



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Veterinary Medicine
and Animal Science**

Department of Animal Environment and Health

Effects of treatment with PiNO (Pulsed Inhaled Nitric-Oxide) on the metabolism in colic horses undergoing abdominal surgery.

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*Uppsala
2014*

Degree Project 30 credits within the Veterinary Medicine Programme

*ISSN 1652-8697
Examensarbete 2014:46*

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Behandlingseffekter av PiNO (Pulsed Inhaled Nitric-Oxide) gällande metabolismen hos hästar som genomgår bukkirurgi till följd av kolik.

Izabella Granswed

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Degree Project in Veterinary Medicine

Credits: 30

Level: Second cycle, A2E

Course code: EX0736

Place of publication: Uppsala

Year of publication: 2014

Number of part of series: Examensarbete 2014:46

ISSN: 1652-8697

Online publication: <http://stud.epsilon.slu.se>

Key words: Equine metabolism, anaesthesia, Pulsed Inhaled Nitric-Oxide, PiNO

Nyckelord: Hästens metabolism, narkos, pulsat inhalerat NO

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SUMMARY

The main objective of this research was to study how increased arterial oxygenation by the use of Pulsed Inhaled Nitric-Oxide (PiNO) influenced the metabolic and cardiovascular parameters on horses undergoing acute abdominal surgery because of colic. The parameters blood lactate, blood glucose, pH and heart rate were evaluated before and during anesthesia and closely after recovery. The study showed that blood lactate concentrations decreased significantly during anaesthesia in horses treated with PiNO compared to non-treated horses. It was also seen that the lactate concentration decreased most in horses with the highest levels before PiNO treatment. Since enhanced oxygen extraction ratio was evident in the PiNO group, improved oxygen delivery to the tissue may be a possible explanation for the improved situation. PiNO did not influence levels of blood glucose, pH or heart rate. It became evident that the majority of horses included in the study were subject to a respiratory acidosis caused by hypoventilation during spontaneous breathing. It also became evident that all horses were hyperglycemic, probably because of a stress-response which may have increased the level of cortisol and resulted in temporary reduced insulin sensitivity. Mechanical ventilation and insulin treatment is suggested for future research to correct the hypercapnia and improve the metabolic situation. Future research is further encouraged to study the effect of PiNO treatment during recovery in the postoperative period and to see if the treatment can influence the long term clinical outcome.

SAMMANFATTNING

Syftet med denna studie var att se hur förbättrad arteriell syresättning vid behandling med Pulsad Inhalerad Kväve-Monoxid (PiNO) påverkade metabola och kardiovaskulära parametrar hos hästar som genomgick akut bukkirurgi till följd av kolik. Parametrarna blodlaktat, blodglukos, pH och hjärtfrekvens mättes före, under och direkt efter narkosen. Studien visar att blodlaktatkoncentrationerna minskade signifikant hos hästar som behandlades med PiNO under operationen jämfört med obehandlade kontrollhästar. Hästar med de högsta värdena av blodlaktat innan kirurgi visade den mest uttalade minskningen vid behandling med PiNO. Eftersom en förbättrad syreextraktionsratio kunde ses i behandlingsgruppen så kan förbättrad syreleverans ut i vävnaden vara en möjlig förklaring till den förbättrade situationen. Behandling med PiNO påverkade inte nivåerna av blodglukos, pH eller hjärtfrekvens. Däremot såg man att flera av hästarna hade en respiratorisk acidosis med sänkt pH och höga nivåer av koldioxid i blodet till följd av hypoventilering under spontanandning. Hästarna var också hyperglykemiska under narkosen, förmodligen på grund av en stress-respons vilket gav höga nivåer av kortisol, som i sin tur ledde till en tillfälligt minskad insulinkänslighet. Mekanisk ventilation tillsammans med PiNO och insulinbehandling föreslås för framtida forskning för att korrigera hyperkapni och förbättra den metabola situationen. Framtida forskning bör även fokuseras på effekterna av PiNO-behandling under uppvak i den post-operativa perioden samt undersöka om behandling kan påverka utfallet i det långa loppet.

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ABBREVIATIONS

ABP	Arterial blood pressure
C	Control
CaO ₂	Oxygen content in arterial blood
C(a-v)O ₂	Arterial-venous oxygen content difference
C(a-v)O ₂ / CaO ₂	Oxygen extraction ratio
ECG	Electrocardiography
EtCO ₂	End-tidal carbon dioxide concentration
EtIso	End-tidal Isoflurane concentration
FiO ₂	Fraction inspired oxygen
HR	Heart rate
iNO	Inhaled nitric oxide
IPPV	Intermittent positive pressure ventilation
NO	Nitric oxide
PaCO ₂	Partial pressure of CO ₂ in arterial blood
PaO ₂	Partial pressure of oxygen in arterial blood
PEEP	Positive end-expiratory pressure
PiNO	Pulse-delivered inhaled nitric oxide
Qs/Qt	Ratio of shunted blood (Qs) to total blood flow

INTRODUCTION

It is already known that pulsed inhaled nitric-oxide (PiNO) is effective and safe to use in equine anaesthesia with the objective to increase arterial oxygenation (PaO₂) in arterial and venous blood of horses during general anaesthesia (Grubb *et al.*, 2008; Nyman *et al.*, 2012; Grubb *et al.*, 2012; Grubb *et al.*, 2013). However, this method has not before been evaluated in critically ill horses, such as horses that undergo acute colic surgery. Colic horses are from the beginning of surgery in a worse clinical condition and are expected to have lower PaO₂ during surgery than healthy horses during general anaesthesia (Edner *et al.*, 2007). We presume that colic horses are in greater need for improvement in arterial oxygenation during anaesthesia than clinically healthy horses.

During 2012 and 2013 me and my graduate colleague Maja Wiklund have evaluated PiNO on critically ill horses that underwent colic surgery at the Equine Clinic at the University Hospital in Uppsala, Sweden. Our work has been a part of professor Görel Nyman's ongoing research on PiNO as a method to treat hypoxemia during equine anaesthesia.

In our work the objective has been to collect arterial and venous blood and interpret the blood gas analysis before surgery, during surgery, in recovery and ten minutes after the horse returned to standing. Metabolic parameters such as blood lactate, blood glucose and pH have also been evaluated.

The horses have been monitored during the whole surgical procedure according to the anaesthesia-protocol used in the clinic. In our work we have collected data from 15 horses treated with PiNO and 15 horses that did not receive any treatment. The non-treated horses are referred to as the control group. The PiNO treated horses also served as their own controls since data was collected before and during treatment with PiNO during anaesthesia. My part in the present study included observing possible effects of improved arterial oxygenation, i.e. the metabolic and cardiovascular parameters during treatment with PiNO.

The hypothesis was that blood lactate and blood glucose would decrease during colic surgery, and even decrease more in the group treated with PiNO compared with the control group. A second hypothesis was that pH and heart rate would normalize faster during surgery in horses treated with PiNO compared with the control group.

LITERATURE REVIEW

Hypoxemia during anaesthesia

It has been showed that colic horses already before surgery have an altered metabolism with decreased cardiovascular function and high levels of blood and muscle lactate (Edner *et al.*, 2007). They usually also have intestines extended with gas which gives the lungs less space for expanding. This combined with the fact that the horse is placed in dorsal recumbency during colic surgery contribute to the formation of lung atelectasis and shunting of deoxygenated blood directly from the venous to the arterial circulation (Qs/Qt). Lung atelectasis and Qs/Qt is the most common reason for development of hypoxemia in horses during anaesthesia (Nyman & Hedenstierna, 1989; Nyman *et al.*, 1990).

Hypoxemia and the formation of lung atelectasis are further exaggerated when horses are positioned in dorsal recumbency (Whitehair & Willits, 1999; Dobson *et al.*, 1985). During surgeries longer than one hour (which is usually the case in equine colic surgery) there is also an increased mortality-risk during anaesthesia. The mortality-risk then increases with time throughout surgery (Johnston *et al.*, 1995). Hypoxemia is only one of the many common complications that may occur during equine anaesthesia but it has been associated with several unwanted events.

Hypoxemia has been connected to the development of cerebral necrosis post anaesthesia (McKay *et al.*, 2002), sudden cardiac arrest during anaesthesia (McGoldrick *et al.*, 1998), decreased skeletal muscle oxygenation, hepatic insult (Whitehair *et al.*, 1996), increased serum lactate (Portier *et al.*, 2009) and lactic acidemia (Taylor, 1999). Furthermore, improved cardiac output and improved oxygen delivery has been shown to decrease mortality in human sepsis patients (Tuchschmidt *et al.*, 1992).

There are several different suggestions of how to increase PaO₂ in the anaesthetized horse. These include breathing of high inspired oxygen fraction, reduction of the pressure from the abdominal content by repositioning the horse, increase of the tidal volume by using intermittent positive-pressure ventilation (IPPV), administration of a β_2 adrenoceptor agonists and the use of positive end-expiratory pressure (PEEP) (Taylor & Clarke, 2007). However, none of these methods has proven to be as effective and simple to use as PiNO, demonstrated in a series of publications (Grubb *et al.*, 2008; Nyman *et al.*, 2012; Grubb *et al.*, 2012; Grubb *et al.*, 2013).

Inhaled Nitric-Oxide (PiNO)

Nitric Oxide (NO) is a molecule produced by endothelial cells in the body itself, causing selective vasodilation in certain organs in the body. By administrating NO into the lungs by inhalation, a selective vasodilation in well ventilated regions occurs which facilitates the transport of oxygen molecules from the inspired gas into the arterial bloodstream. The drug is widely used in the neonatal care in humans as treatment of hypoxemia and respiratory failure in both newborn babies and children (Dobyns *et al.*, 1999). The reason to why NO is superior to other vasodilators in this content is the drugs potent ability to create “sustained pulmonary vasodilation without decreasing systemic vascular tone” (Kinsella & Abman, 1995).

Inhaled NO in horses was for the first time tried by Young *et al.*, (1999). In their study, 10 ppm of NO was added to the inhalation gas for 20 minutes during anaesthesia in seven horses anaesthetized at two different occasions, with and without NO-treatment respectively. NO was given continuously during the whole inspiration, and was thus not given as a pulse as in the PiNO method. The initial study showed no improvement in either oxygenation or shunt fraction. Heinonen *et al.*, (2000) then investigated and developed the method of pulsing NO during a specific time of the breath in anaesthetized pigs. Their hypothesis was that pulsed delivery of NO would cause selective vasodilation in the upper parts of the lung field that still was well ventilated, without affecting

atelectatic lung areas. The method was then evaluated and further developed for the use in horses in collaboration with professor Görel Nyman at the Swedish University of Agricultural Sciences (Heinonen *et al.*, 2001). The PiNO method has been studied in healthy horses and proved to be both effective, safe to use and ready for clinical use by a series of published papers (Grubb *et al.*, 2008; Nyman *et al.*, 2012; Grubb *et al.*, 2012; Grubb *et al.*, 2013). The first clinical trial investigating the effects of PiNO on critically ill horses undergoing abdominal surgery is presented in this study, and in the study presented by my colleague Maja Wiklund.

Metabolism during anaesthesia

Lactate and glucose

When investigating the metabolism of colic horses, it is shown that inhalation anaesthesia alone causes a stress-response in the healthy horse which gives rise to increased levels of cortisol, glucose and lactate concentrations in the blood (Luna *et al.*, 1996). It has also been shown that glucose and lactate are significantly higher in horses subjected to hypoxemia during anaesthesia (Taylor, 1999). Edner *et al.*, (2007) concluded that cortisol, glucose and lactate were elevated already before surgery in colic horses. These horses also had higher values of cortisol, glucose and lactate during the whole surgical procedure compared to healthy horses, since they were entering the surgery with an already altered metabolism. Increased levels of cortisol and glucose in anaesthetized horses can be explained by a massive sympathetic outflow which causes a stress-response with increased levels of adrenocorticotrophic hormone and cortisol. Cortisol has anti-insulin properties and results in temporary insulin-resistance in humans, leading to increased levels of glucose (Clerk *et al.*, 1986). This is also the theory to why horses enter surgery with increased levels of cortisol and glucose (Edner *et al.*, 2007). In several human studies, the link between severe stress-responses in critically ill trauma patients and temporary insulin-resistance with hyperglycemia is well documented (Andrews & Walker, 1999). In humans it has been shown that intensive insulin therapy in those patients reduces morbidity and mortality (Thorell *et al.*, 2004; Van Den Berghe *et al.*, 2001). Normal concentration of blood glucose in horses is 5.5-6.5 mmol/L (Reed *et al.*, 2010).

Lactate has since the mid 1970's been used as a prognostic indicator in equine medicine and it has been documented in several studies that it has a prognostic value in both human critical care and in critically ill foals and horses (Franklin & Peloso, 1996). Lactate is produced by skeletal muscle, erythrocytes, the brain and the gut. In normal body function with aerobic metabolism, pyruvate is converted to acetyl-coenzyme A (acetyl-CoA) through the citric acid cycle to generate ATP, facilitated by the enzyme pyruvate dehydrogenase together with oxygen and glucose. In anaerobic metabolism, pyruvate fails to convert to acetyl-CoA and instead converts to lactate with help from the enzyme lactate dehydrogenase (Franklin & Peloso, 1996).

Not only anaerobic metabolism alone is responsible for the formation of lactate. Lactate can also form from any mechanism that result from tissue hypoxemia such as pulmonary dysfunction, circulatory failure, lack of oxygen delivery secondary to anemia, hepatic failure and endotoxemia (Franklin & Peloso, 1996). Furthermore, hyperlactatemia has been connected to increased levels of insulin and glucose in human studies as production of lactate by adipocytes increases (Henry *et al.*, 1996). Blood lactate levels leading to lactic acidosis in the horse are suggested by Franklin & Peloso (1996) to occur when the measured lactate exceeds 5 mmol/L. Studies have also suggested that lactic acidosis does not only form from tissue hypoxemia, but from increased pyruvate production that occurs during sepsis (Gore *et al.*, 1996; Wolfe & Martini, 2000).

In a healthy resting horse the blood lactate should not exceed 2 mmol/L. 2-5 mmol/L is seen as a moderate rise and colic horses with blood lactate levels over 8 mmol/L is said to have a guarded

prognosis and will require “expensive intensive care” (Franklin & Peloso, 1996). When Radcliffe *et al.*, (2012) looked at the plasma level of lactate in 33 horses that underwent acute colic surgery they found significantly higher levels in non-surviving horses. In non-surviving horses the preoperative median plasma lactate level measured was 7.56 mmol/ L compared to a median of 3 mmol/L in surviving horses.

In the study by Edner *et al.*, (2007) colic horses that underwent acute abdominal surgery had all increased levels of lactate and glucose, which was one reason for us to investigate if levels were lower when horses that underwent similar surgeries were treated with PiNO.

pH

To keep normal body function, the body is striving to keep physiological pH between 7.35 and 7.45. Alterations in pH can have profound physiological effects such as altered cardiac contractility, arrhythmias, diminished responsiveness to catecholamines, insulin resistance, electrolyte disturbances and dysfunction of enzyme systems (Monnig, 2013). By the use of buffer systems, the body is able to keep physiological pH despite small acid-base disturbances, but when those disturbances exceeds the buffer capacity a drop or increase in pH will result in acidosis or alkalosis. The lungs and the kidneys are the primary organs regulating pH in the body (Monnig, 2013).

Acidemia is defined as a blood pH below 7.35. This can either be caused by a respiratory acidosis, a metabolic acidosis or a combination of these two. Metabolic acidosis is caused by an increase in non-volatile acids, increased loss of HCO_3^- or impaired renal excretion of H^+ . Increased production of non-volatile acids, and especially lactate, can occur due to anaerobic muscle work during heavy exercise or due to insufficient supply of oxygen to the tissues by other reasons, for example hypoxemia during anaesthesia. Other types of non-volatile acids that can contribute to lowered pH and the formation of a metabolic acidosis is B-hydroxybutyric acid and acetoacetic acid which is produced when ketosis and diabetes mellitus is present (Sjaastad, 2003).

Respiratory acidosis arises when pulmonary ventilation fails to transport CO_2 out from the body, at the same speed as it is produced. CO_2 is accumulated in the blood which results in a decrease in pH (Sjaastad, 2003). In horses breathing spontaneously during general anaesthesia this is an invariably event. The reason is hypoventilation caused by respiratory depression by the anesthetic used (Taylor & Clarke, 2007). Increased CO_2 can also be caused by different lung disorders such as pneumonia, pulmonary edema or pulmonary obstruction (Sjaastad, 2003).

Since acidemia can be caused by either a respiratory or metabolic acidosis, and it is well known in several studies that colic horses have increased levels of lactate (Edner *et al.*, 2007; Dunkel *et al.*, 2012), we choose to measure pH to see if altered lactate would influence pH through the metabolic pathway.

Cardiovascular function

Heart rate

Normal heart rate in horses is considered by most literature to be between 28 and 40 beats per minute. The occurrence of heart arrhythmias during anaesthesia is well known and the prevalence of arrhythmias after anaesthesia in both healthy horses and horses that underwent colic surgery was recently documented and shown to be very common (Morgan *et al.*, 2011). In humans, there is documented evidence that tachycardia occur together with hypoxemia and low saturation of oxygen after abdominal surgery (Gögenur *et al.*, 2004). Thus, this was the reason for us to study if heart rate during anaesthesia by any means was affected by changes in PaO_2 during treatment with PiNO.

MATERIAL AND METHODS

Study design

This was a prospective clinical study of horses referred to the Equine Clinic at the Swedish University of Agricultural Sciences because of colic. The criterion for horses to be included in the study was that the colic could not be treated medically and the horses therefore needed acute abdominal surgery. Further, the owners needed to give an informed consent before the horse could be subjected to PiNO treatment during the anaesthesia. The horses were studied as two different groups; 15 horses were given treatment with PiNO during surgery and 15 horses were not treated with PiNO and worked as a control group. Both groups were subjected to measurements of arterial and venous blood gases, blood lactate, blood glucose, pH and heart rate. Beyond this, all parameters that are included in the equine hospitals regular anaesthesia monitoring protocol was also recorded. The samples were collected before surgery when the horse still was conscious and standing, during surgery, after surgery in the recovery room when it was still recumbent and ten minutes after recovery when the horse had regained a standing position. Each horse in the treated group also worked as its own control, since data was collected both before and after PiNO was introduced during surgery.

Horses

Table 1: Individual facts about included horses.

S.W = Swedish Warmblood

S.T = Standardbred Trotter

Horse	Age	Breed	Weight (kg)
NO 1	13	S.T	520
NO 2	6	Unknown breed	590
NO 3	5	S.W	656
NO 4	5	S.T	492
NO 5	20	S.T	572
NO 6	5	S.W	515
NO 7	15	S.W	645
NO 8	4	S.W	640
NO 9	10	Tinker	528
NO 10	7	Icelandic horse	340
NO 11	7	S.W	688
NO 12	21	S.W	643
NO 13	1	Belgian Draft	595
NO 14	16	Icelandic horse	389
NO 15	17	Welsh Pony	337
C 1	1	Connemara	330
C 2	12	Swedish Riding Pony	438
C 3	15	Hannover	563
C 4	18	S.T	572
C 5	5	S.T	453
C 6	20	S.W	610
C 7	3	S.W	550
C 8	12	S.T	616
C 9	16	S.W	515
C 10	15	S.W	571
C 11	15	S.W	584
C 12	5	S.W	490

C 13	11	S.T	585
C 14	8 months	S.W	250
C 15	9	S.T	500

Thirty horses of different breed (fourteen Swedish Warmblood, seven Standardbred trotters, two Icelandic horses, one Tinker, one Belgian draft horse, one Hannover, one Swedish Riding pony, one Welsh pony, one Connemara pony and one horse of unknown breed arrived to the Equine Clinic at the Swedish University of Agricultural Sciences between May 2012 and December 2013 because of colic. The majority of the horses had been examined and treated in the field by a veterinarian before referred to the clinic. The horses were referred if they did not respond to treatment or if the treating veterinarian thought that the colic was a case for surgery. All of the horses were examined again by the veterinarian on duty after arrival to the clinic. Clinical and rectal examination was made before decision of abdominal surgery was taken. The age of the horses varied between 8 months and 21 years (mean 10.3 years).

Anaesthesia

The majority of the horses had been treated with metamizol (20-50 mg/kg IV, Vetalgin vet, Intervet, Sweden) or flunixin meglumine (0.25-1.1 mg/kg IV, Flunixin, N-vet, Sweden) by the referring veterinarian before arrival. If the horses were referred without any treatment from a referring veterinarian the first drug of choice was metamizol and later flunixin meglumine for pain-management. Other treatments given before surgery and prior to rectal examination were a low dose of detomidin (0.004-0.01 mg/kg IV, Domosedan vet, Orion Pharma Animal Health), butorphanol (0.01-0.02 mg/kg IV, Dolorex vet, Intervet, Sweden) and butylskopolamin (0.1-0.3 mg/kg IV, Buscopan, Boehringer & Ingelheim, Sweden).

All horses received intravenous electrolytes (Ringer acetat, Pharmacia & Upjohn, Sweden) and/or a dextran colloid (Macrodex®, Meda AB, Solna, Sweden) prior to and during surgery. Other treatment before surgery consisted of broad-spectrum antibiotics and a booster dose of tetanus vaccine.

The horses were anaesthetized following the standard protocol used at the clinic. Premedication existed of acepromazine (0.03 mg/kg IM, Plegicil, Pharmaxim, Sweden), romifidin (0.1 mg/kg IV, Sedivet vet, Boehringer Ingelheim Vetmedica, Sweden) and butorphanol (0.025 mg/kg IV, Butador, Vetoquinol, Sweden). The majority of the horses did not receive any acepromazine prior to surgery because of the risk to aggravate preexisting hypotension.

Horses were induced with diazepam (0.03 mg/kg IV, Diazepam-ratiopharm10, PharmaMedics, Switzerland) and ketamine (2.2 mg/kg IV, Ketaminol®vet, Intervet, Sweden). After induction the horses were intubated with a cuffed endotracheal tube and connected to a semiclosed large animal anaesthetic circuit. Anaesthesia was maintained with isoflurane (IsoFlo; Orion Pharma Animal Health, Sweden) in oxygen. Breathing was spontaneous during the whole anaesthetic procedure. If mean ABP decreased below 60 mmHg during surgery the horses received dobutamin (2-5 µg/kg/min IV, Dobutamin Carino, Carinopharm, Germany). For additional pain-management during surgery the horses were given lidocaine (2 mg/kg IV, bolus during 15 minutes) followed by CRI (2 mg/kg/h IV, Xylocain, Astra Zeneca, Sweden). For a calmer and safer recovery horses were sedated with xylazine (0.2 mg/kg IV, Narcoxyl vet, Intervet, Sweden) when turned in the recovery room after surgery.

Delivery of PiNO

PiNO was started immediately after the first arterial blood sample was collected. The time from start of surgery to commencement of PiNO varied between the horses since the time to place the arterial catheter and collect the baseline sample varied (approximately 5-30 minutes). PiNO was delivered via a line connected between the delivery device and the proximal end of the endotracheal tube. PiNO was delivered during the initial portion of each breath by the use of a special device developed by Datex-Ohmeda Research Unit, Helsinki, Finland (Heinonen *et al.*, 2001, Heinonen *et al.*, 2002). Hence, NO was pulsed during approximately the first third of each inspiration period (instead over the entire breath). In our work, the PiNO horses were first treated with NO during the first 30 % of inspiration and the time of PiNO increased to 45% or 60% of the inspiration, if the response in arterial PaO₂ was insufficient. We adjusted the dose of PiNO until we reached what we defined as the best possible (highest) arterial PaO₂. This dose was continued during the entire anaesthesia and surgical procedure. The NO was supplied in a cylinder of 2,000 ppm medical grade NO in N₂ (AGA AB, Sweden).

Instrumentation

All horses were instrumented with ECG-electrodes for analysis of heart rhythm and pulse frequency, pulse oximetry for clinical measurement of oxygen saturation, arterial catheter in the facial artery for measurement of invasive arterial blood pressure and for collection of blood samples for blood gas analysis in a blood gas analyzing machine (ABL 500 or ABL 90, Radiometer, Copenhagen). The arterial catheter was placed in the facial artery and secured with stitches so that it would be secured in the facial artery during recovery. The horses were also instrumented with a venous catheter, either a central venous or a jugular venous for the collection of blood for blood gas analysis. This part of the study is presented by my colleague Maja Wiklund. Beyond this, respiratory rate, FiO₂, EtCO₂ and EtIso were recorded from an anaesthesia monitor and capnograph (Mindray, BeneView T5).

Collected data

Arterial and venous blood samples were collected in pre-heparinized syringes and stored on ice before analysis. The first venous sample was collected before surgery in the standing horse for measurement of blood gases, blood lactate and blood glucose. After anaesthesia had been induced and the horse was maintained on isoflurane anaesthesia, arterial and venous blood samples were collected as soon as the catheter in the facial artery had been placed. When these blood samples had been collected, the delivery of PiNO was started in the treatment group. In this way, a baseline value was collected in each treated horse which could work as a control sample. Arterial and venous blood was then collected every fifteenth minute during the entire surgery. Arterial and venous samples were also collected in the recovery period, five minutes after isoflurane and NO had been discontinued. Arterial and venous blood was also collected ten minutes after the horse had regained a standing position. In some horses the arterial catheter was displaced during recovery which made further postoperative arterial sampling impossible.

Blood gases were either analyzed immediately or stored on ice and analyzed as soon as possible during surgery.

Calculated data

From the values obtained the following calculations were made using standard equations.

Arterial and central venous oxygen content:

$$C_{xO_2} = (Hb \times 1.36 \times S_{yO_2}) + (0.227 \times P_{yO_2}) \quad y = a, v$$

Arterial-central venous oxygen content difference:

$$C(a-v)O_2 = CaO_2 - CvO_2$$

Oxygen extraction ratio:

$$C(a-v)O_2 \times CaO_2^{-1}$$

Statistical analysis

Data are presented as mean \pm standard deviation (SD). Raw data was first processed in Microsoft Excel and progressed to the statistical analysis program GraphPad Prism 5 where all statistical analysis was made. The statistical analysis used were t-tests to evaluate differences between treated versus non treated groups. Correlation analysis was also used to determine if changes in arterial PaO₂ influenced any of the studied metabolic parameters. Statistical difference was considered when $p < 0.05$ with a 95% confidence interval.

RESULTS

Out of the 30 horses entering the study, 21 horses were alive one week after surgery and discharged from the hospital. Nine horses were either euthanized during surgery due to bad prognosis or euthanized after recovery because of poor clinical condition and bad prognosis.

Several metabolic parameters in this section are correlated to the percentile change in PaO₂. The analysis of changes in PaO₂ with and without PiNO treatment was the main aim of my colleague Maja Wiklund's work. Changes in PaO₂ will therefore not be discussed here, but will be presented and correlated to the metabolic and cardiovascular parameters in the present work; i.e. blood lactate, blood glucose, arterial pH and heart rate.

Table 2: Individual data, diagnosis and outcome of surgery.

Horse	Age	Breed	Weight (kg)	Diagnosis	Outcome
NO 1	13	S.T	520	2	E. post-surgery
NO 2	6	Unknown breed	590	5	Survived
NO 3	5	S.W	656	2	Survived
NO 4	5	S.T	492	5	Survived
NO 5	20	S.T	572	6	Survived
NO 6	5	S.W	515	7	Survived
NO 7	15	S.W	645	3	Survived
NO 8	4	S.W	640	9	E. D. S
NO 9	10	Tinker	528	1	E. post-surgery
NO 10	7	Icelandic horse	340	1	Survived
NO 11	7	S.W	688	9	Survived
NO 12	21	S.W	643	9	E. post-surgery
NO 13	1	Belgian Draft	595	5	E. D. S
NO 14	16	Icelandic horse	389	11	Survived
NO 15	17	Welsh Pony	337	8	Survived
C 1	1	Connemara	330	5	E. D. S
C 2	12	S. Riding Pony	438	3	Survived
C 3	15	Hannover	563	4	Survived
C 4	18	S.T	572	3	Survived
C 5	5	S.T	453	5	Survived
C 6	20	S.W	610	2	E. post-surgery
C 7	3	S.W	550	2	E. D. S
C 8	12	S.T	616	12	Survived
C 9	16	S.W	515	7	Survived
C 10	15	S.W	571	4	Survived
C 11	15	S.W	584	5	Survived
C 12	5	S.W	490	4	Survived
C 13	11	S.T	585	4	E. D. S
C 14	8 months	S.W	250	10	Survived
C 15	9	S.T	500	4	Survived

- 1 = Colon displacement
- 2 = Colon torsion
- 3 = Small intestine incarceration
- 4 = Nephrosplenic entrapment
- 5 = Small intestine volvulus
- 6 = Proximal enteritis
- 7 = Strangulating lipoma
- 8 = Ileum impaction
- 9 = Invagination of small intestine
- 10 = Strangulation of small colon
- 11 = Caecal impaction
- 12 = Proximal jejunal impaction

Survived = Survived and resigned from hospital

E.D.S = Euthanized during surgery due to bad prognosis

E. post-surgery = Euthanized post-surgery due to bad prognosis

Blood lactate during surgery

Levels of blood lactate were increased (0.9-9.8 mmol/L) in all horses before induction of anaesthesia. Three horses in the treated group and one horse in the control group had blood lactate over 5 mmol/L before induction of anaesthesia. Levels of blood lactate at the start of anaesthesia varied between 1-11.8 mmol/L. The highest measured blood lactate at the start of anaesthesia (11.8 mmol/L) was in one horse in the PiNO group. In the figure below, the change in blood lactate during anaesthesia is presented as percentile change in blood lactate mmol/L between the first and the last sample measured during anaesthesia. We chose to present the relative differences since the individual blood lactate concentrations varied largely between horses. One horse had a non-explainable rise in blood lactate during surgery and was considered as an outlier. Data from that horse was removed and the analysis of blood lactate in the PiNO group therefore only includes 14 horses.

The blood lactate concentration decreased significantly in horses treated with PiNO compared to the control group. Ten out of 14 horses in the PiNO group showed a decrease in blood lactate concentration at the end of surgery compared to the first sample during anaesthesia. Two horses in the PiNO group had similar concentrations of blood lactate both at the beginning of and at the end of surgery. In two horses the blood lactate concentration increased during surgery. In ten of the fifteen control horses the blood lactate concentration decreased during anaesthesia and in five horses blood lactate concentration increased during anaesthesia.

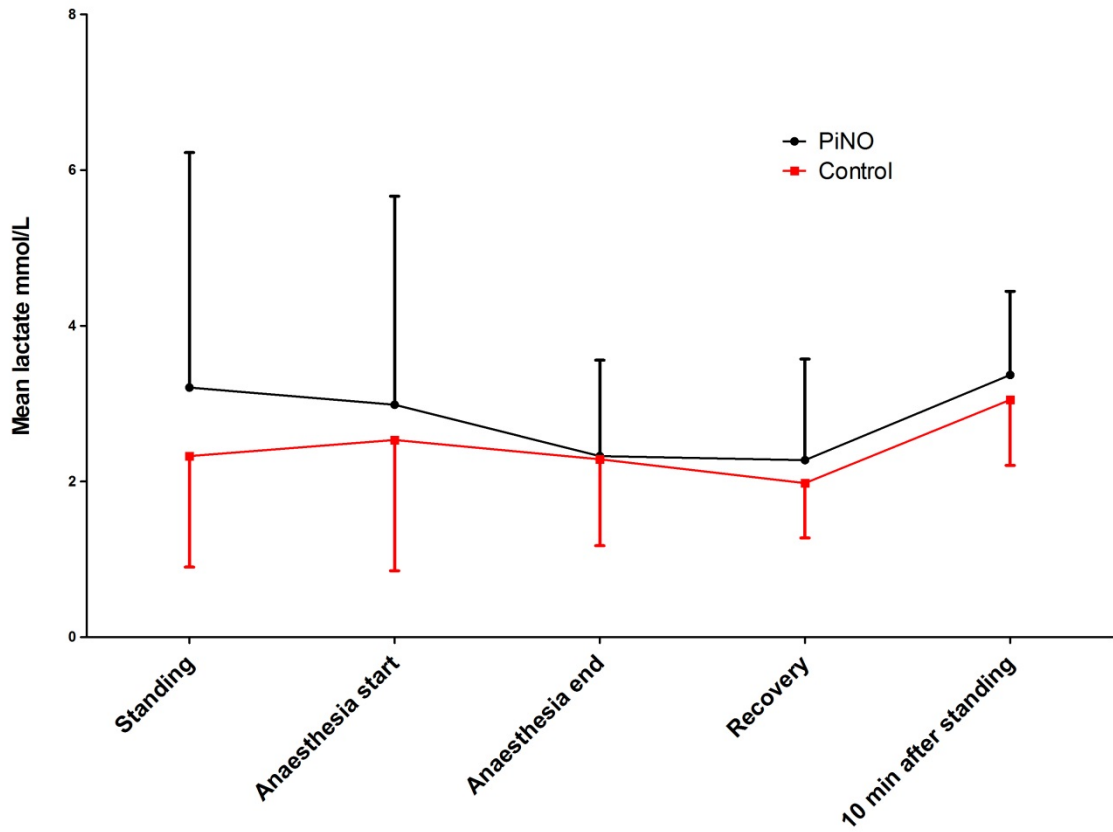


Figure 1: Mean blood lactate concentration \pm SD for the PiNO group and the control group before, during and after surgery. Values were measured at the start of anaesthesia, during anaesthesia, at the end of anaesthesia, five minutes into recovery and ten minutes after the horse had regained a standing position.

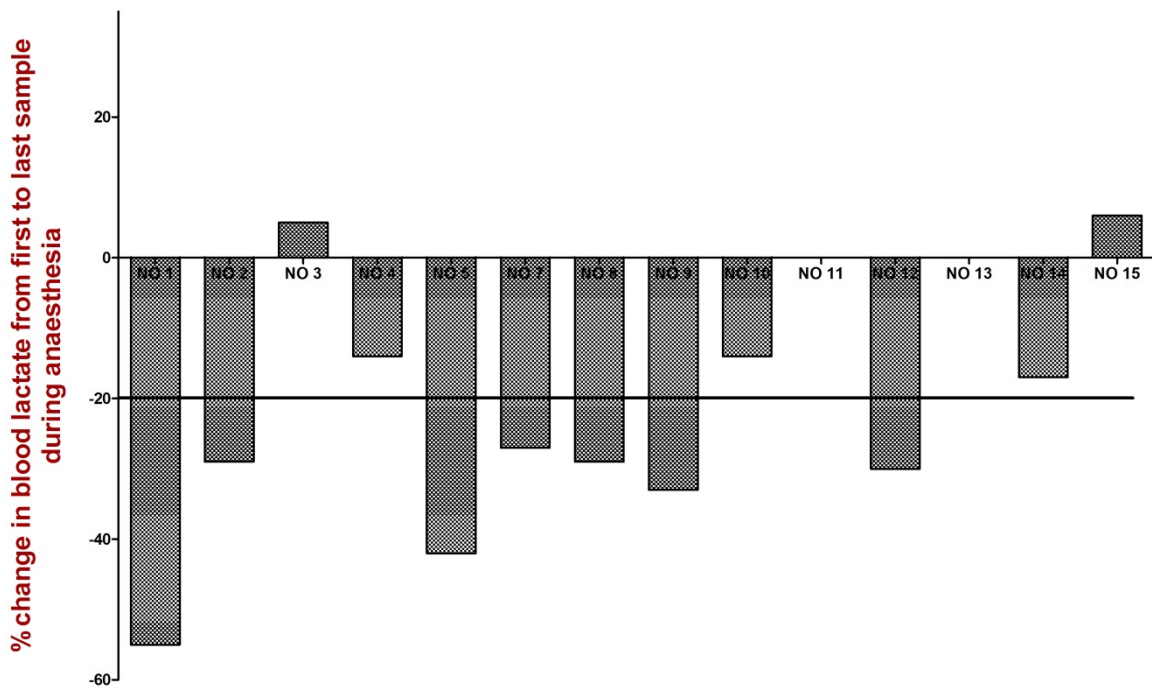


Figure 2: Individual change in blood lactate concentration (%) in horses between the first and the last sample during anaesthesia in the PiNO group.

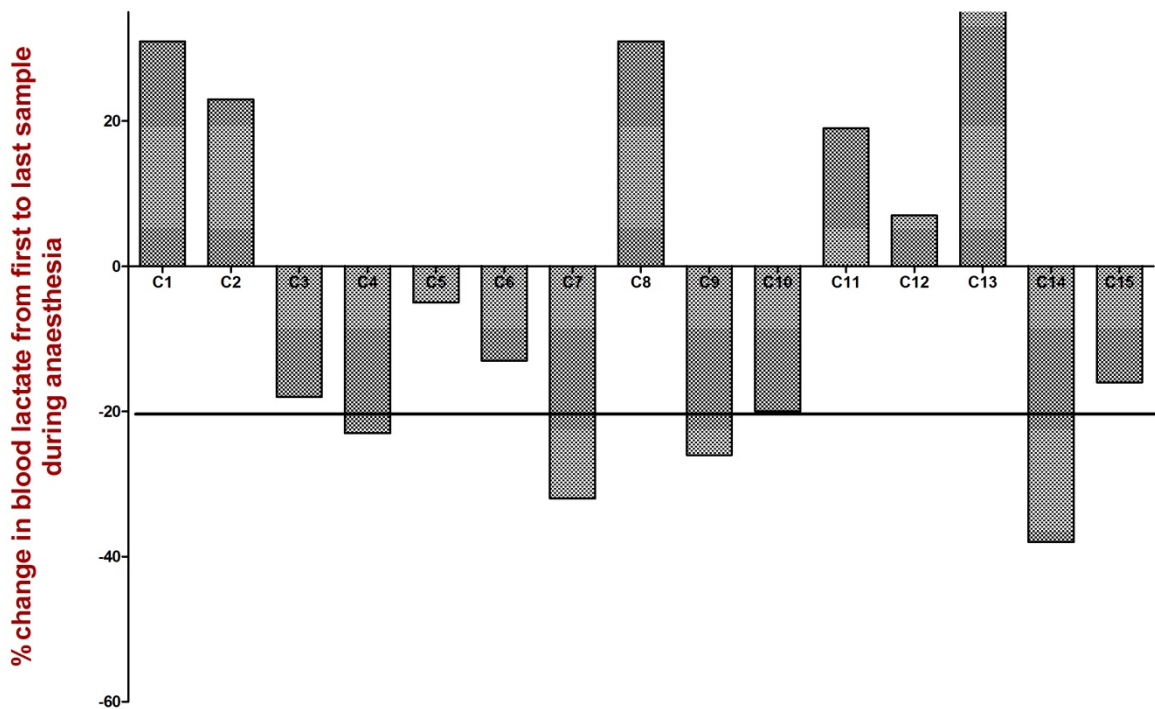


Figure 3: Individual change in blood lactate concentration (%) in horses between the first and the last sample during anaesthesia in the non-treated control group.

The PiNO group showed a significant decrease in blood lactate concentration (mmol/L) during anaesthesia ($p=0.0461$) compared to the control group ($p=0.1336$) which had similar blood lactate concentration throughout the entire anaesthesia. This was confirmed with a paired one-tailed t -test and compared the first and the last sample measured during surgery for each group. The two groups also differed significantly from each other when comparing the percentile change in blood lactate concentration during surgery by the use of a one-tailed non-paired t -test ($p=0.0251$). The mean percentile change + SD for the two groups were -19.99 ± 4.89 mmol/L (PiNO group) and 0.05 ± 8.26 (control group).

In the PiNO group seven of the 15 horses had a 20 % decrease or more in blood lactate concentration from start to end of anaesthesia. In the control group six of 15 horses had a 20% decrease or more in blood lactate concentration from start to end of anaesthesia. Six out of eight horses that showed an increase in blood lactate concentration during anaesthesia are found in the control group which did not receive any PiNO treatment.

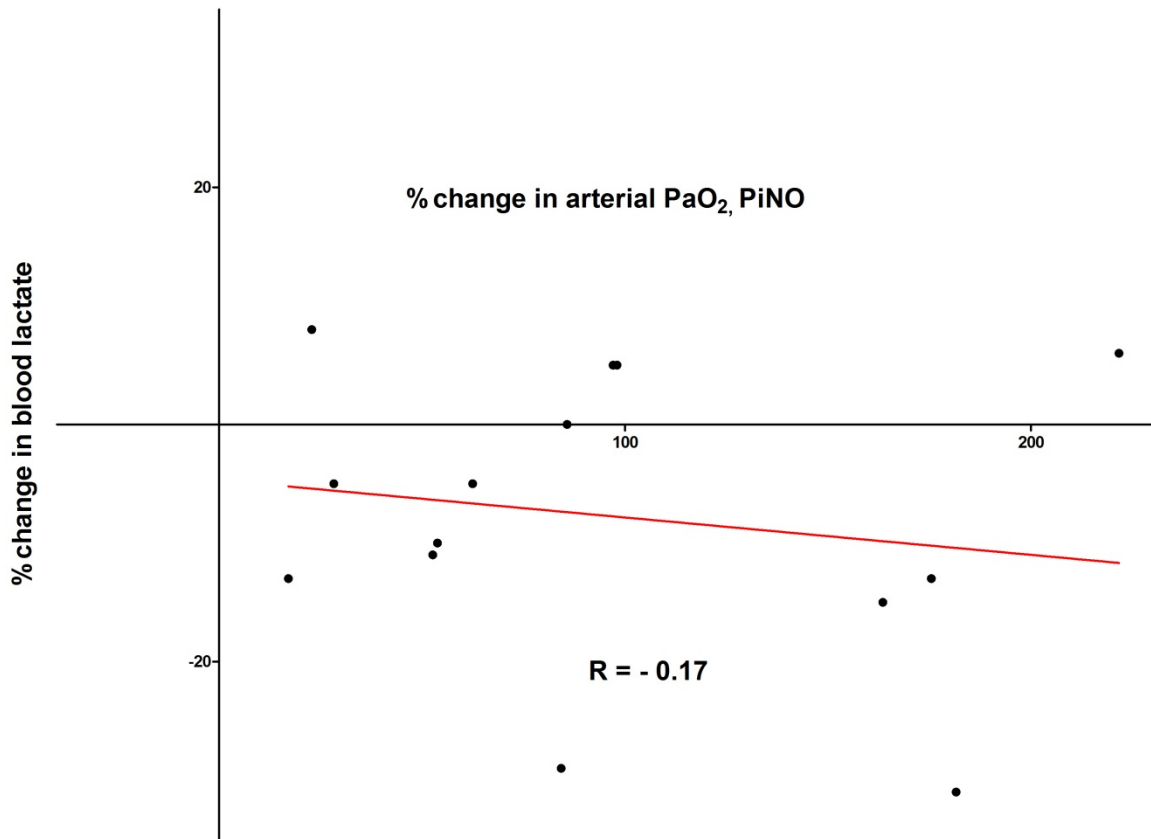


Figure 4: Correlation between the change in blood lactate concentration (%) to the change in PaO₂ (%) during anaesthesia in the treated PiNO group. PaO₂ increased in all treated horses and blood lactate decreased in the majority of horses. The figure indicates a slight negative correlation but it is not statistically significant when a one-tailed Pearson correlation analysis is calculated ($p=0.28$). Since large variations in this data were present the correlation was also tried using Spearman correlation analysis, but the correlation was not significant ($p=0.35$). Note the value above the x-axis and most far to the right. That horse responded differently to the treatment and clearly elevate the R and p-value.

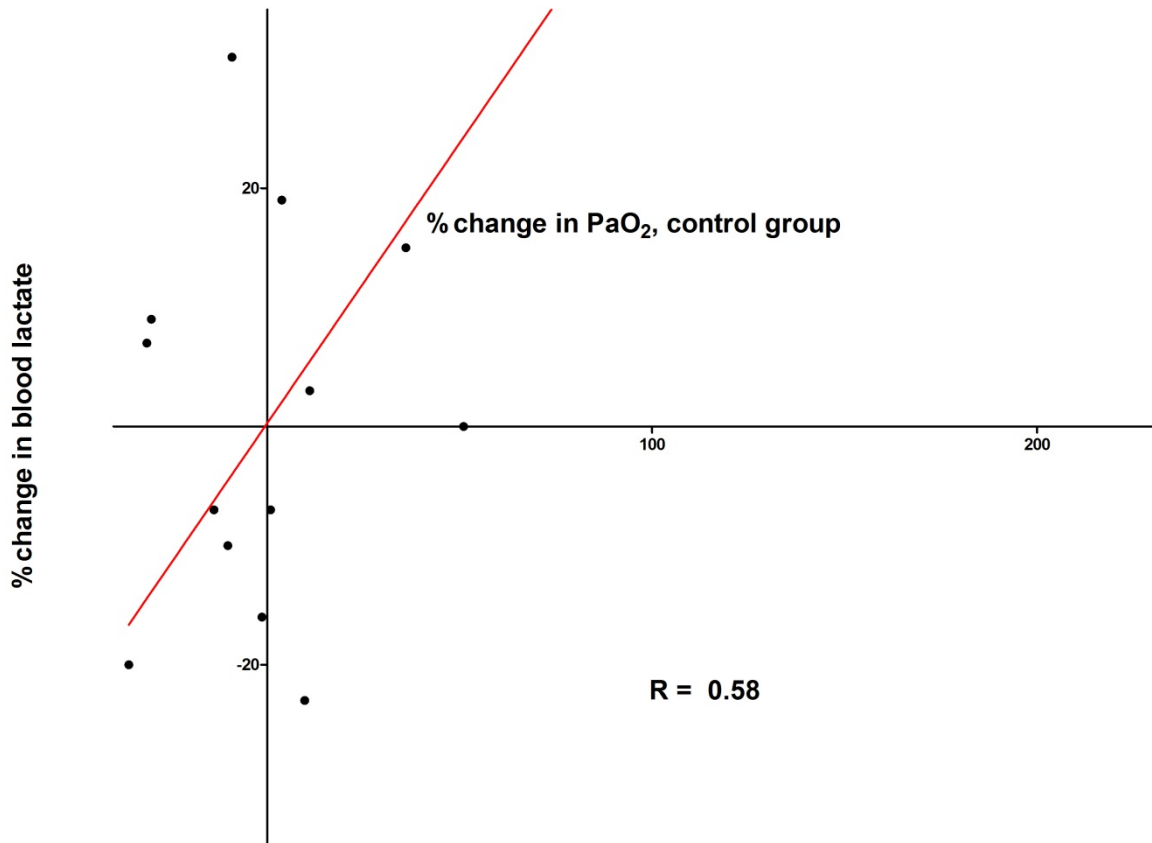


Figure 5: Correlation between the change in blood lactate concentration (%) to the change in PaO₂ (%) during anaesthesia in the non-treated control group. PaO₂ decreased in the majority of horses and blood lactate increased in the majority of horses and statistical analysis shows a positive correlation when a one-tailed Pearson correlation analysis is calculated ($p=0.01$).

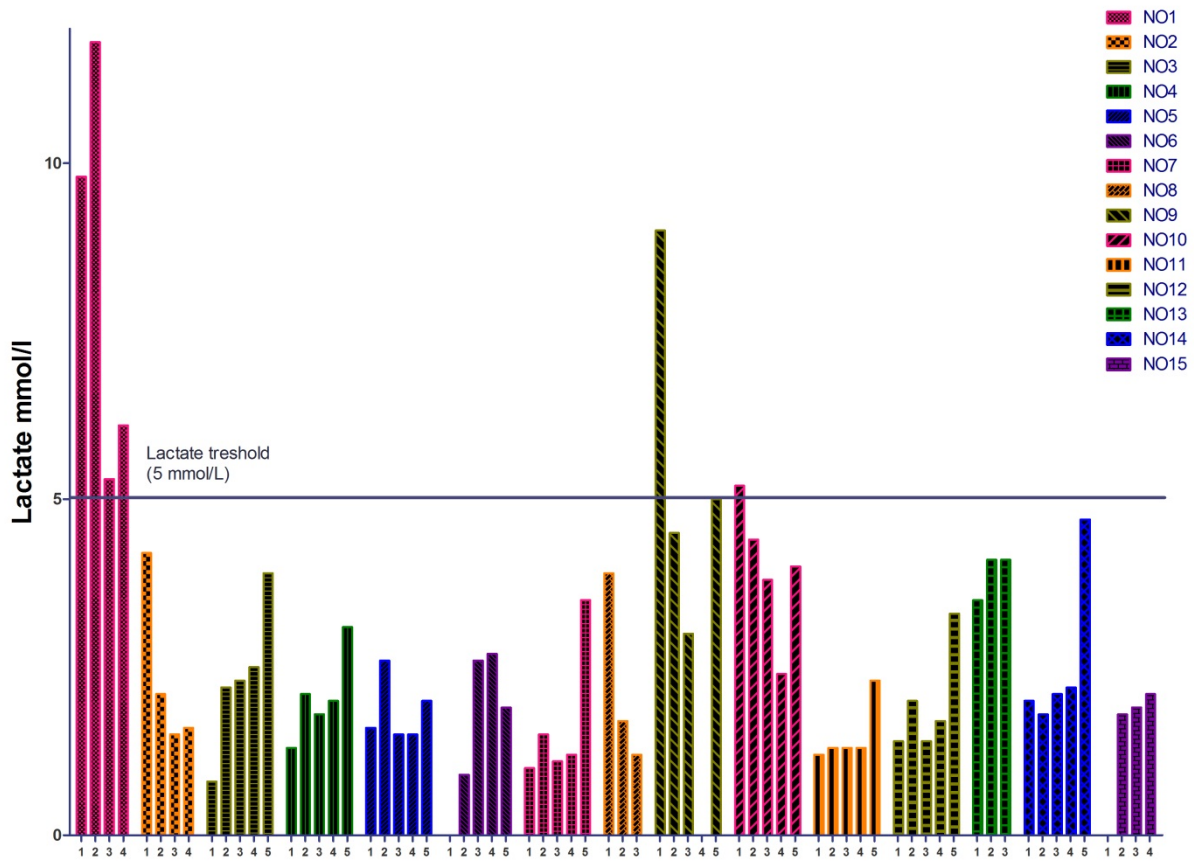


Figure 6: Values of blood lactate before surgery (1), during surgery (2), at the end of surgery (3), in recovery (4) and ten minutes after the horse regained standing position (5) can be seen for the individual horses in the treated PiNO group. The lactate threshold of 5 mmol/L is drawn with a line to show which horses that had the highest and most critical levels of blood lactate. When blood lactate concentration exceeds 5 mmol/L metabolic acidosis because of lactate production can occur (Franklin and Peloso, 2006).

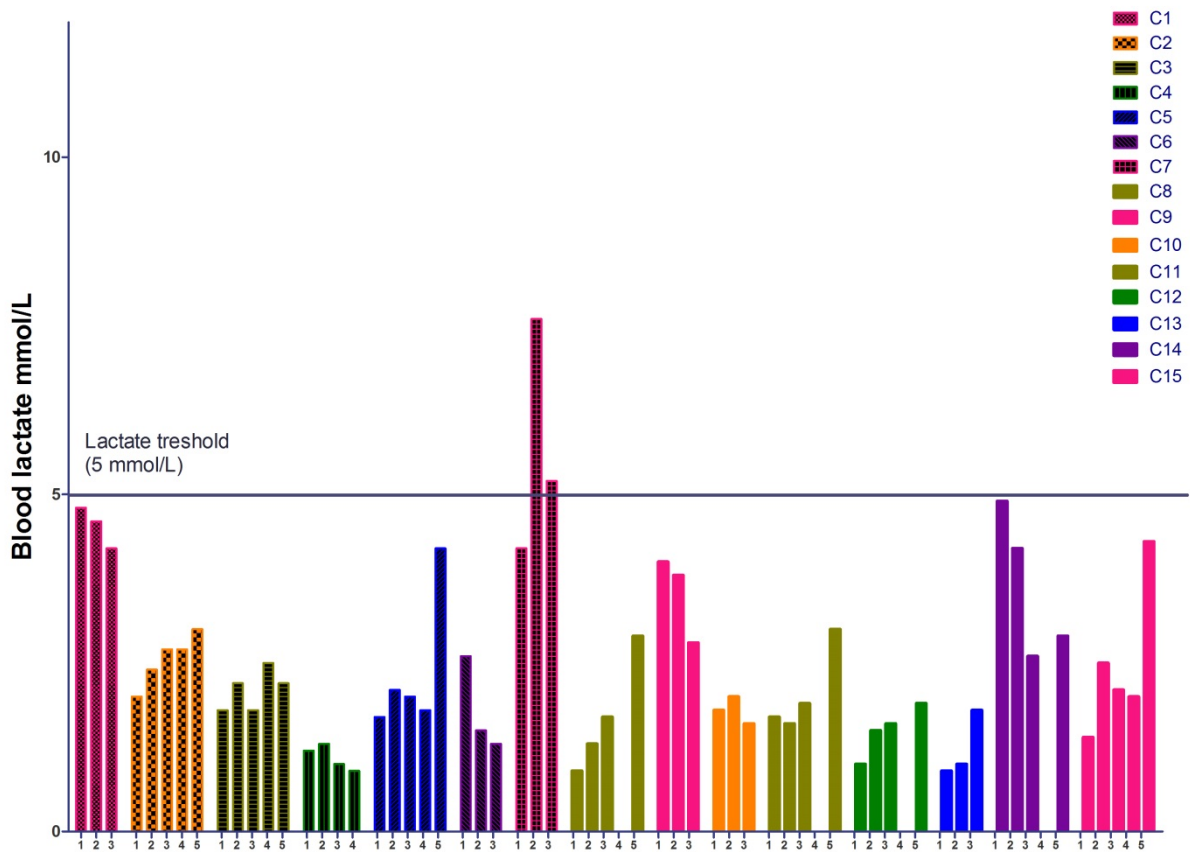


Figure 7: Values of blood lactate before surgery (1), during surgery (2), at the end of surgery (3), in recovery (4) and ten minutes after the horse regained standing position (5) can be seen for the individual horses in the non-treated control group. The lactate threshold of 5 mmol/L is drawn with a line to show which horses that had the highest and most critical levels of blood lactate. When blood lactate concentration exceeds 5 mmol/L metabolic acidosis because of lactate production can occur (Franklin and Peloso. 2006).

When comparing the diagrams, it can be visualized that horses from both groups show a gradual decrease in blood lactate during surgery. Thus, it appears that the blood lactate concentration in two of three horses with values over the suggested lactate threshold in the treated PiNO group, decrease below 5 mmol/L after treatment. The horse with a blood lactate concentration over the threshold value in the control group does not decrease below 5 mmol/L and is subject to a presumed metabolic acidosis.

Blood lactate during recovery

Five minutes into recovery the blood lactate concentration did not change significantly in either the PiNO group or the control group. Hence, no sudden increase in blood lactate could be seen after the inhalation anaesthesia and PiNO was discontinued. A significant rise in blood lactate concentration was measured in both groups after the horse had regained a standing position, compared to the value collected five minutes into recovery. This was confirmed with an unpaired two-tailed *t*-test ($p < 0.05$ for both groups). The rise in blood lactate in recovery can be seen in both figure 1 (mean) and figure 6 and 7 (individual values).

Blood glucose during anaesthesia

Blood glucose levels were increased over 6.5 mmol/L in both groups after induction of anaesthesia and gradually decreased during anaesthesia.

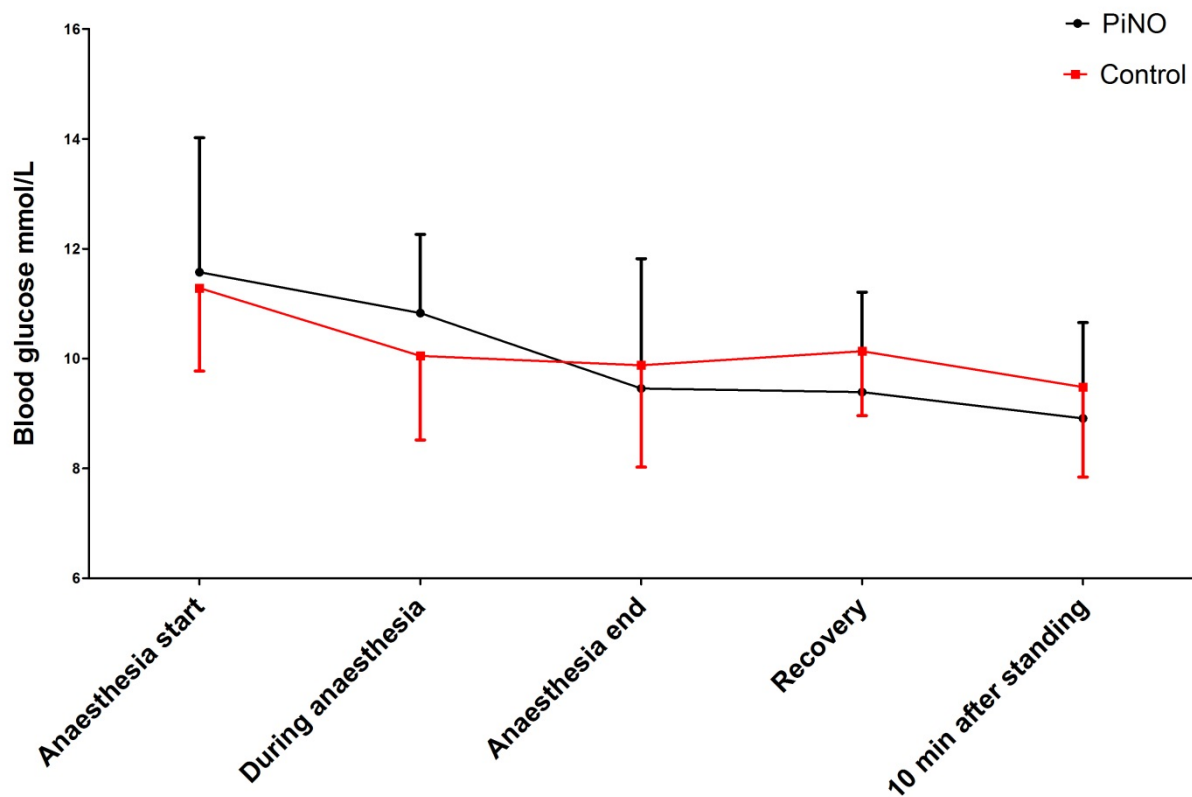


Figure 8: Mean values \pm SD of blood glucose in both the PiNO group and the control group.

When performing a *t*-test there was no significant difference between the groups, indicating that the use of PiNO does not affect the levels of blood glucose differently in the two groups during anaesthesia. Further statistical analysis was not continued since it was clear that there were no differences between the groups.

Blood glucose during recovery

Blood glucose stayed on the approximate same level in recovery and after the horse had regained a standing position. There was no significant change in levels according to a performed *t*-test. There was no significant difference in blood glucose levels between the PiNO group and the control group.

pH during anaesthesia

All horses in both the PiNO group and in the control group had a pH below 7.35 at the start of anaesthesia. The PiNO group started at a mean pH lower than the control group, indicating that horses in the PiNO group were more acidemic from the beginning of surgery. The pH decreased in both groups during anaesthesia, indicating that both groups were facing further acidosis.

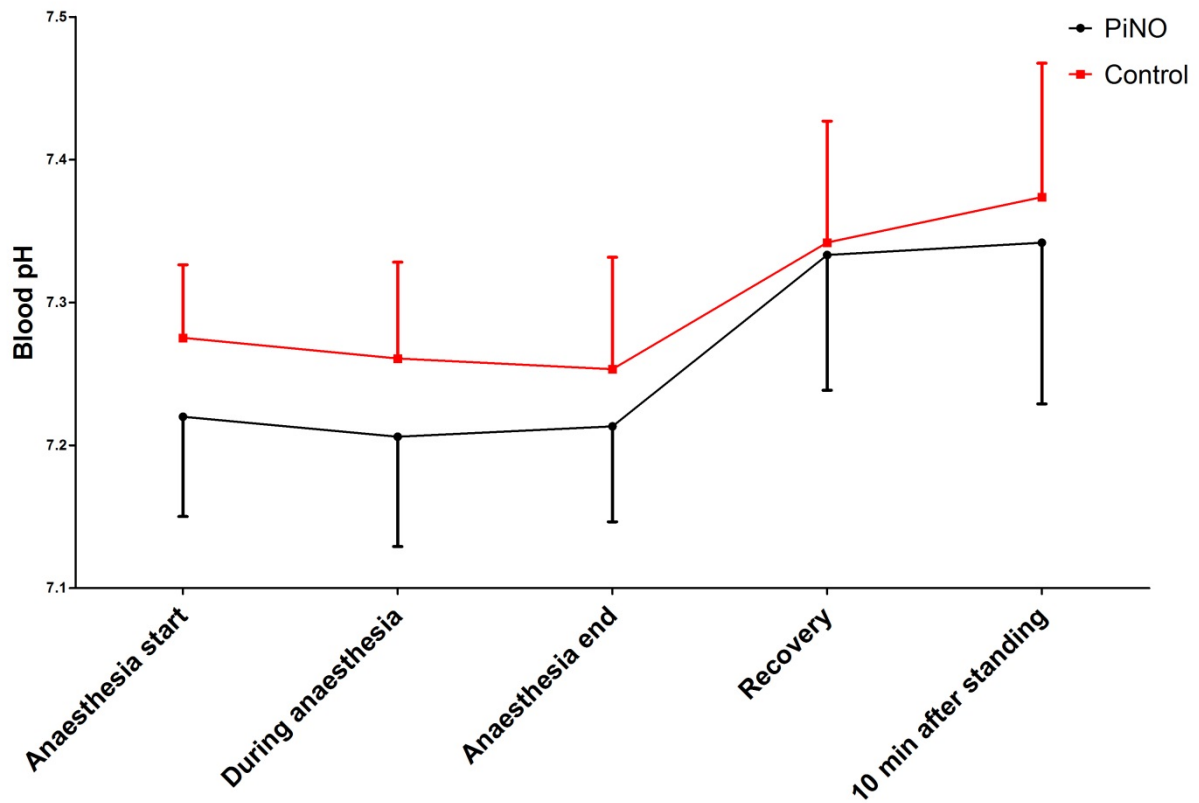


Figure 9: Mean pH \pm SD is shown for the PiNO group and the non-treated control group from the beginning of surgery to the last sample when the horse had regained a standing position.

There was no correlation between differences in pH simultaneously with the increase in PaO₂. On the contrary, pH decreased further during anaesthesia. However, when correlating decrease in pH to the increase in PaCO₂, a correlation observation was made.

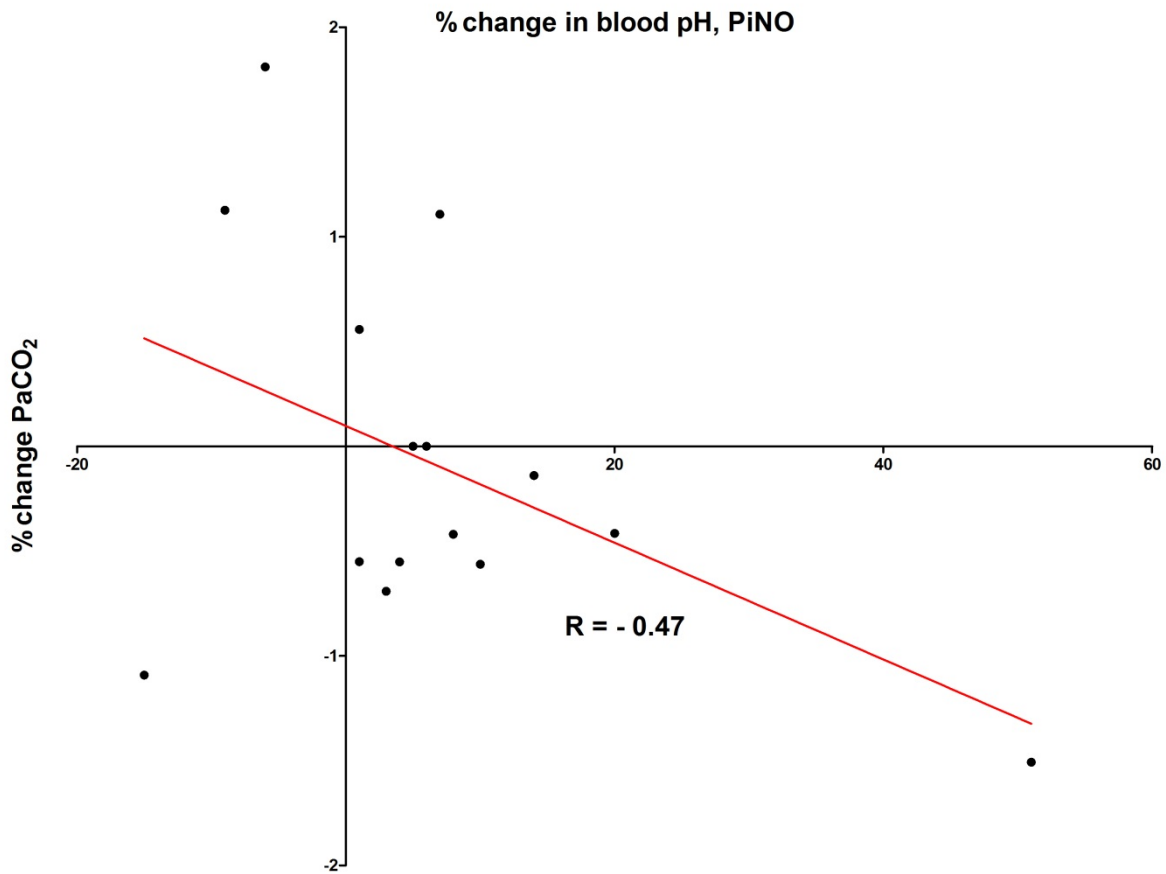


Figure 10: Correlation between the change in pH (%) to the change in PaCO₂(%) during anaesthesia for the PiNO group. The figure shows a significant negative correlation, confirmed with a one-tailed Pearson correlation analysis ($p < 0.04$).

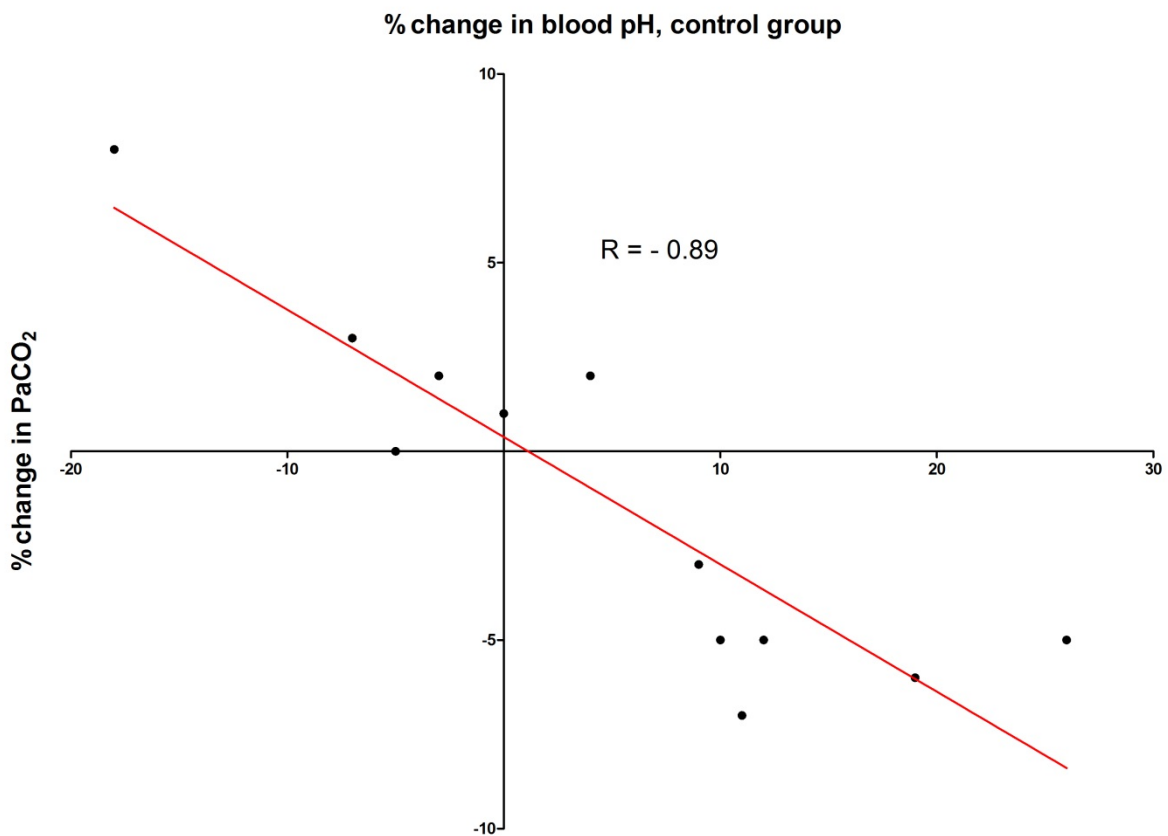


Figure 11: Correlation between the change in pH (%) to the change in PaCO₂ (%) during anaesthesia for the non-treated control group. The figure shows a significant negative correlation, confirmed with a one-tailed Pearson correlation analysis ($p > 0.01$).

pH during recovery

There was no significant difference between the groups in pH during recovery or after the horse had regained a standing position. For both the PiNO group and the control group, pH increased as soon as the horse was removed from the anaesthesia circuit and positioned in lateral recumbency in the recovery box; pH increased from the last sample taken during anaesthesia to the sample taken five minutes into recovery, confirmed with a paired two-tailed t -test $p < 0.01$ for the PiNO group. For the control group a paired t -test could not be performed since blood samples from only 5 horses were collected in recovery. However, the mean values from the last samples taken during anaesthesia and the samples collected five minutes into recovery were significantly different $p < 0.05$, confirmed with a non-paired two-tailed t -test. The pH increased further in both groups after the horses had regained a standing position (see mean values \pm SD during recovery in Figure 9).

Heart rate during anaesthesia

There was no difference in heart rate between the PiNO group and the control group; a mean value of 40 beats/min for both groups at the start of anaesthesia. At the end of anaesthesia the mean value for both groups was 38 beats/min. Changes in heart rate during anaesthesia did not correlate to changes in PaO₂ and seemed to be independent of the metabolic variables measured. Heart rate was not measured in recovery or after recovery.

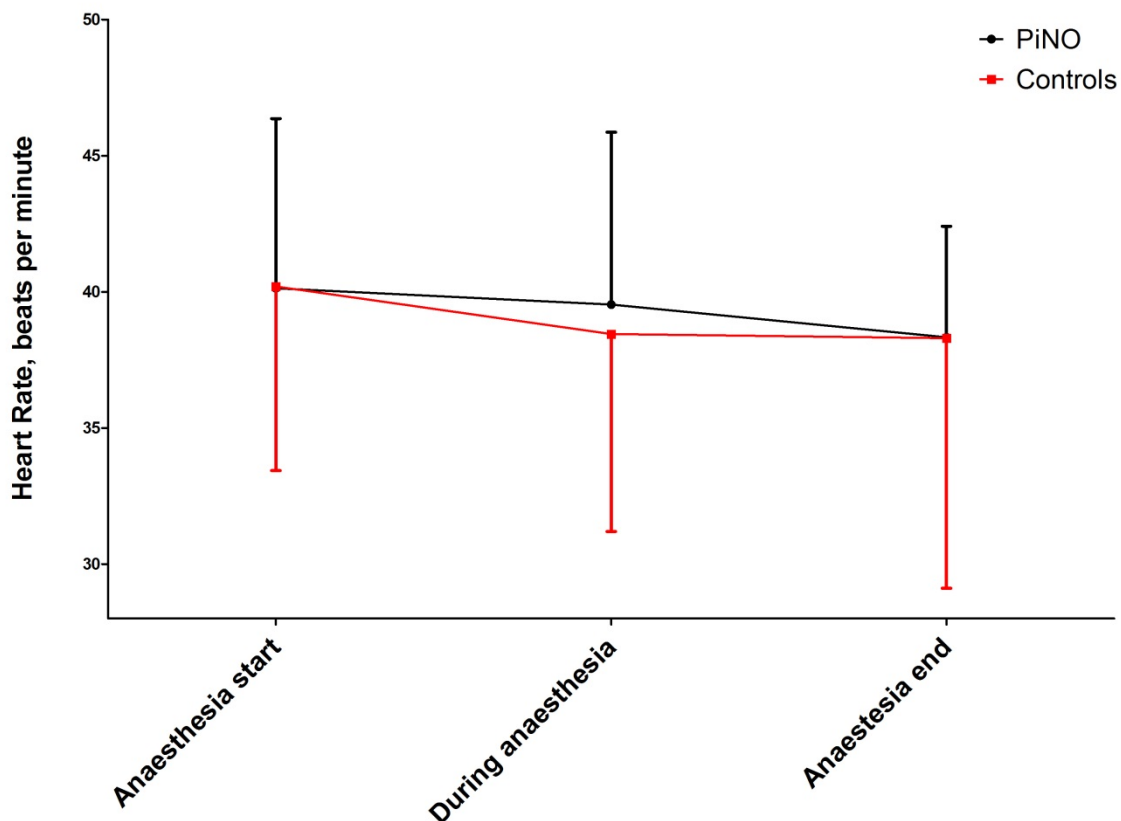


Figure 12: Mean \pm SD of heart rate for the PiNO group and the non-treated control group at the start of anaesthesia, during anaesthesia and at the end of anaesthesia.

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 ($p < 0.005$) difference between the individuals that received PiNO (N=14) and individuals in the control group (N=13), this is illustrated in Figure 13.

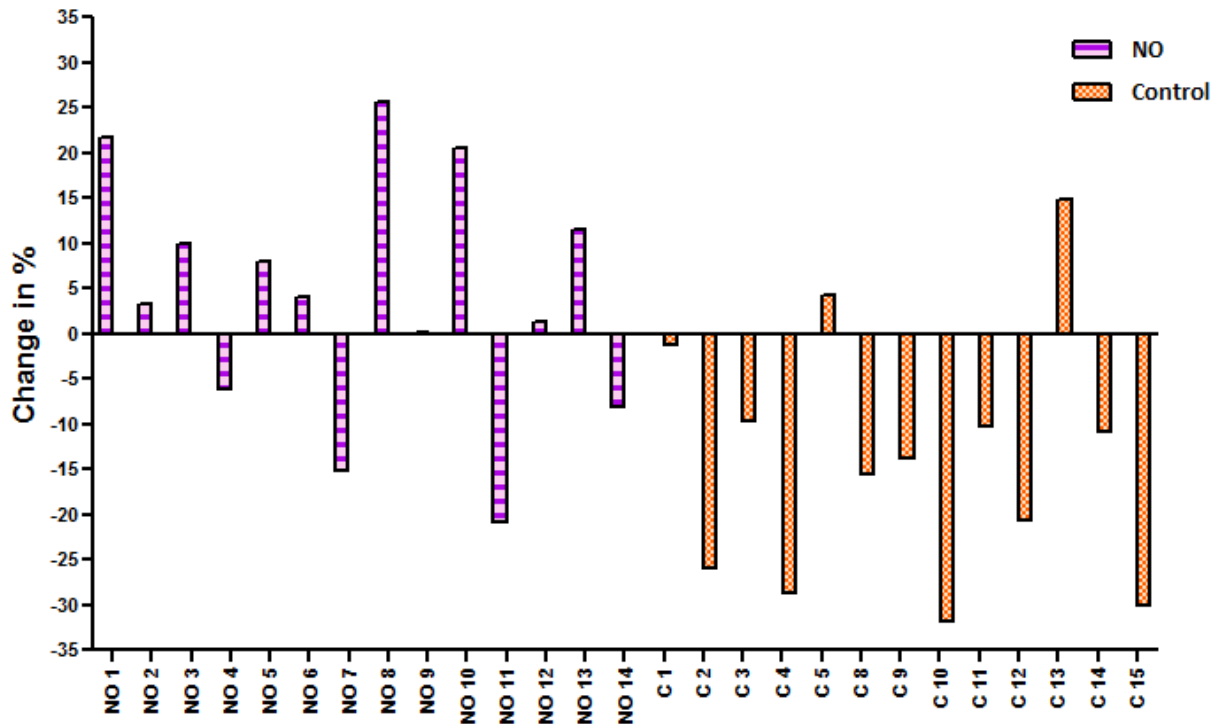


Figure 13. Individual changes in CaO₂ in % when comparing the highest measured CaO₂ during anaesthesia with the baseline value in individual horses.

Arterial- venous oxygen content difference C(a-v)O₂

No significant differences in C(a-v)O₂ was measured between the first to the last measurement during anaesthesia between the two groups.

Oxygen extraction ratio: C(a -v)O₂ / CaO₂

Significant differences in oxygen extraction ratio was calculated between the PiNO group and the control group between the first and the last measurement during anaesthesia (one-tailed *t*-test $p < 0.039$).

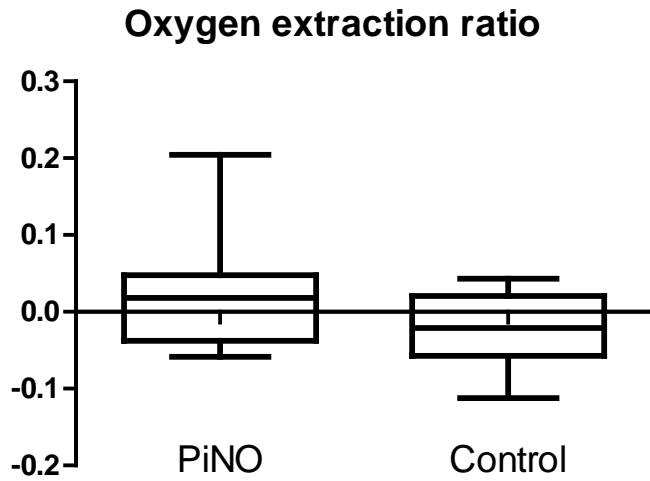


Figure 13. *Change in oxygen extraction ratio between the first and the last measurement during anaesthesia. Note that the oxygen extraction ratio increased 2% in the PiNO group and decreased 2% in the control group.*

DISCUSSION

The main finding in this study was a significantly more pronounced decrease in blood lactate during treatment with PiNO compared to untreated colic horses during anaesthesia and abdominal surgery. In line with previous research, the majority of the horses treated with PiNO showed the highest PaO₂ during inhalation of NO during the first 30% of inspiration. In the earlier work by Nyman one could find that the most effective dose for increasing PaO₂ was when iNO was delivered during 30-43% of the inspiration which was the main reason for us to start on a dose of 30% of the inspiration period (Nyman *et al.*, 2012).

The data and analysis of PaO₂, SaO₂ and the change in the degree of alveolar shunt (Qt/Qs) is found in my colleague Maja Wiklund's work. In summary, Wiklund came to the conclusion that all horses receiving PiNO improved their oxygenation; they had an increased PaO₂ and SaO₂ which was an effect of the decreased shunt (Qs/Qt) caused by the inhalation of NO. No side effects were detected and the conclusion is that PiNO is a safe and effective way to treat hypoxemia in colic horses undergoing abdominal surgery.

My hypothesis that blood lactate and blood glucose would decrease during colic surgery, and decrease to a greater extent in the PiNO group compared to the control group was based on the assumption that increased arterial oxygenation would have a direct effect on the production of lactate since reduced oxygen delivery and hypoxia in the organs and tissues would be less likely with higher PaO₂. I also presumed that the stress-response resulting in hyperglycemia, would be lower in the horses treated with PiNO compared to the control-group, since higher levels of oxygen would decrease the risk of hypoxemia, which is another potential stress-factor for the anaesthetized horse.

The correlation between the change in blood lactate concentrations and PaO₂ (figure 4 and 5) indicate that the blood lactate concentration over time in the two groups respond to the change in arterial oxygenation in opposite ways. This is visible as the regression lines for the two groups have opposite directions. In the PiNO group the negative R-value (-0.11) indicates a slight negative correlation even if not strong enough to consider significant. However, the control group has a positive R-value (0.58) and shows a significant positive correlation with arterial PaO₂. When looking at the data in detail it also becomes evident that one horse in the PiNO group (NO15) is mainly responsible for the reason to not achieve a significant negative correlation between change in blood lactate and PaO₂. This specific horse has an increase of 222% in PaO₂ and a 6% increase in blood lactate concentration during surgery. The reason for the high PaO₂ can be due to an extreme response to PiNO treatment. Another reason could be a possible development of endotoxaemia during surgery. In humans it has been seen that endotoxaemia and inflammation leads to higher oxygen demands (Oudemans-van Straaten *et al.*, 1996). Endotoxaemia and high oxygen demands could be the reason for increased lactate despite high PaO₂. If removing the result from NO15 the p-value changes from 0.28 to 0.07 and the correlation is then stronger. It could be argued that with more horses included in the study, the correlation between changes in PaO₂ and blood lactate might have been robust. It can thus be concluded that horses treated with PiNO gets a significant rise in PaO₂ and a significant decrease in blood lactate, and that a correlation between the two is indicative.

The major change in blood lactate was seen in horses with the highest values, i.e. horses with values over or near the lactate threshold of 5 mmol/L. Those horses were also the most critically ill which would benefit the most of a reduction in lactate concentration before recovery. The risk of persisting metabolic acidosis could further complicate their state of illness and possibly both the recovery and the outcome of surgery, as discussed by (Franklin & Peloso, 1996).

The increase in PaO₂ and SaO₂ during the PiNO treatment also resulted in an improvement of CaO₂ in this group. Further, although no significant change in C(a-v)O₂ was calculated, the oxygen extraction ratio increased slightly but significantly in the PiNO group which may explain the faster and more pronounced decrease in lactate concentration. It was not possible to measure cardiac output in the clinical patients but the most attractive and plausible hypothesis is that the oxygen delivery to the tissues also improved in the PiNO treated colic horses. Previous studies have shown that the cardiac output is not affected by the PiNO treatment itself (Grubb *et al.*, 2012, Nyman *et al.*, 2012). Although large variations was seen in the C(a-v)O₂ no differences was calculated between the treated and non-treated group indicating that the cardiac output was sufficient in relation to the metabolic demand in many of the colic horses.

The high levels of blood glucose found in the colic horses show the same pattern as seen in earlier studies (Edner *et al.*, 2007). The increased levels of blood glucose are a result from increased levels of cortisol which have anti-insulin properties, and can be explained by a massive sympathetic outflow as a stress-response to trauma. This response to stress is also seen in critically ill human patients (Clerk *et al.*, 1986).

Blood glucose was not affected by treatment with PiNO and was presumably high because of the heavy stress-situation for the horse; e.g. the colic situation and also anaesthesia. Endotoxemia which certainly was present in some of the horses could also have played a role in hyperglycemia as it has been concluded that horses with endotoxemia (even if experimentally induced) induces reduced insulin sensitivity (Tóth *et al.*, 2008; Sundström, 2011). Hence, several different reasons for hyperglycemia and reduced insulin sensitivity during the time of critical illness are present. In the human research field, it is widely accepted that increased levels of cortisol undoubtedly is linked to insulin resistance as discussed in the article by Andrews & Walker (1999). Their review article summarizes the different pathways that glucocorticoids interfere with the insulin metabolism and concludes that glucocorticoids make an important contribution to the pathophysiology of insulin resistance. Since increased levels of cortisol are present already in healthy horses during anaesthesia due to stress (Luna *et al.*, 1996), elevating levels of cortisol might be challenging. The stress that a colic horse is subject to with anaesthesia and also colic is something we cannot prevent. However, what we presumably can prevent or control is the hyperglycemia occurring before, during and after anaesthesia. In the human study by Thorell *et al.*, (2004) it is stated that hyperglycemia is dangerous and that treatment with insulin have major impact on the clinical outcome.

In the human study by Van Der Berghe *et al.*, (2001) in 1548 critically ill human patients, the mortality was reduced from 8 to 4.6 percent with intensive insulin treatment of hyperglycemic critically ill patients in the intensive care unit. The greatest reduction in mortality was seen in multiple organ failure with a proven septic focus, a situation arguably not totally different from a critically ill colic horse post-surgery with endotoxemia. In a study by Holly *et al.*, (2007), records from 269 horses presented with acute abdominal pain showed that over 50 % had increased levels of blood glucose on admission. Horses that did not survive had a higher mean glucose level on admission and up to 48 hours after admission. It would therefore be interesting to study if the outcome of surgery and survival of colic horses could be improved by treatment with insulin. Furthermore, as hyperinsulinemia and hyperglycemia has been connected to increased lactate production by adipose tissue in human studies (Henry S *et al.*, 1996) an additional reason for studying insulin treatment in colic horses is entitled.

It is known from earlier work that horses breathing spontaneously during long surgeries usually are subjected to a respiratory acidosis and decreased blood pH (Edner *et al.*, 2007). My next hypothesis was that horses treated with PiNO, to a greater extent than the control group, would have increased pH or a pH returning towards more normal levels. This could be a result of decreased blood lactate since

high blood lactate can decrease pH through metabolic acidosis. However, pH was not directly affected by treatment with PiNO, probably because pH is not influenced by high lactate until the lactate exceeds 5mmol/L (Franklin & Peloso, 1996). In the present study we did not have enough horses to qualify into such a group. The horses in this study were subject to a respiratory acidosis which was the reason for the low pH during spontaneous anaesthesia under general anaesthesia. This was confirmed with a correlation analysis between pH and PaCO₂, with a p<0.05 in both groups. Hence, increased PaCO₂ caused by hypoventilation was mainly responsible for decreased pH. As soon as the anaesthesia was discontinued and the horses were positioned in lateral recumbency in recovery, the ventilation and pH increased. It is concluded that these horses would have required mechanical ventilation during surgery to correct the levels of PaCO₂. This can be achieved in the future with a new PiNO device adapted for positive pressure ventilation.

My last hypothesis was that horses with tachycardia during surgery would benefit from treatment with PiNO, compared to the control group, since sufficient oxygen supply would reduce the stress during anaesthesia. In humans there is documented evidence of tachycardia occur together with hypoxemia and low oxygen saturation after abdominal surgery (Gögenur *et al.*, 2004). Heart rate was not affected by treatment with PiNO in this study. However, the SD of the heart rate in both groups was very high with makes it more uncertain to evaluate the results. Furthermore, during equine anaesthesia many other factors play important roles in affecting the heart rate. The horses were subject to the use of several different drugs and the majority of the horses were also given inotrope drugs (dobutamine) to improve blood-pressure at the same time as they were medicated with analgesic drugs such as lidocaine. Both these drugs have a strong potential to influence the heart rate which makes it challenging for us to draw any conclusions based on the data collected from this study.

CONCLUSIONS

The main finding of the study was an enhanced oxygen extraction ratio and a concomitant reduction of blood lactate concentration during treatment with PiNO in colic horses undergoing anaesthesia and abdominal surgery. A significant increase in PaO₂ during pulsed inhalation of NO during the first part of inspiration resulted in increased oxygen content in arterial blood. A possible explanation for the enhanced metabolic condition may be improved oxygen delivery to the tissues. Future research is encouraged to study the effect of PiNO treatment on the long term clinical outcome.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Görel Nyman for the best support and lessons in equine anaesthesia and physiology. The knowledge and inspiration that this work has given me is exceptional and will undoubtedly help me in my future work as an equine veterinarian. The deeper understanding of equine anaesthesia cannot be achieved in any other way than a combination of theoretical and practical work, which I have received through the PiNO-project. I would also like to thank Anneli Rydén for fantastic additional support and help with collecting data. Last but not least, thanks to my graduate colleague Maja Wiklund for great team-work and friendship.

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