Sveriges lantbruksuniversitet

Slutrapport

Kan förbättrad syresättning med pulsad inhalerad NO under hästanestesi öka patientsäkerheten?

Does improved oxygenation with pulsed inhaled NO during equine anaesthesia increase patient safety?

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Part 1: Utförlig sammanfattning

I veterinärmedicinsk praktik har det visat sig riskfyllt att söva hästar och syrebrist under anestesin är ett känt problem. Konventionella lösningar som används inom humanmedicinen är i de flesta fall ogynnsamma pga att hästlungan trycks samman av vikten från de tunga bukorganen, s.k. kompressionsatelektas uppstår i de lägst belägna lungdelarna. När gasen kvävemonoxid (NO) tillförs som en puls under första delen av inandning (PiNO) förbättras syresättningen tack vare att blodet omfördelas från de lägst belägna sammanfallna lungdelarna till ventilerade lungdelar. PiNO fungerar vid anestesi av friska hästar och kolikhästar under spontanandning men i kliniken behöver ofta sövda hästar ventileras med övertrycksandning.

Vårt långsiktiga mål är att behandling med PiNO ska förbättra patientsäkerheten och minska komplikationer i samband med operation av hästar.
Syftet i detta forskningsprojekt har varit att undersöka om PiNO, som ges i form av en gas tillsammans med narkos- och syrgas under anestesi, resulterar i förbättrar syresättning när blodet som passerar genom lungan anpassas till ventilationen. Om graden av shunt i lungan kan minskas även när hästen ventileras med övertrycksandning vid mekanisk ventilation (MV) eller assisterade ventilation vid spontanandning kan både normalt syre- och koldioxidtryck upprätthållas under anestesin. Ett andras syfte var att undersöka om komplikationer under uppvakningen och i den tidiga postoperativa perioden kunde minskas hos hästar som var väl syresatta under anestesi. Ett tredje syfte syfte var att anpassa och utveckla en metoden ”perfusion datortomografi” för att i detalj kunna studera blodflödesfördelningen i lungan baserat på tillförlitliga mätningar.

Resultaten visar att en kombination av övertrycksandning via mekanisk ventilation (MV) och pulserad inhalerad kväveoxid (PiNO) optimerar matchningen av perfusion och ventilation och förbättrar arteriell oxygenering under anestesi av både friska hästar och kolikhästar. Med perfusions-CT-metoden som utvecklats i detta projekt, är det uppenbart att PiNO omfördelar blodflödet mot gravitation från atelektatiska till ventilerade lungdelar och skiftninge ske momentant när PiNO tillförs till inandningen. Ett lite oväntat resultat var att PiNO inte lyckades förbättra syresättningen om blodtrycket var för lågt. Sannolikt beror det på att blodflöde i de ventilerade lungregioner minskar pga av högt tryck i luftvägarna i de ventilerade lungdelarna. Lyckligtvis förbättrade samtidig administrering av PiNO och dobutamin den arteriella syresättning och även syreleveransen till vävnaden.
Vi har också visat positiva effekter av PiNO för förbättrad kvalitet på uppvakningen. I projektet visas att hästar som har bättre syresättning under anestesi har bättre uppvakningskvalitet och ffa mer koordinerade resningar efter narkosen jämfört med kontroller. Vår hypotes är att hästar som är bättre syresatta under operationen återfår muskelfunktionen fortare och blir även fortare klara i hjärnan efter medvetslösheten. För att utvärdera PiNOs effekt under hela postoperativa perioden efter kirurgi krävs resultat från ett stort antal hästnarkoser i multicenterstudier.

Efter omfattande studier av PiNO anser vi att PiNO är en enkel, effektiv och säker metod för behandling av hypoxemi hos sövda hästar. PiNO fungerar både vid övertrycksandning med mekanisk ventilation, vid assisterad andning och under spontanandning. För optimalt gasutbyte behöver blodtrycket upprätthållas genom vätsketerapi och hjärtaktiva läkemedel, t.ex. dobutamin.

Det är hög tid att PiNO-utrustningen förädlas och produceras. Idag finns det bara en utrustning för leverans av PiNO (en s.k. NOrse) och vi söker nu finansiering för att utveckla och tillverka fler utrustningar. Långsiktigt är det vår förhoppning att överlevnaden av hästar under den perioperativa perioden kan förbättras.

Part 2. Final report

Background and aim of the project

In veterinary practice, it has proved to be risky to anaesthetize horses because of oxygen deficiency during anesthesia. Conventional solutions used in human medicine are unfavorable because the horse lung is compressed by the weight of the heavy abdominal organs. When nitric oxide (NO) is introduced as a pulse during the first part of the inhalation, pulsed inhaled nitric oxide (PiNO), oxygenation improves due to the redistribution of the blood from the dependent collapsed and airless (atelectatic) lung regions into well-ventilated areas, due to the local vasodilation caused by the NO. PiNO is efficient during anaesthesia in healthy horses as well as in colic horses breathing spontaneously. However, under clinical circumstances equine patients need to be ventilated with positive pressure to avoid hypoventilation. The intention of the present investigation, by means of the grant from the Swedish-Norwegian Foundation for Equine Research, was to study the effect of PiNO during during and after positive pressure ventilation in anaesthetised horses. The distribution of perfusion was measured with "perfusion computed tomography". The long-term effect of improved oxygenation during anesthesia was studied during recovery in the early postoperative period in both healthy and compromised horses after arthroscopy or abdominal surgery.

Hypothesis and Objectives

Our long-term goal is that treatment with pulsed inhaled nitric oxide should improve patient safety and nursing care associated with the surgery in horses.

Our hypothesis is that nitric oxide added as a pulse in the early breathing phase (PiNO):

1) results in improved oxygenation and oxygen delivery to the tissue even when the horse is ventilated with positive pressure ventilation or when assisted ventilation is applied to spontaneous breathing, i.e., mechanical ventilation with positive pressure ventilation (MV),

2) improves matching of pulmonary flow and ventilation which reduces the amount of shunt and improves the gas exchange in the lung also during MV.

3) reduces complications during the recovery and early postoperative period of healthy horses and in horses with colic undergoing abdominal surgery.

Materials and methods, including statistical analysis

The current research project included three different parts.

1) Development of a method, "perfusion CT", for studies of distribution of perfusion and blood flow in the lung.

2) Experimental studies performed during anaesthesia of 30 warmblood horses and 7 ponies.

3) Clinical studies conducted on 80 horses undergoing elective or emergency surgery (50 healthy horses and 30 compromised horses with colic).

The design of the different studies were prospective randomized non-blinded or blinded experimental or clinical trials. The local ethics committee for animal experiments, Uppsala, Sweden (C 201/14), approved the project. Informed owner consents were obtained in the clinical studies. Determination of sample size was based on results of previous studies, e.g. the number of horses included in the clinical study were needed to give a power of 90%. Physiology data were tested for Gaussian distribution using the Shapiro–Wilk test. For individual changes, Mann–Whitney tests and unpaired t tests were used to compare the difference between groups and Wilcoxon tests and paired t tests to compare differences within groups. The difference was considered significant when $P<0.05$ (confidence interval of 95%). Data are presented as mean ± SD. Bland-Altman plots were used for comparison of inter- and intraobserver variability.

Results and Discussion

Research questions in the application to be addressed:

1) Adaptation of equipment for delivery of PiNO for mechanical ventilation with positive pressure ventilation (MV).

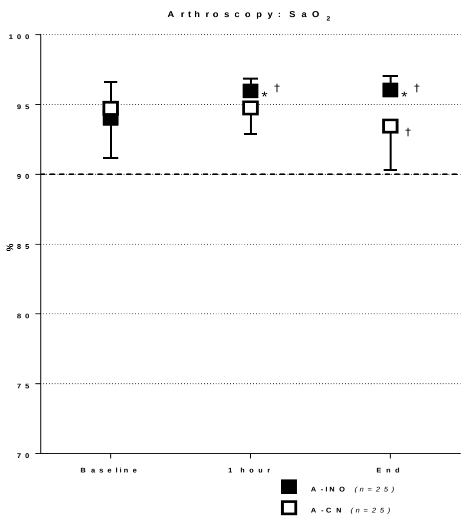
The equipment used for delivery of nitric oxide has been rebuilt and adapted to start the delivery of pulses of NO triggered by positive airway pressure during mechanical ventilation. However, delivery of NO can still be triggered by negative inspiratory pressure during spontaneous breathing.

2) Comparison of physiological effects during prolonged (about 3 hours) treatment with pulsed inhaled nitric oxide (PiNO) under mechanical ventilation (MV) and MV without PiNO (control group).

The objective of this part of the study was to determine if PiNO has the same effects during MV as during spontaneous breathing in healthy and compromised horses. Positive pressure ventilation in horse anaesthesia is necessary in clinical practice to counteract hypoventilation and prevent hypercarbia. Fifty horses underwent elective arthroscopy (Group A, healthy horses) and 30 horses with colic had abdominal surgery (Group C, compromised horses) in dorsal recumbency. Every second horse in each group received PiNO. Premedication included standard doses of flunixine meglumine, romifidine and butorphanol. Horses in Group A also received acepromazine. Anaesthesia was induced with diazepam/ and ketamine and maintained with isoflurane in oxygen. Horses in Group C received lidocaine during anaesthesia. All horses were mechanically ventilated with the goal of maintaining normocarbia. PiNO was mechanically delivered at the proximal end of the endotracheal tube as a pulse during the first part of each inspiration. Blood was collected at the start (before PiNO) and at the end of inhalation anaesthesia for determination of PaO2 and other blood gas components. Calculated data included F-shunt for determination of ventilation/perfusion matching. For statistics, unpaired and paired t tests were used with $P<0.05$ considered significant (*).

Results: During PiNO, arterial oxygen tension (PaO2) increased from 18.6±10.4 and 9.4±7.2 to 26.7±10.6* and 18.4±10.4* kPa, and the F-shunt decreased by 15±11%* and 23±16%* in Group A and C, respectively. In controls, PaO2 changed insignificantly from 17.9±10.3 and 12.5±14.5 to 16.7±11.5 and 12.6±12.4 kPa, and the F-shunt increased by 11±16%* and 5±13% in Group A and C, respectively. Since it was a clinical study cardiac output could not be measured, and thus O2 delivery could not be calculated.

Conclusions: The combination of mechanical ventilation and PiNO improved the pulmonary gas exchange during anaesthesia by a simultaneous decrease in F-shunt and improved alveolar ventilation.



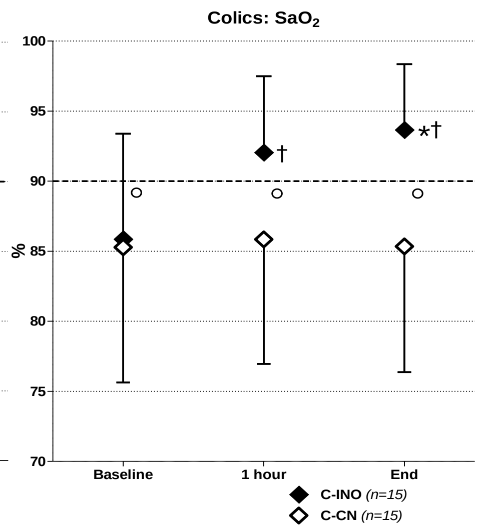


Figure: Arterial oxygen saturation (SaO2) in horses that were administered pulsed inhaled nitric oxide (PiNO) and underwent arthroscopy (A-INO) or abdominal surgery (C-INO) and those were not (A-CN, C-CN) during anaesthesia. Data is presented as mean ± SD at baseline (beginning of anaesthesia before PiNO delivery commenced), at 1 hour and at end of anaesthesia. The dotted lines indicate 90% SaO2. Mean values and SD. †Significantly different from baseline value; *Significant difference between horses receiving PiNO and controls in each group; ºSignificantly different from arthroscopies.

The objective of the next part of the study was to determine the impact of different ventilation modes and blood pressure and cardiac output (CO) on the efficacy of pulsed inhaled nitric oxide (PiNO) to improve oxygenation. Twenty-four healthy adult horses were anesthetized and divided into 4 groups: spontaneous breathing (SB), mean arterial blood pressure (MAP) < 70 mmHg (SB-L, 7 horses); SB, MAP > 70 mmHg (SB-N, 8); mechanical ventilation (MV), MAP < 70 mmHg (MV-L, 6); and MV MAP > 70 mmHg (MV-N, 6). Dobutamine was used in the groups to create MAP > 70 mmHg. Baseline data were collected after a 60-minute equilibration period. PiNO was administered and data collected at 15 and 30 minutes. Data included PaO_2, SaO_2, DO_2 and Vd/Vt (dead space ventilation). Data were analyzed with Shapiro-Wilk, Mann–Whitney and Friedman ANOVA tests with $p < 0.05$ considered significant.
Results: Oxygenation indices (PaO_2, SaO_2, CaO_2 and DO_2) increased significantly with PiNO in SB-L, SB-N and MV-N groups. These indices were significantly lower in the MV-L than the MV-N group at all time points and lower than SB groups during PiNO. There were no or few differences between SB-L and SB-N. Vd/Vt was highest in the MV-L group.
Conclusions and Clinical Relevance: Pulmonary blood flow (CO and MAP > 70 mmHg) had an important impact on PiNO efficacy during MV but not during SB. PiNO failed to increase oxygenation indices in the MV-L group, likely due to profound ventilation-perfusion mismatch. During SB, PiNO improved arterial oxygenation irrespective of the magnitude of blood flow but hypoventilation and hypercarbia persisted. When using MV, maintaining systemic blood pressure > 70 mm Hg to ensure good pulmonary blood flow is necessary for PiNO´s effect to be manifested.

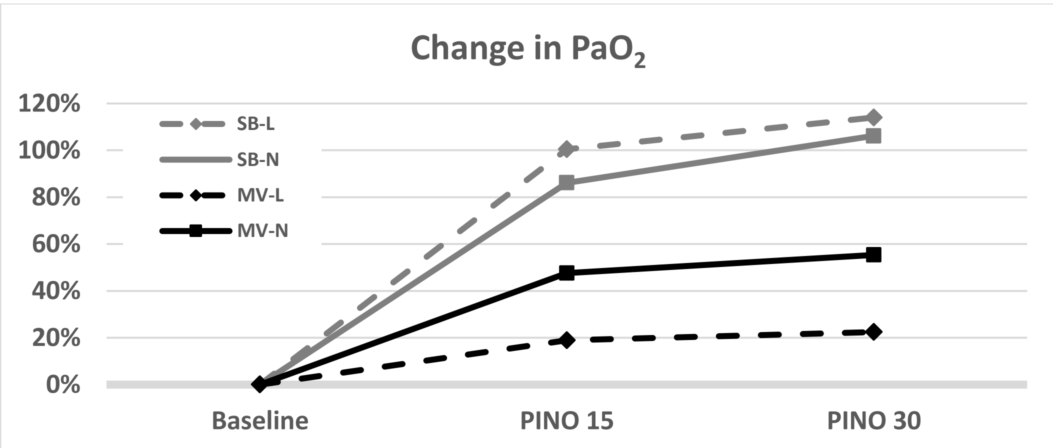


Figure: Percentage (%) change in arterial oxygen tension (PaO2) over time from baseline and at 15 and 30 minutes of pulsed inhaled nitric oxide (PiNO) during spontaneous breathing (SB) and mechanical ventilation (MV) with mean arterial blood pressure < 70 mm Hg (L) and > 70 mmHg (N). The effect of PiNO was greater with SB than MV. Data presented as mean values.

Besides positive pressure ventilation (mechanical ventilation) other ventilation modes can be used. Assisted ventilation, in which a spontaneous breath (negative airway pressure) triggers the ventilator to deliver a preset tidal volume, has been proposed to decrease the risk of alveolar rupture, improve pulmonary gas distribution and preserve blood flow. The effects of PiNO during patient-triggered, assisted ventilation was studied in an experimental study on nine horses anaesthetized with inhalation anaesthesia. During assisted ventilation, flow required to trigger the ventilator, tidal volume and inspiratory time were tailored to each individual horse's breathing pattern. Data, including arterial and mixed venous blood analysis, were recorded before delivery of PiNO (baseline) and at 15 and 30 minutes of PiNO administration.

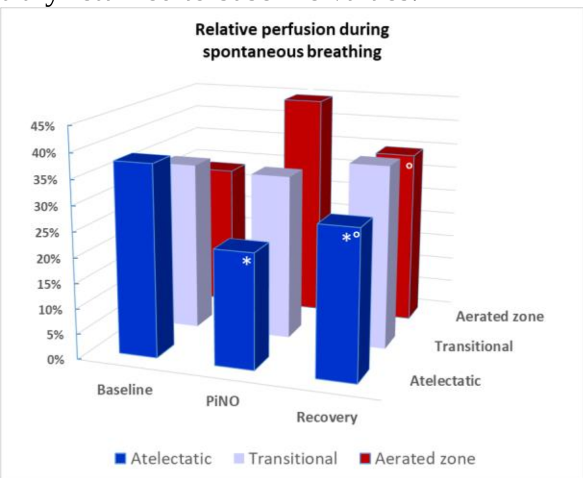
Parameter		*Baseline*	*PiNO 15'*	*PiNO 30'*
PaO2	kPa	12 (7-43)	18 (11-59)*	21 (10-58)*†
P(A-a)O2	kPa	58 (33-72)	52 (17-67)*	50 (17-68)*
SaO2	%	93 (86-97)	95 (93-97)*	96 (94-97)*
Qs/Qt	%	53 (33-57)	41 (25-45)*	41 (26-49)*

Data presented as median (range) * significantly different from baseline, † significantly different from PiNO 15 minutes. In conclusion, PiNO improved oxygenation in anaesthetised horses during patient-triggered, assisted ventilation.

3) Comparison of physiological effects during prolonged (about 3 hours) treatment with PiNO under MV and MV without PiNO (control group) with "perfusion computer tomography" (PCT).

The data presented in this study demonstrate that the "perfusion CT" (PCT) method is suitable for measurement of lung perfusion in spontaneously breathing and mechanically ventilated anaesthetised ponies. We also showed that the CT images can be accurately analysed semi-automatically using computer software. Physiological data verifies that aerated and atelectatic lung perfusion and blood flow changes as expected with PiNO and mirrors alterations in oxygenation.

A comprehensive survey of the distribution of PCT showed that PiNO redistributes blood from unventilated atelectatic regions of lung to better ventilated lung via selective vasodilation. This improves arterial oxygenation. This mechanism is similar during spontaneous breathing and mechanical ventilation. The effect of PiNO persists beyond the end of treatment as PaO_2 never truly returned to baseline values.



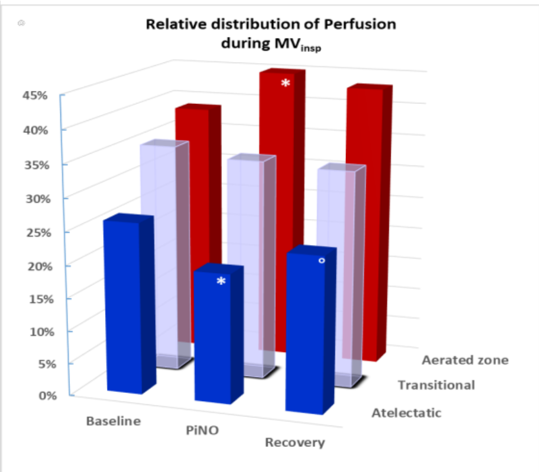


Figure: Relative mean perfusion values to the aerated, transitional and atelectatic lung before, during and after PiNO. The first figure shows the relative perfusion during spontaneous breathing (SB) and the second figure during the inspiratory phase of mechanical ventilation (MV_{Insp}). The blue bar shows the perfusion of the atelectatic lung, the lilac bar transitional, poorly inflated lung and the red bar shows the perfusion of the aerated lung. * Significantly different from baseline, ° significantly different from PiNO.

Perfusion to atelectatic lung decreases and perfusion to well-aerated lung increases with PiNO. Perfusion in poorly aerated lung (transitional region) did not change.

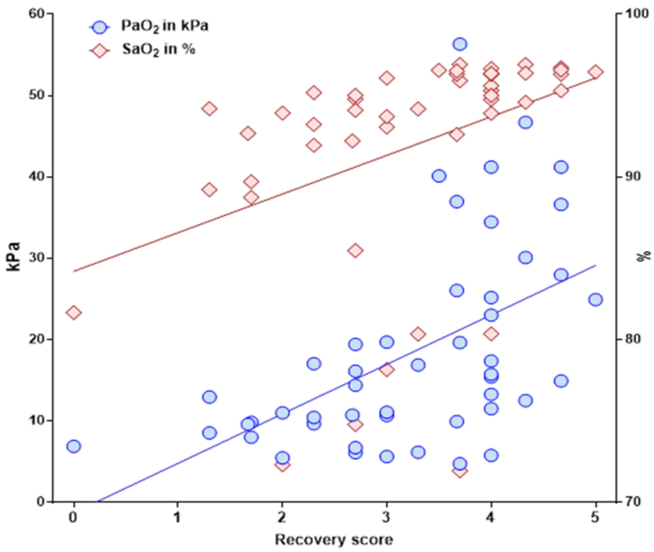
4) Effects of improved oxygenation during the recovery in the early postoperative period of colic horses.

The purpose of the study was to investigate if the time under anaesthesia during an emergency laparotomy, to treat colic, affects the quality of recovery. The study also investigated whether treatment with PiNO during anaesthesia, affected the quality of the recovery. Data was collected over a 13 month period at the University Animal Hospital's horse clinic, Uppsala, and included a total of 24 patients that had undergone acute surgery due to colic.

A shorter time spent under general anaesthesia resulted in a better quality of recovery. The recovery time spent in the recovery box was found to affect the recovery quality. Additional parameters that were found to affect the recovery quality were; age, duration of surgery and size of recovery box. Further studies of horses that have undergone acute laparotomy are required, but the results of this study indicate that reducing time under general anaesthesia leads to better recovery quality.

Preliminary results from 26 healthy horses which had undergone arthroscopy, and 24 horses which had undergone abdominal surgery for colic showed that the quality of the recovery is better if arterial oxygenation is good during anaesthesia. Horses that receives PiNO during anaesthesia have a significant better oxygenation during anaesthesia than controls. A significant correlation between arterial oxygenation and recovery quality score was found. Further studies are required to evaluate PiNO´s long-term effect on the postoperative complications. Because of the large number of horses required, multicenter collaboration is necessary.

Figure: Mean values of arterial oxygenation (PaO_2 and SaO_2) during anesthesia plotted in relation to the quality of recovery (Recovery score). The recovery was videotaped and scored (0-5, 5 being the best recovery) by three anaesthetists who were blinded to the treatment. A linear regression line is inserted to better illustrate the relationship.



5) Measuring regional blood flow and perfusion in the anaesthetised horse lung with perfusion CT (method for 3), above)

Background

For this project, we chose a different method to measure regional changes in pulmonary blood flow than the gamma camera method we used previously. The gamma camera method is not the best: the detail seen is poor, and many repeated injections of radioactive tracer into the lungs make later injections difficult to measure due to the accumulation of radioactivity in the lungs, which becomes an increasing background ("noise"). Contrast perfusion CT offers high resolution and a 3-dimensional picture of the lungs to determine the extent (areas) of the atelectasis, and ability to study movement of the lungs and diaphragm. The kidneys rapidly excrete the contrast medium (the same as used in routine clinical radiography), allowing many repeated injections during a day of an experiment, In humans the method is used routinely to evaluate blood flow in the brain, kidney and liver, and lung tumours, but has not been used to measure flow in aerated lung. Our aim was to develop and use a new method with contrast perfusion CT.

Theory

The principle is simple. CT changes in density are measured in Hounsfield units (HU), where water is 0 and air is -1000. The relationship of HU to density is linear, making the calculations simple. CT machine manufacturers sell a program for their controlling software which automatically measures regional flow or perfusion. We tried it for three months, but as was expensive (90.000 SEK), and a closed "black box", and did not give reliable results, we had to develop our own methods. As no studies have been published on measuring perfusion and flow in the aerated lung, this is pioneering research to find a good method. The perfusion in ml/min blood/ml tissue volume is simply the maximum upslope of the curve of contrast concentration on the blood passing through the region of interest (ROI) of the organ (aerated AER and atelectatic ATEL), divided by the height of an arterial reference curve (aorta) as HU of density of contrast medium, and normalised to soft tissue volume. Flow in the ROI is perfusion x area of ROI. For absolute results in physiologic units, and comparison of different physiologic conditions, flow has to be corrected for changes in cardiac output by simple division so the units become flow per litre CO. However, these are not necessary to assess redistribution of blood flow and perfusion in our experiments.

Problems we encountered while developing the method

Before starting on the investigations, we used three trial days with the CT scanner to test the method. Three horses were studied with different injection rates. Contrast medium could not be injected fast enough to make a good time-density curve. The injection tubing was too small. We changed to a larger one and warmed the contrast liquid to reduce its viscosity. We made test studies of different methods of breath holding in expiration and peak inspiration with SB and IPPV, and made injections to get data to test. Controlling breathing rate for the SB studies required adjustments to the CO_2 levels. Many other practical problems were solved.

Defining well aerated (AER), poorly aerated (TRANSitional) and atelectatic (ATEL) regions of interest (ROI). Freehand drawing on the computer using the digital tool to define the AER and ATEL ROIs resulted in too large variability, measured statistically, especially between measurers (3 people). We tried special programs designed to define regions within a range of densities, but none worked on the lungs. We then tested a threshold method using a range of densities to define AER and ATEL regions to guide our drawing by hand. We set thresholds of -1000 – 350 HU for AER ROI and -350 – -100 HU for the TRANS ROI, and -100 – +100 for the ATEL ROI in the first experiments. We need to find out how NO affected the TRANS region. Two people measured each of these ROIs twice. Statistical calculations (Bland-Altman plots) for inter and intra measurer variability were done to see how reliable they were. As the TRANS ROIs had no significant redistribution of blood flow or perfusion due to NO, we subsequently combined the two AER ROIs to one of -1000 – -100 HU.

<u>Making curves from Aorta, ATEL and AER ROIs</u>. The series of pictures of the slice though the thorax made at 3 per second, were saved as DICOM files, the standard method for radiologic images. They were then opened in a free programme, ImageJ, which was used to draw the aorta, ATEL and AER ROIs. The data (time-density curves) from each was saved as Excel files.

We found the following things which disturbed the curves:

1. Movement of thoracic wall due to attempts to breathe, during studies of spontaneous breathing. If this occurred during passage of contrast medium through the ROI, the injection was repeated.
2. In the AER curves, decreasing volume of AER lung ROI due to leakage in anaesthetic tubing or resorption of gas volume into blood during contrast passage through the lungs, a low frequency disturbance called “trend slope”. Even after changing from clamping to using a plumber’s brass tap with no leakage, the trend slope was present, indicating that the loss of volume was mostly due to resorption of oxygen in the alveoli.
3. In the AER curves, high frequency pulses caused by arterial pulses being transmitted though the compliant aerated lung, but not through the more solid atelactatic lung (see Figure).

   All the above are “noise” as opposed to “signal”, the true curve of contrast density, from which the maximum slope is measured.

<u>Methods of measuring aorta amplitude and maximum slope</u>

We used the following method

1. Manual measuring from the time-density curves. X and Y values were used to calculate maximum upslope and net increase in aortic density. This method had more and not acceptable interobserver variability than the methods in the curve was smoothed (noise removed).
2. Using the statistical and data analysis program Prism, all curves were smoothed. Aorta baseline and maximum were measured on the curve, maximum slopes were measured by maximum first derivative of the curves. Two people (AA and PL) measured each of these twice for inter and intra measurer variability statistical calculations to see how reliable they were. Manual copying of Excel files to Prism and results to an Excel database with all calculations in it was very time-consuming and errors were easily made when typing titles of the files and transferring them to the database. We needed a more automatic method.
3. Automatic smoothing of curves, automatic measuring of maximum and trend slopes, automatic transferring of results to Excel database. This was done with an add-in program to the statistical program MatLab, written this year by a colleague (Dr. Alessandro Beda) of our co-doctoral supervisor at the Hedenstierna Laboratory at Akademiska Sjukhuset, Dr Gaetano Perchiazzi. This program first smooths the curves

similarly to Prism, then uses vertical time cursors to automatically set the values of baseline and peak of the aorta curve and slope and trend slope of the ATEL and AER curves. The methods paper or the physiologic paper comparing SB with IPPV will include a comparison of results using Prism and Matlab.

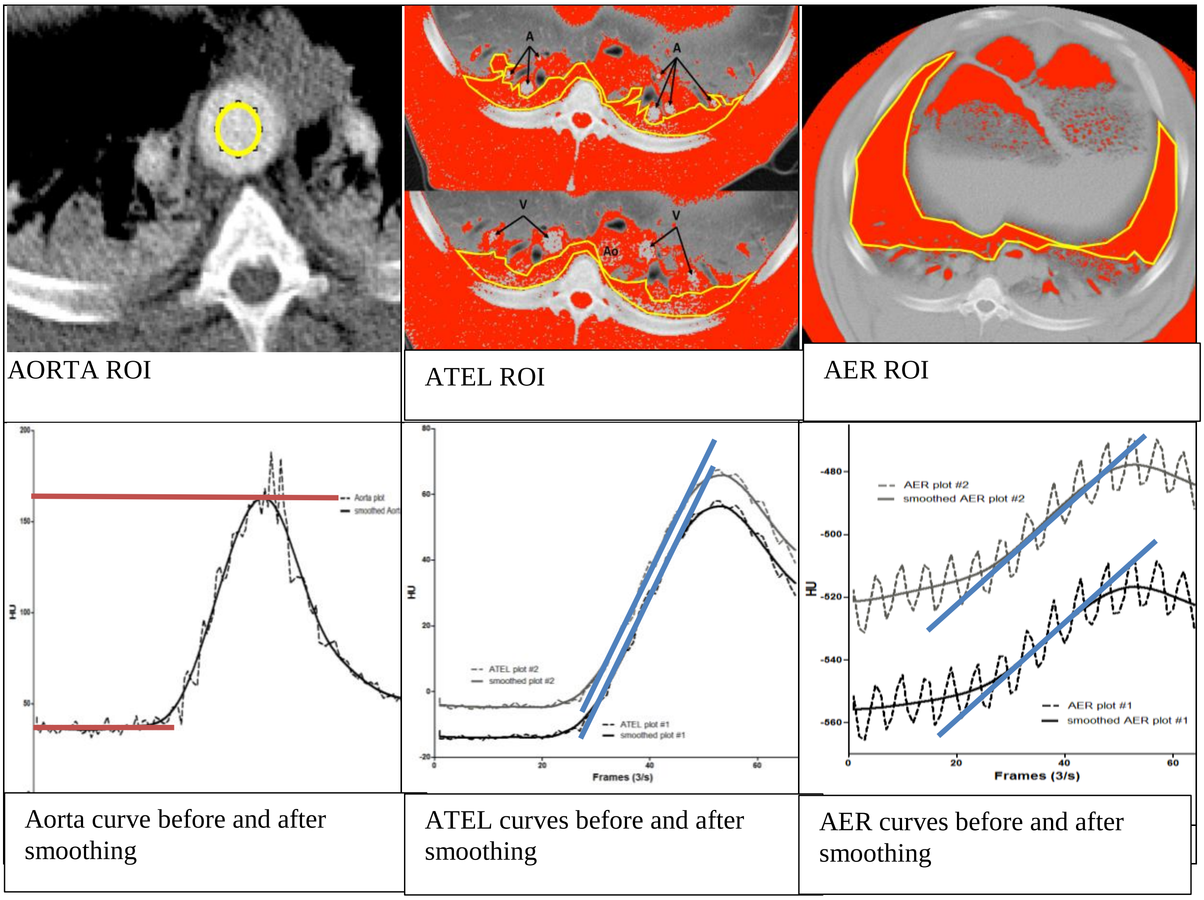


Figure: *Upper figures.* Regions of interest (ROI) created for the aorta and atelectatic and aerated lung regions using region) with upper image demonstrating pulmonary arteries (A) and lower image demonstrating pulmonary veins (V) opacified by contrast medium and excluded from the ROI. Aerated ROI (-1000 to -100 HU) *Lower figures.* The corresponding raw and smoothed time-density curves from single aorta and pairs of ROI drawings from one person. Baseline and peak aorta HU are indicated by red lines. The maximum upslopes of AER and ATEL (blue lines) are the same. Note the pulses in all unsmoothed curves.

Results

We compared baseline densities from injections during the study day and found no increase in aortic HU after the first injection. We could make at least 7 injections during a full day of anaesthesia, as contrast medium was rapidly excreted by the kidneys. Therefore multiple physiologic interventions such as change of ventilation method or drug effects can be studied consecutively.
Statistical analysis by Bland-Altman plots showed that agreement of repeated measurements by the same person was very good, and satisfactory between two different persons. Smoothing the curves reduced variability and should be done. The biggest sources of variability of measurement were the areas and the maximum upslopes of the aerated ROI curves. We used an average of three measurements of area to reduce variability, as is done in echocardiography. Flow results were more variable than perfusion results because ROI area is used. The trend slope found in nearly all AER curves had to be corrected, but

added variability to these results. In the comparison of SB with IPPV the ATEL redistribution results were highly significant with only 6 horses, while the AER results were not. As the hypothesis is proved with only the ATEL results, the method is satisfactory and was used for the experiment comparing the effect of NO in hypotensive and normotensive states.

Summary methodology development

We developed a new contrast CT method for measuring regional blood perfusion and flow in the horse lung. This was used to study differences in oxygen saturation between SB and IPPV with PiNO, and the effect of normotension versus hypotension on the effect of PiNO with IPPV. Many other studies of ventilation are now possible. In addition to the above studies, we routinely made CT scans at 3/sec without contrast during cycles of spontaneous breathing and IPPV for future analysis. The measurements of areas of atelectasis and aerated lung, and the high temporal resolution will enable us to study the effects of different kinds of ventilation on regional lung inflation, perfusion and arterial oxygenation (see above comments).

Conclusions

The results of this research project shows a combination of positive pressure ventilation and PiNO optimize the matching the perfusion of well-ventilated lung regions and improve the ventilation and arterial oxygenation during anaesthesia in both healthy and colic horses. The different method validates our previous result using the gamma camera. Based on PiNO-induced increases in arterial oxygenation and decreases in shunt, the delivery of PiNO is an effective method to improve oxygenation in both healthy and compromised (colic) horses that are mechanically ventilated during anaesthesia. The improvement in arterial oxygenation and decrease in shunt was not unexpected based on the results from previous studies. However, horses in those studies were hypercapnic due to hypoventilation. During inspiration, positive pressure mechanical ventilation (MV) increases intrathoracic pressure, which forces pulmonary blood from non-dependent to dependent areas of the lung, and hence it was not certain whether or not PiNO would be effective.

With the method for measuring regional blood perfusion and flow in the horse lung developed in the present research project, it is possible to study the distribution of perfusion in the lung under different modes of ventilation and physiological states. The perfusion CT studies clearly show that PiNO effectively redistributes the pulmonary blood flow against gravity from atelectatic lung region to aerated regions. Unexpectedly, with MV, PiNO failed to increase the indices of oxygenation when the blood flow was low, likely due to decreased perfusion of ventilated lung regions caused by high airway pressure during positive pressure ventilation. Fortunately, simultaneous administration of PiNO and dobutamine dramatically improved the arterial oxygenation and oxygen delivery during MV. In spontaneously breathing horses, PiNO improved arterial oxygenation irrespective of the magnitude of blood flow but hypoventilation and hypercarbia persisted.

Use of PiNO during anaesthesia appears to maintain the increase in oxygenation during the early postoperative period. Preliminary results from recovery of both healthy horses and compromised horses indicated that the quality of recovery improved when PiNO was used during anaesthesia and surgery. Further studies are required to evaluate PiNO´s effect on the postoperative period.

Benefits for the industry and recommendations

We are confident that pulsed inhaled nitic oxide (PiNO) is ready for clinical use in anaesthetised horses. PiNO is an effective and safe method to improve oxygenation and oxygen delivery during anesthesia in anaesthetised horses irrespective of ventilation mode. We have also demonstrate positive effects in terms of improved quality of recovery in the early postoperative period and positively postoperative survival will be greater. Futher studies are required to evaluate PiNO´s effect on the postoperative period.
Most urgently, the PiNO equipment needs to be refined and produced. Today there is only one equipment for delivery of PiNO (the NOrse). We are now seeking funding to develop the equipment with the hope to improve the survival of horses in the perioperative period.

References

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Part 3: Dissemination of results of the project

Vetenskapliga publiceringar	**Effects of pulsed inhaled nitric oxide (PiNO) on arterial oxygenation during IPPV in anaesthetized horses undergoing elective arthroscopy or emergency abdominal surgery** Maja Wiklund DVM, Malin Kellgren DVM, Sofia Wulcan DVM, Tamara Grubb DVM, PhD, DACVAA and Görel Nyman DVM, PhD, DECVAA, Equine Veterinary Journal accepted 2019, ISSN 0425-1644, DOI: 10.1111/evj.13129
	Effects of ventilation mode and blood flow on arterial oxygenation during pulse-delivered inhaled nitric oxide in anesthetized horses Adam Auckburally DVM, Tamara Grubb DVM, PhD, DACVAA, Maja Wiklund DVM and Görel Nyman DVM, PhD, DECVAA. American Journal Veterinary Research, 2019, 80, 3, Pages 275-283.
	Measurement of regional perfusion and blood flow of the lung using CT angiography in anaesthetised horses Adam Auckburally DVM, Maja Wiklund DVM, Anna Straube DVM, Charles Ley DVM, PhD, Görel Nyman DVM, PhD and Peter Lord DVM, PhD. Manuscript in preparation
	Determination of the effect of pulsed inhaled nitric oxide on pulmonary perfusion in spontaneously breathing and mechanically ventilated anaesthetised ponies using CT angiography Adam Auckburally DVM, Maja Wiklund DVM, Anna Straube DVM, Peter Lord DVM, PhD and Görel Nyman DVM, PhD. Manuscript in preparation
	Effect of cardiac output and systemic blood pressure on regional perfusion of the lung in anaesthetised horses measured using CT angiography. Adam Auckburally DVM, Maja Wiklund DVM, Anna Straube DVM, Peter Lord DVM, PhD and Görel Nyman DVM, PhD Data collection completed. Images are currently being analysed.
	Effects of pulsed inhaled nitric oxide (PiNO) on quality of recovery after elective arthroscopy or emergency abdominal surgery Maja Wiklund DVM, Catrine Strid BSc, Kristina Wallin BSc and Görel Nyman DVM, PhD, DECVAA Data collection completed. Data are currently being analysed.
Övriga vetenskapliga publiceringar	**Effects of blood pressure on oxygenation in mechanically ventilated anaesthetised horses administered pulsed inhaled nitric oxide.** Auckburally A, Grubb T, Wiklund M, Nyman G (2018) Abstract, 2018 Spring AVA conference, Grenada, West Indies
	Effects of PiNO on arterial oxygenation during IPPV in horses undergoing elective arthroscopy or abdominal surgery under general anaesthesia. (2018) **.** Wiklund M, Kellgren M, Wulcan , Grubb T, Nyman G. Abstract, 2018 Spring AVA conference, Grenada, West Indies
	Pulsed inhaled nitric oxide improves arterial oxygenation in colic horses undergoing abdominal surgery

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	Effects of pulsed inhaled nitric oxide (PiNO) on oxygenation during assisted ventilation in anaesthetised horses, Maja Wiklund, Veterinärkongressen 2018-11-09
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Studentarbete	**Pulsed Inhaled Nitric Oxide during Mechanical Ventilation in Horses undergoing Abdominal Surgery - The effect on arterial oxygenation.** Sofia Wulcan, Uppsala 2017, Degree Project 30 credits within the Veterinary Medicine Programme, ISSN 1652-8697, Examensarbete 2017:9
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