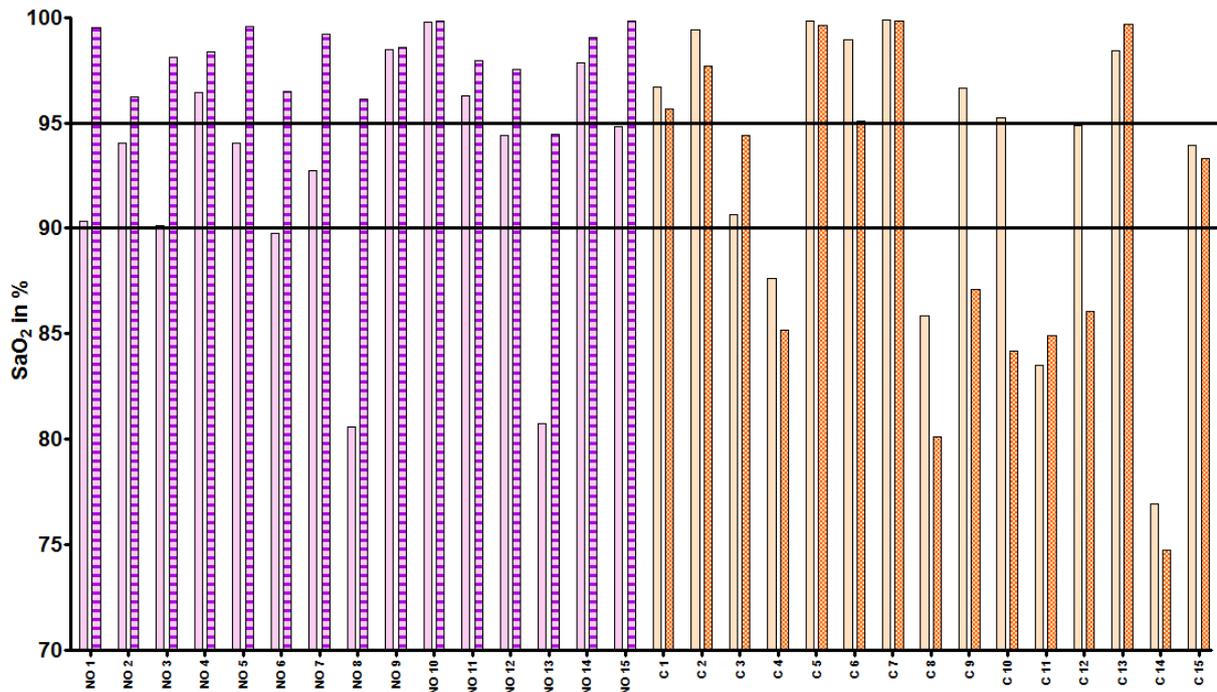


Figure 5. Illustration of the change in SaO₂ in individual horses, 15 receiving PiNO and 15 controls. For every horse the bar to the left is the baseline, SaO₂ and the bar to the right is the last measured SaO₂ during anaesthesia.

There was a large difference in oxygen saturation at the beginning of anaesthesia between the individual horses. Change over time in SaO₂ for horses receiving PiNO and control horses is illustrated in Figure 6 and 7. In Figure 6 the dotted lines represent an oxygen saturation of 95% and 90%. The difference between the slopes seen in Figure 7 were significant (P=0.014) and the P values by the lines indicate whether the slopes was deviated from zero or not. For horses receiving PiNO the slope was significantly (P<0.004) deviated from zero, in the control group the slope was not.

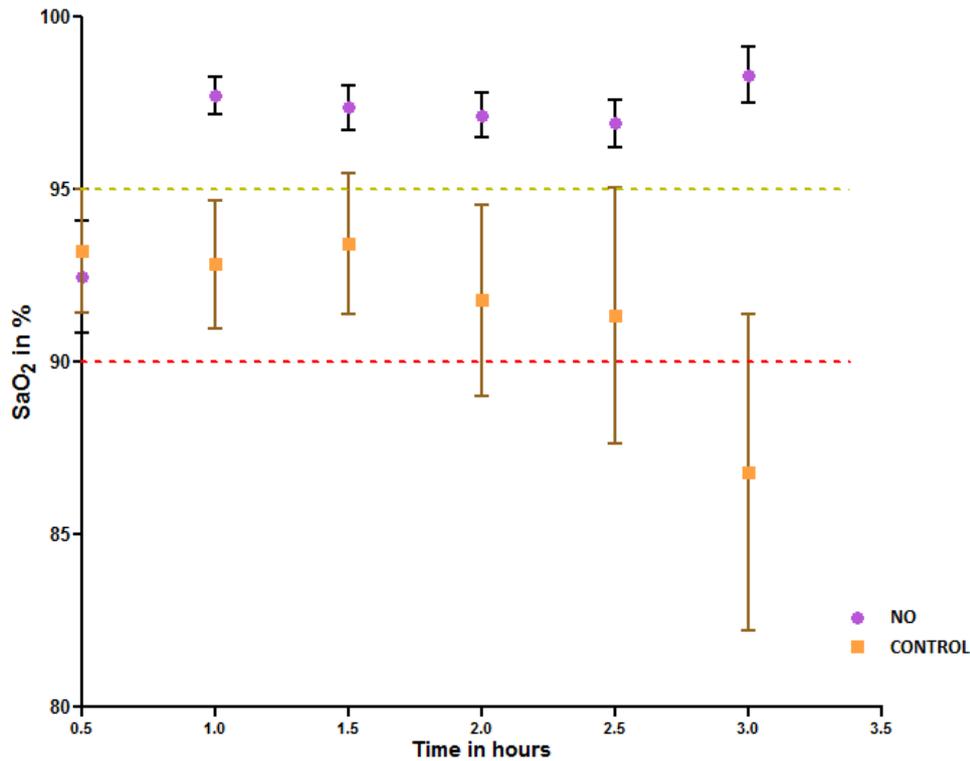


Figure 6. SaO₂ at different times during anaesthesia. Values are mean \pm SEM for horses receiving PiNO and controls.

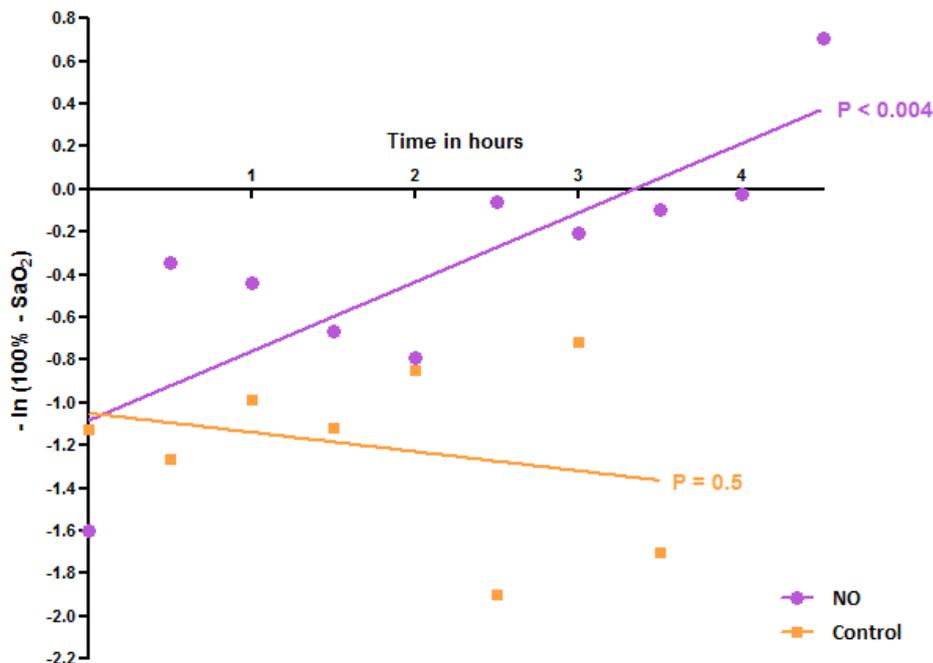


Figure 7. Change in SaO₂ over time, comparison between horses receiving PiNO (N=15) and controls (N=15). Note the significantly improved oxygen saturation over time in horses receiving PiNO. The P values indicate if the slopes are deviated from zero or not.

Oxygen content in arterial blood, CaO₂

The individual change in CaO₂, when comparing the baseline value with the highest measured value during anaesthesia, was analyzed using a one-tailed unpaired t test. There was a significant (P=0.0013) difference between the individuals that received PiNO (N=14) and individuals in the control group (N=13), this is illustrated in Figure 8.

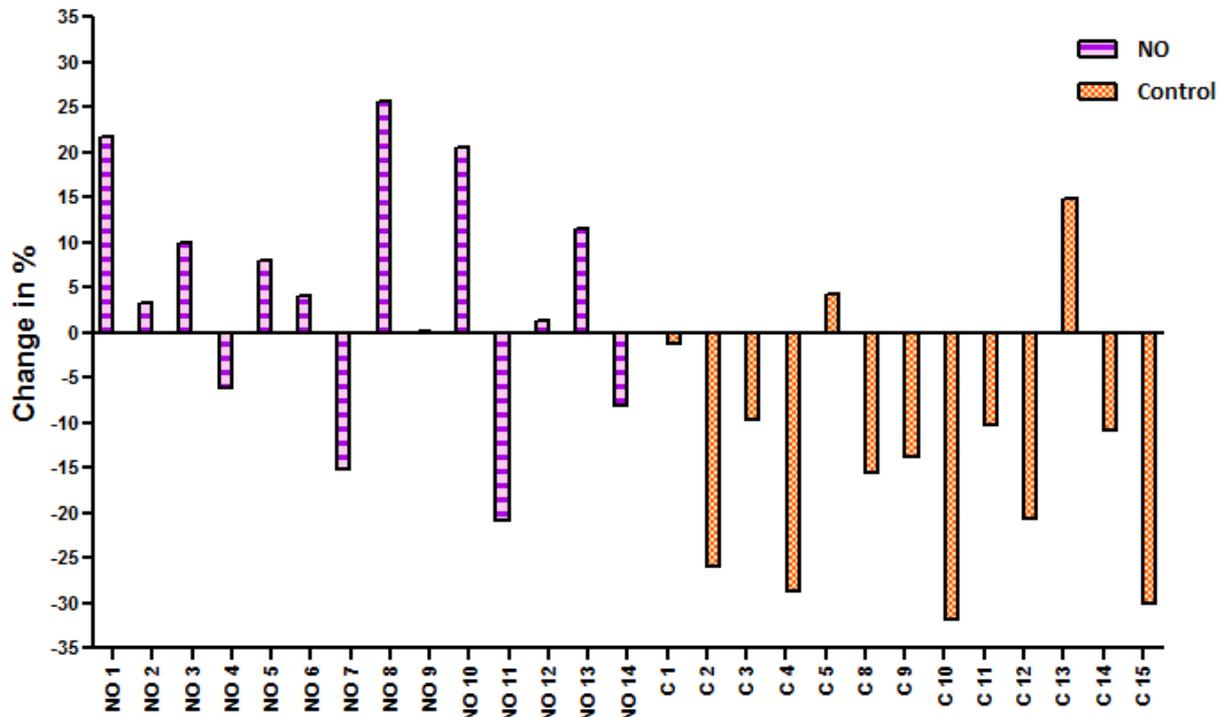


Figure 8. Change in CaO₂ in % when comparing the highest measured CaO₂ during anaesthesia with the baseline value in individual horses. Not all horses are included in this calculation since Hb was not obtained in all cases.

Shunt, Qs/Qt

Comparison of each individual's change in Qs/Qt during anaesthesia, calculated in percent and analyzed with a two-tailed unpaired t test, showed a significant (P<0.0001) difference between horses receiving PiNO and controls (Figure 9). The difference in each horse is between its lowest calculated Qs/Qt during anaesthesia and its baseline value. Horses receiving PiNO showed a decrease in Qs/Qt with a mean \pm SEM of $-30.8\% \pm 5.0$ (N=14). All these horses except one, which had a 5.8% increase in Qs/Qt, showed a decrease in Qs/Qt. Among the horses in the control group there was an increase in Qs/Qt with a mean \pm SEM of $27.6\% \pm 7.6$ (N=12). One of the horses in the control group showed a decrease in Qs/Qt, -13.4%, the rest (N=11) showed an increase in Qs/Qt varying from 5.2% to 89.3%. The difference between the average value for horses receiving PiNO and controls was 58.4 ± 8.9 percentages.

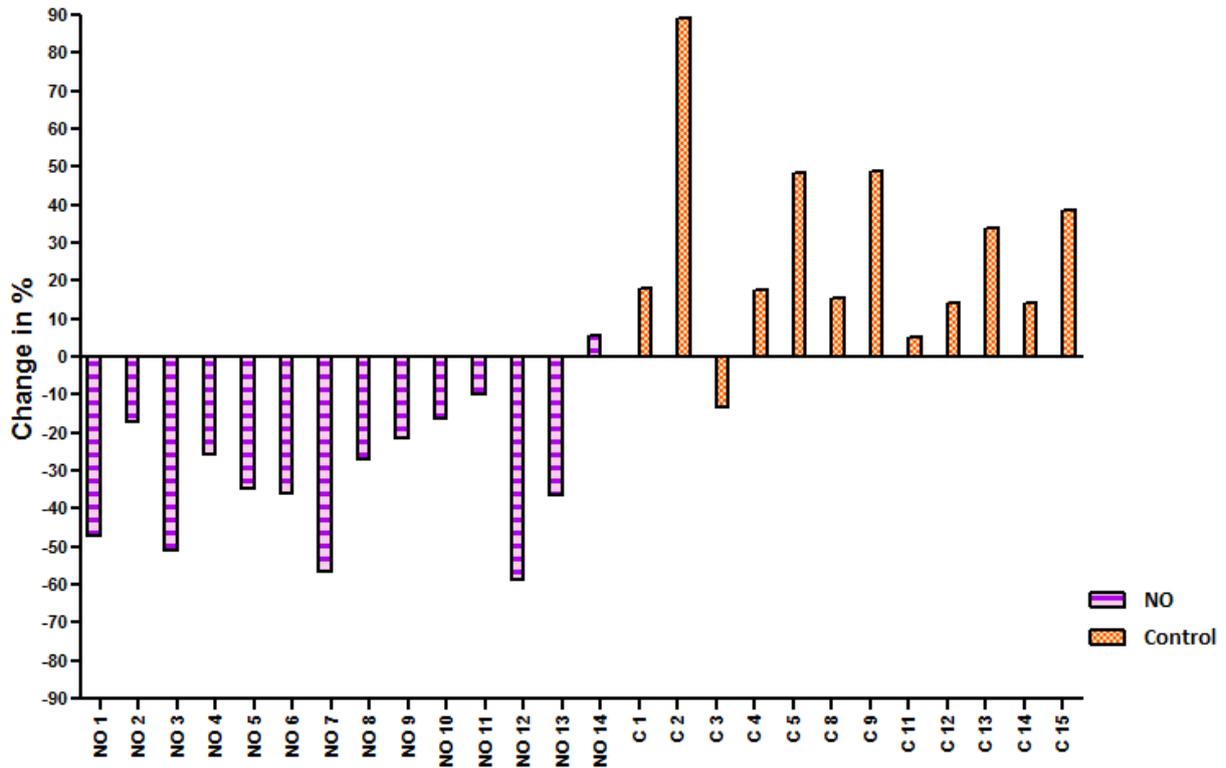


Figure 9. Each individual horse's change in shunt, Q_s/Q_t , during anaesthesia, both horses receiving PiNO and controls. The change is shown in percent, comparing each individual horse's baseline Q_s/Q_t with its lowest calculated Q_s/Q_t after the baseline value. Not all horses are included in calculation of the shunt since venous blood samples were not obtained in all cases.

Alveolar-arterial oxygen difference $P(A-a)O_2$

The individual change in $P(A-a)O_2$, when comparing the baseline value with the last calculated value during anaesthesia, was analyzed using a one-tailed unpaired t test. There was a highly significant ($P < 0.0001$) difference between the horses that received PiNO ($N=15$) and horses in the control group ($N=15$), this is illustrated in Figure 10. In Figure 11 the difference between the baseline value and the lowest calculated $P(A-a)O_2$ after the baseline is displayed. The difference between the two groups was analyzed using a one-tailed unpaired t test and the difference was significant ($P=0.0002$). There was no statistical significant difference in baseline value when comparing horses receiving PiNO and controls. The difference was analyzed with a two-tailed unpaired t test.

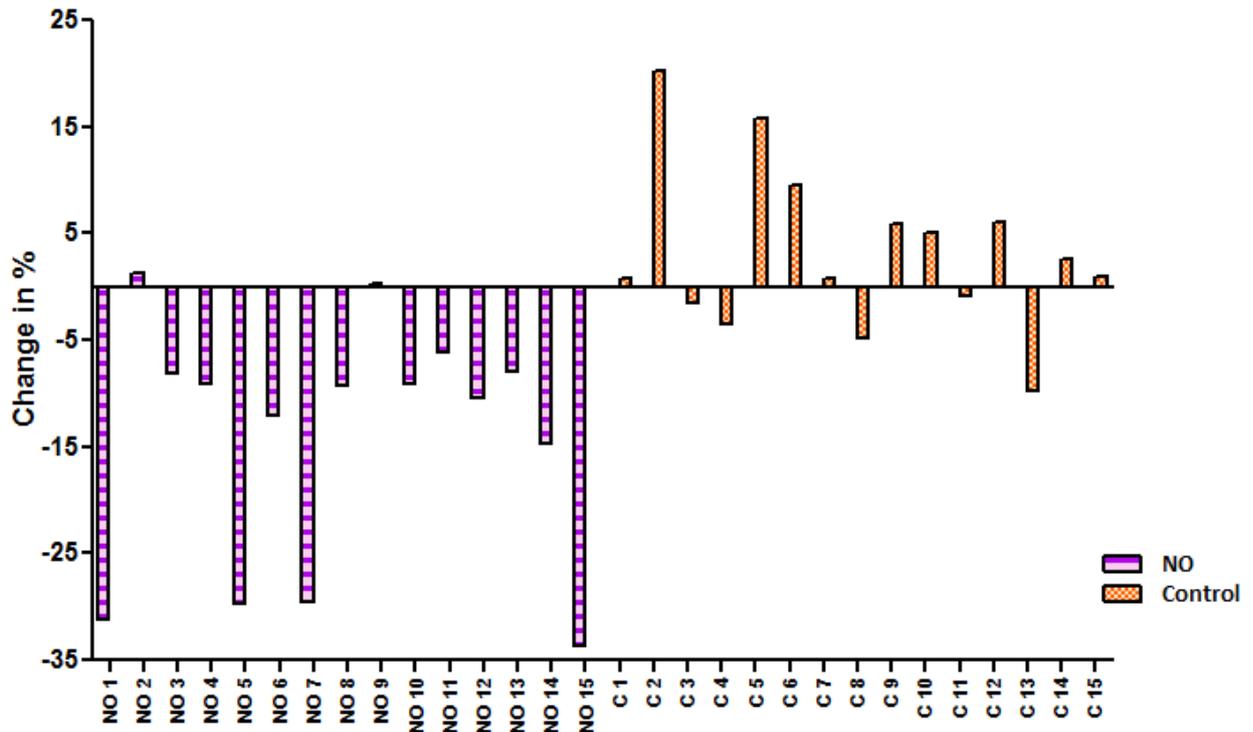


Figure 10. Change in $P(A-a)O_2$ in % when comparing the last calculated $P(A-a)O_2$ during anaesthesia with the baseline value in individual horses.

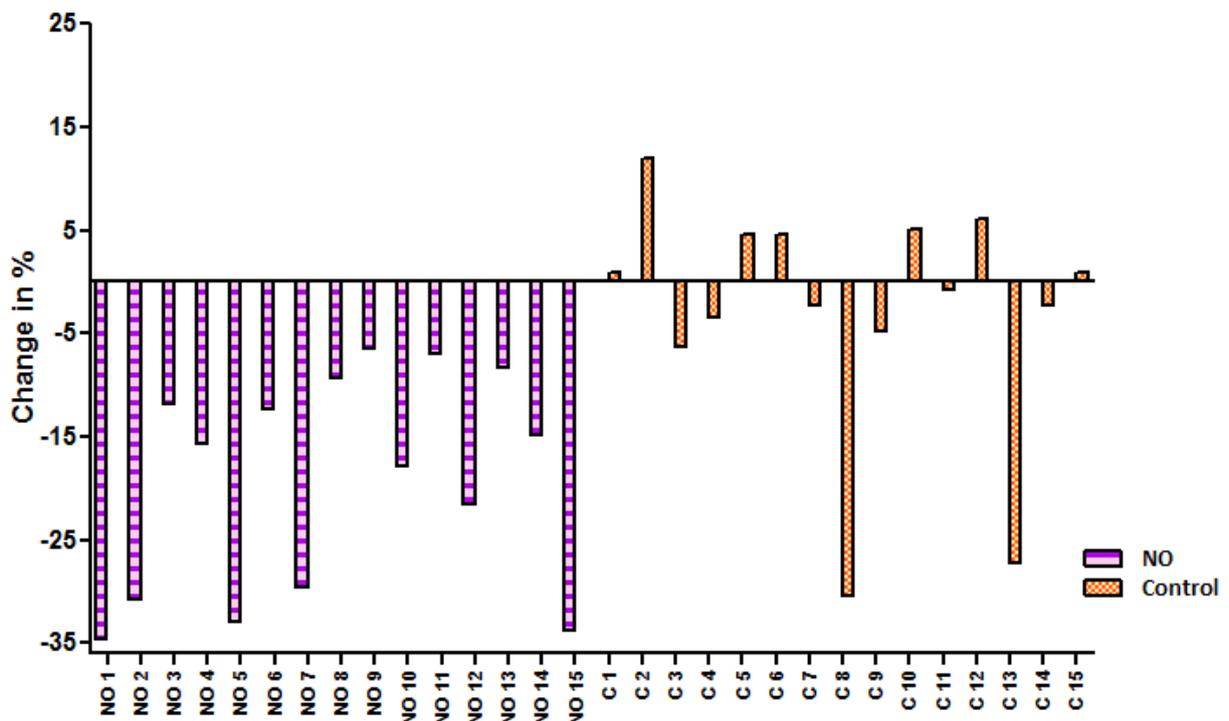


Figure 11. Change in $P(A-a)O_2$ in % when comparing the lowest calculated $P(A-a)O_2$ during anaesthesia with the baseline value in individual horses.

For analyze of $P(A-a)O_2$ over time the mean value for the two groups was calculated and for the statistics a two-way ANOVA was used. It showed a highly significant ($P<0.0001$) difference between the horses that received PiNO and the horses in the control group. The mean value of $P(A-a)O_2$ over time for the two groups is illustrated in Figure 12.

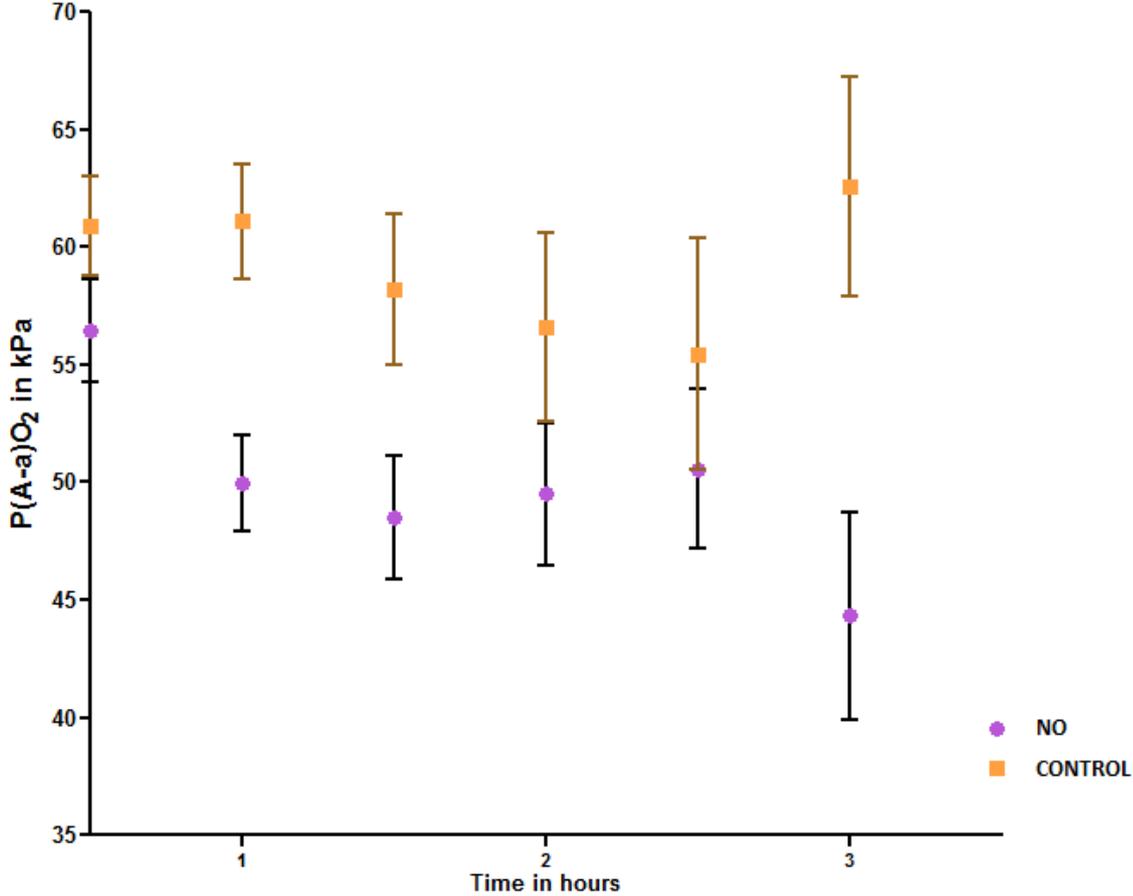


Figure 12. $P(A-a)O_2$ at different times during anaesthesia. Values are mean \pm SEM for horses receiving PiNO and controls.

Partial pressure of carbon dioxide in arterial blood, PaCO₂

The change in PaCO₂ over time was analyzed using a two-way ANOVA. There was no difference in PaCO₂ between horses receiving PiNO (N=15) and horses in the control group (N=15), this is illustrated in Figure 13.

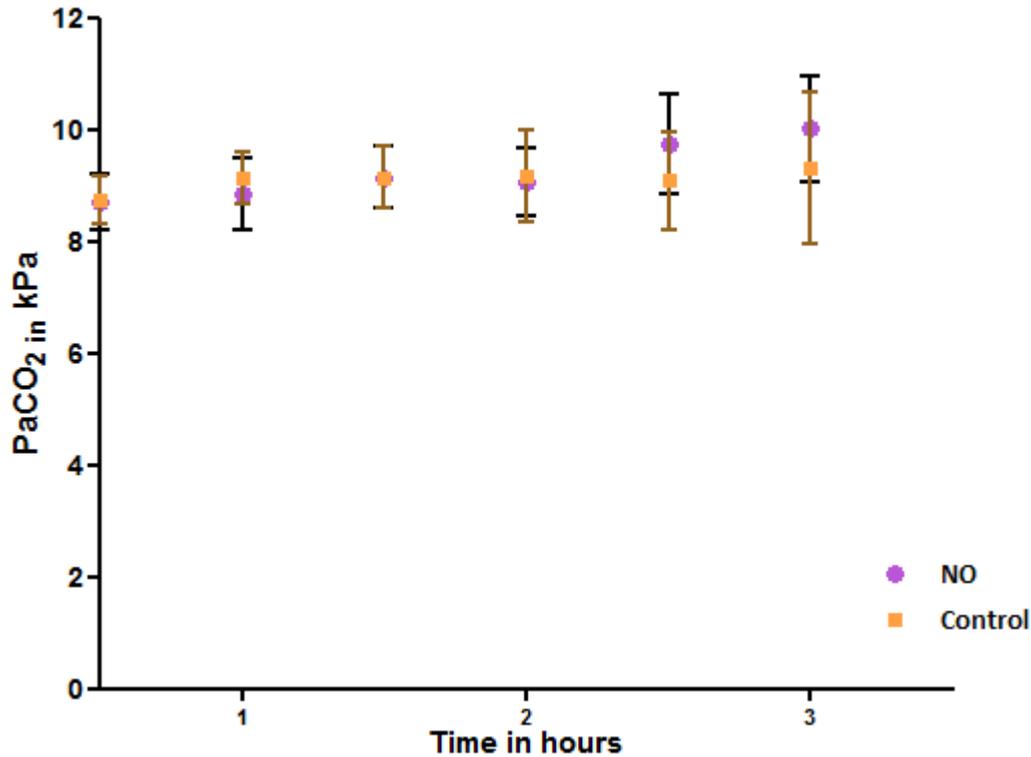
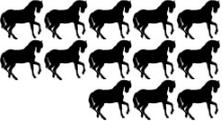
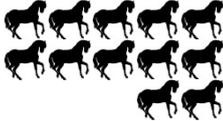
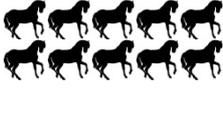
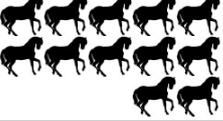
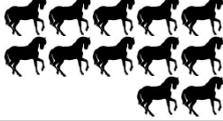
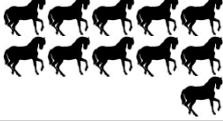


Figure 13. PaCO₂ at different times during anaesthesia. Values are mean ± SEM for horses receiving PiNO and controls.

Survival

Table 5 shows the survival for horses in the two groups, horses receiving PiNO and controls. 13 horses in the PiNO group survived surgery and in the end 10 of them were able to go home. In the control group 12 horses survived surgery and 11 were able to go home after some time in the clinic.

Table 5. Survival for the horses in this present study. Each horse represents one individual.

	Surgery	Recovery	Early postop	Leaving clinic
PiNO				
Control				

DISCUSSION

In the present study the aim was to evaluate if PiNO could be used for treatment of hypoxemia during general anaesthesia in colic horses undergoing abdominal surgery. The results showed that although all the horses were different regarding level of sickness and what caused the colic, they all responded to PiNO in a positive way. There was a wide distribution of horses both in age, breed and weight but also regarding the degree of pain and the existing disease. Thus it is difficult to compare one individual horse with another. Having that in mind most statistic calculations and comparisons was made in relation to the individual baseline values. On the other hand it is an advantage to have all this different horses since it reflects the clinic situation and this study showed that regardless baseline values, all horses receiving PiNO improved the arterial oxygen partial pressure, arterial oxygen saturation, arterial oxygen content, alveolar-arterial gradient and degree of shunt.

PiNO was delivered in pulses starting at 30% of the beginning of inspiration, if the positive effect did not occur the pulse length was increased to 45% and if still no response it was increased to 60%. Most horses (N=7) showed the best effect at 30%, some (N=6) required 45% and one horse showed best effect at 60%. This result is in line with what has been shown in previous studies (Heinonen *et al.*, 2002; Grubb *et al.*, 2013). When PiNO is delivered in the early phase of inspiration it reaches only the dependent areas of the lung compared to if iNO is given continuously, it will then also reach the poorly ventilated areas, called transitional or border zone alveoli (Heinonen *et al.*, 2000). NO has an immediate effect on the vascular bed, causing vasodilatation (Ignarro *et al.*, 1987; Frostell *et al.*, 1991). Treatment with PiNO results in a vasodilatation in dependent lung regions which leads to a change in blood flow where more blood is distributed to these well ventilated areas due to less resistance. Also when only given at the beginning of inspiration the total amount of inhaled NO is reduced and the risk of rebound effects decreases (Heinonen *et al.*, 2000).

From the horses in this study other parameters that was received and collected was analyzed in another paper (Granswed, 2014). The results from that study showed that there was no difference in heart rate, pH or glucose between horses receiving PiNO and controls. This is important since it shows that PiNO did not have any side effects on these parameters.

PaO₂

The response of PaO₂ to PiNO was in, all but one, immediate. Already after 10 to 15 minutes of receiving PiNO an increase in PaO₂ was seen. The effect over time varied since other incidents also have an influence on PaO₂, for example lifting the large intestines out of the abdomen lowers the pressure on the lungs and results in an improved gas exchange. In some cases during surgery the surgeon tilts the table so the horse's head drops, this is to get more room to evaluate and take out the intestines. Dropping the head and lift the rear end, results in an increased pressure on the lungs from the intestines and affect the gas exchange in a negative way (Nyman *et al.*, 1990).

In this study the positive effect of PiNO lasted throughout the anaesthesia and even when the anaesthesia lasted more than two hours the measured PaO₂ was still higher than the baseline value. In comparison with the controls the difference became even more obvious. Some

horses in the control group had an increase in PaO₂ at the start of anaesthesia since the inspired oxygen fraction in the breathing circuit was higher than in air but in prolonged surgeries the PaO₂ decreased below baseline value. Instead of comparing baseline value with highest measured value for each horse, the baseline value was also compared to the last measured PaO₂ during anaesthesia. Then the difference between horses receiving PiNO and controls became even more obvious, since most of the controls had their highest measured PaO₂ the sample after the baseline value. At the end of anaesthesia most controls (N=10) had a PaO₂ of 10 kPa or lower and seven of these horses had a PaO₂ below 8 kPa, which is critically low. Amongst horses receiving PiNO there was one horse that had a PaO₂ below 10 kPa (9.73 kPa) at the last sample taken, this horse was then euthanized on the table due to bad prognosis.

Horses with a PaO₂ below 10 kPa as baseline value increased more than horses with a baseline value above 10 kPa after receiving PiNO. This support the hypothesis that a lower arterial oxygen tension at the start of anaesthesia results in a larger relative increase in arterial oxygen tension within the group of horses treated with PiNO.

SaO₂

In addition to the improved PaO₂, treatment with PiNO during general anaesthesia improved SaO₂, which is in line with a previous study done on healthy horses (Grubb *et al.*, 2012). Since there is a maximum value for SaO₂ (100%) looking at each individual horse is more valuable than calculating the change. The most interesting individuals are the one that had a baseline value below 95% as this is considered to be a critical limit for the arterial oxygen saturation. All horses receiving PiNO showed improved SaO₂ and the two individuals with the lowest SaO₂ at the beginning of anaesthesia, 81%, improved the most. All horses benefit from improved SaO₂ since it determines the delivery of oxygen to the tissues but it is most essential that the horses with critical low SaO₂ do respond to treatment with PiNO.

CaO₂

Oxygen content in arterial blood increased significantly in horses receiving PiNO in comparison to controls. Some horses are not included in the calculation and the reason is that Hb was not obtained in these cases during anaesthesia. Although cardiac output was not measured in this clinical study, no differences in cardiac output were seen between horses receiving PiNO and controls in a previous study (Grubb *et al.*, 2013). Interestingly, improved oxygen content in horses receiving PiNO may also result in a better oxygen delivery provided that PiNO does not affect cardiac output.

Shunt

The reason why some horses is not included in calculation of the shunt is that no venous blood samples were obtained in these cases during anaesthesia. Since only central venous or jugular venous blood could be taken in these clinical cases only the individual differences in shunt and no absolute values are presented. No calculation on shunt was done during recovery in this study but a previous study showed that horses receiving PiNO not only had a decreased shunt during anaesthesia but also had a smaller shunt during recovery in comparison with

horses not receiving PiNO (Grubb *et al.*, 2012). This study shows that PiNO was effective in every case and the magnitude of shunt measured at baseline did not make any difference in treatment effect. The length of anaesthesia varied, from approximately 1 hour to 4 hours, between the horses which showed that the onset and effect of a continuous delivery of PiNO is immediate and also long lasting.

P(A-a)O₂

There was no significant difference in baseline value between horses receiving PiNO and horses in the control group. Since horses receiving PiNO had a larger percentage change in P(A-a)O₂ in comparison to controls, the final result showed that these horses had a smaller alveolar-arterial oxygen difference. In a study by Grubb *et al.* (2013) the horses receiving PiNO showed no difference in P(A-a)O₂ in comparison to horses in a control group. The horses that received PiNO had a higher PaCO₂ which affected the P(A-a)O₂. However, they discussed that if the PaCO₂ would have been similar in the two groups the horses receiving PiNO would have a lower P(A-a)O₂, which is in line with this study.

PaCO₂

Although a respiratory acidosis was evident in most cases due to spontaneous breathing, there was no difference regarding the partial pressure of carbon dioxide in the blood between horses receiving PiNO and controls. This is due to the fact that PiNO do not affect respiratory rate or the volume of the breaths and those two factors is what influences how well CO₂ can be ventilated (Nyman *et al.*, 2012). The next step after this study is to combine PiNO with mechanical ventilation to receive both improved ventilation and oxygenation.

CONCLUSION

In conclusion, the present study showed that a continuous delivery of pulsed inhaled nitric oxide during the first part of inspiration is an effective method to improve arterial oxygenation in colic horses during abdominal surgery.

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