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Optimising pulmonary gas exchange in anaesthetised horses

Unravelling the role of pulsed inhaled nitric oxide using computed tomography angiography of the lung

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Abstract

Mortality rates in healthy, anaesthetised horses are higher than in most other species. Hypotension, hypoxaemia and hypoventilation are implicated as risk factors, which develop due to the combined effects of general anaesthesia and recumbency. Hypoxaemia is largely a consequence of ventilation perfusion ($\dot{V}/\dot{Q}$) mismatch. When horses are recumbent, dependent areas of lung become compressed and collapse. These atelectatic lung regions receive a large proportion of pulmonary blood, do not participate in gas exchange and contribute significantly to venous admixture. Whilst the hypercapnia associated with hypoventilation is easy to manage, treatment of hypoxaemia is more challenging. Mechanical ventilation (MV) is often employed, but can have detrimental effects, and the response to it is unpredictable. Pulsed inhaled nitric oxide (PiNO), has been used successfully to manage hypoxaemia in anaesthetised horses. The presumptive mechanism of action is via redistribution of pulmonary perfusion, from dependent areas of lung, to better ventilated, non-dependent lung regions. This movement of blood occurs due to the selective, pulmonary vasodilatory effect of PiNO. However, it necessary to further elucidate the mechanism of action of PiNO. The aims of these studies were to: develop a CT method to quantify regional pulmonary perfusion in the equine lung; measure changes in regional pulmonary perfusion when PiNO is administered, during spontaneous breathing (SB) and MV and; measure changes in pulmonary perfusion in response to PiNO during SB and MV in hypotensive and normotensive horses.

The CT method could reliably measure changes in aerated and atelectatic regions of lung, and compared well to previously reported values measured using microspheres. During SB and MV in the normotensive horse, PiNO caused a redistribution of blood to non-dependent lung regions which led to improvements in gas exchange. Unexpectedly, PiNO was ineffective during MV if horses were hypotensive. However, during SB, the response to PiNO was similar regardless of blood pressure.

By developing a new CT method with angiography for studies of the distribution of pulmonary perfusion, these experiments have shown that PiNO is an effective and safe treatment option for hypoxaemic horses, but blood flow and blood pressure must be supported if horses are mechanically ventilated.

Keywords: computed tomography angiography, horse, pulmonary perfusion, nitric oxide.

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Dedication

For Derek. Without his love, encouragement and patience, I never would have achieved this. For my parents, whose unwavering support have shaped my life and career. Finally, for Görel and for Peter - their expertise, enthusiasm and faith in me, finally got me to the end, and I am immensely grateful for having found them.

In memory of Göran Hedenstierna, who sadly passed away July 12th 2021

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Auckburally A** & Nyman G (2017) Review of hypoxaemia in anaesthetised horses: predisposing factors, consequences and management. *Vet Anaes Analg*, 44, 397-408.

II. **Auckburally A**, Nyman G, Wiklund M, Straube A, Perchiazzi G, Beda A, Ley C, Lord P. Development of a method to measure regional perfusion of the lung in anesthetized ponies using computed tomography angiography and the maximum slope model. *Am J Vet Res*. *Accepted.*

III. **Auckburally A**, Wiklund M, Lord P, Hedenstierna G, Nyman G. Comparison of the effect of pulsed inhaled nitric oxide on the distribution of pulmonary perfusion in spontaneously breathing and mechanically ventilated anesthetized ponies. *Am J Vet Res. Accepted.*

IV. **Auckburally A**, Grubb TL, Wiklund M, Nyman G (2019) Effects of ventilation mode and blood flow on arterial oxygenation during pulse-delivered inhaled nitric oxide in anesthetized horses. *Am J Vet Res*, 80 (3), 275-283.

V. **Auckburally A**, Wiklund M, Lord P, Nyman G. Cardiac output affects the response to pulsed inhaled nitric oxide in mechanically ventilated anesthetized ponies determined by computed tomography angiography of the lung. (*Manuscript)*

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The contribution of Adam Auckburally to the papers included in this thesis was as follows:

I. Literature review, manuscript preparation, critical revision of the manuscript.

II. Study design, data collection, analysis and interpretation, statistical analysis, manuscript preparation, critical revision of the manuscript.

III. Study design, data collection, analysis and interpretation, manuscript preparation, critical revision of the manuscript.

IV. Study design, data collection, analysis and interpretation, manuscript preparation, critical revision of the manuscript.

V. Study design, data collection, analysis and interpretation, manuscript preparation, critical revision of the manuscript.

Abbreviations

ABP	Arterial blood pressure
AIF	Arterial input function
ARDS	Acute respiratory distress syndrome
CaO_2	Oxygen content of arterial blood
$Cc'O_2$	Oxygen content of end capillary blood
CI	Cardiac index
COR	Coefficient of repeatability
CPAP	Continuous positive airway pressure
CT	Computed tomography
CTA	Computed tomography angiography
CV	Coefficient of variation
$C\bar{v}O_2$	Oxygen content of mixed venous blood
DAP	Diastolic arterial blood pressure
DICOM	Digital Imaging and Communications in Medicine
DO_2	Oxygen delivery
ECG	Electrocardiogram
$ETCO_2$	End-tidal carbon dioxide partial pressure
FIO_2	Inspired oxygen fraction
*f*R	Respiratory rate
FRC	Functional residual capacity
[Hb]	Haemoglobin concentration
HR	Heart rate
ICC	Intraclass correlation coefficient
iNO	Inhaled nitric oxide
LOA	Limits of agreement
MAP	Mean arterial blood pressure
MDCT	Multi-detector computed tomography
MIGET	Multiple inert gas elimination technique
MPAP	Mean pulmonary arterial pressure
MSM	Maximum slope model
MV	Mechanical ventilation
NO	Nitric oxide

OER	oxygen extraction ratio
$P(A\text{-}a)O_2$	Alveolar to arterial oxygen partial pressure difference
$PaCO_2$	Arterial carbon dioxide partial pressure
$PACO_2$	Alveolar carbon dioxide partial pressure
PaO_2	Arterial oxygen partial pressure
PAO_2	Alveolar oxygen partial pressure
PCWP	Pulmonary capillary wedge pressure
PEEP	Positive end expiratory pressure
PET	Positron emission tomography
PiNO	Pulsed inhaled nitric oxide
PIP	Peak inspiratory pressure
$P\bar{v}O_2$	Mixed venous oxygen partial pressure
PVR	Pulmonary vascular resistance
$\dot{Q}s/\dot{Q}t$	Venous admixture
$\dot{Q}t$	Cardiac output
ROI	Region of interest
RQ	Respiratory quotient
SAP	Systolic arterial blood pressure
SB	Spontaneous breathing
SaO_2	Arterial haemoglobin oxygen saturation
SPECT	Single-photon emission computed tomography
SpO_2	Arterial haemoglobin oxygen saturation using pulse oximetry
$S\bar{v}O_2$	Mixed venous haemoglobin oxygen saturation
TAC	Time attenuation curve
TV	Tidal volume
Vd/Vt	Alveolar dead space
VE	Minute ventilation
$\dot{V}/\dot{Q}$	Ventilation perfusion

1. Introduction

1.1 Hypoxaemia in anaesthetised horses

Minimising patient mortality and morbidity is a priority for anaesthetists, regardless of species. Identification of the causes, is key to appropriate case management, to reduce the risks as far as possible. Mortality associated with anaesthesia in healthy horses varies between reports, but can be as high as 0.9% (Young & Taylor 1993; Johnston et al. 2002; Bidwell et al. 2007; Dugdale et al. 2016). When systemic disease is present, the risk becomes greater, and mortality rates are much higher (Pascoe et al. 1983; Johnston et al. 2002). For horses, the cumulative effects of hypotension, hypoxaemia and acid-base derangements that frequently occur, are implicated in the high mortality rates (Johnston et al. 2002). For this reason, numerous researchers have investigated treatment strategies to manage these complications and the effect they have on oxygen delivery (DO_2), often with frustrating and discouraging results. In 1968, Hall et al., published data demonstrating the effects of anaesthesia and recumbency in the horse, on the arterial partial pressure of oxygen (PaO_2). Since its publication, and subsequent further work, it is now widely accepted that anaesthetised horses, whether lying in lateral or dorsal recumbency, will develop an elevated alveolar to arterial partial pressure gradient or tension ($P(A\text{-}a)O_2$), which may lead to hypoxaemia (Mitchell & Littlejohn 1974; Steffey et al. 1977; Schatzmann et al. 1982; Stegmann & Littlejohn 1987; Gleed & Dobson 1988; Nyman et al. 1988; Nyman et al. 1990; Steffey et al. 1990; Day et al. 1995). Clinical experience would suggest that hypoxaemia occurs commonly, even in healthy horses, and is difficult to prevent, and challenging to treat (Nyman & Hedenstierna 1989; Nyman et al. 2012). Although the frequency at which hypoxaemia occurs during anaesthesia in the general horse population is unreported, approximately 13% of horses anaesthetised for colic surgery (using a definition of $PaO_2 < 80$ mmHg (10.7 kPa)) (Pascoe et al. 1983), and up to 55% of healthy anaesthetised horses in dorsal recumbency (using a definition of $PaO_2 < 60$ mmHg (8 kPa)) (Day et al. 1995), will develop it. Therefore, identifying a safe and effective treatment to manage hypoxaemia, is key to reducing the risk of general anaesthesia in the horse.

1.2 Causes of hypoxaemia

Generally speaking, there are five accepted causes of hypoxaemia: a low inspired fraction of oxygen (FIO_2); hypoventilation; ventilation perfusion ($\dot{V}/\dot{Q}$) inequality or mismatch; diffusion impairment; and a right to left shunt. Low FIO_2 can result from the delivery of hypoxic gas mixtures i.e. when there is insufficient oxygen in the gas mixture through the addition of other carrier gases (nitrous oxide or air). This is generally caused by human error, and can be quickly ruled out as a cause. Hypoventilation often occurs during general anaesthesia, induced by the depressant effects of anaesthetic drugs and recumbency (Hubbell & Muir 2015). It is easily identified due to the development of hypercapnia and rectified by the institution of mechanical ventilation (MV). However, hypoventilation in animals breathing enriched oxygen mixtures, as occurs during inhalational anaesthesia, can never be the primary cause of hypoxaemia. Diffusion impairment within the lung, results from pulmonary diseases that alter the gas exchange barrier from the alveolar lumen to the red blood cells within the pulmonary capillaries. Animals with severe enough disease (e.g. severe pulmonary oedema), to cause a diffusion impairment would be clinically unwell, and would generally be unsuitable candidates for general anaesthesia. So again, this as a cause of hypoxaemia in healthy horses can usually be eliminated. An intracardiac right to left shunt, for example as occurs in an animal with a direct communication between the right and left ventricles or atria of the heart, is an unusual finding. Hypoxaemia as a consequence of a right to left intracardiac shunt, is usually a diagnosis of exclusion and, again, would not be a common cause of this problem.

Of the five causes, $\dot{V}/\dot{Q}$ mismatch, and in particular, a right to left intrapulmonary shunt, is the predominant reason why horses become hypoxaemic during anaesthesia (Nyman & Hedenstierna 1989). Ventilation perfusion mismatch is a condition that occurs for a variety of reasons. Ideally, alveolar ventilation is perfectly matched to the amount of blood flowing past each alveolus, such that near perfect gas exchange occurs ($\dot{V}/\dot{Q}$ ratio of one). Two extremes of mismatch can occur: areas of lung that are optimally ventilated but have no blood supply; and areas of lung that are not ventilated at all, but have a normal or increased amount of blood flowing past them. The first scenario is known as alveolar dead space, and this contributes little to oxygen levels in the blood, since there is no blood joining the systemic circulation ($\dot{V}/\dot{Q}$ ratio of ∞). The second scenario leads to a large amount of blood that has not participated in gas exchange, and returns to the left atrium to join the systemic circulation ($\dot{V}/\dot{Q}$ ratio of zero). This is, in effect, a right to left shunt, and is confusingly termed intrapulmonary shunt, or venous admixture (venous admixture is the amount of mixed venous

blood that would be required to produce the observed difference between the arterial and pulmonary end-capillary oxygen partial pressures – end capillary partial pressure of oxygen is usually taken as equal to ideal alveolar oxygen partial pressure (Lumb & Pearl 2010)). Consequently, this extreme of $\dot{V}/\dot{Q}$ mismatch is a major cause of hypoxaemia when it occurs. The magnitude of venous admixture is calculated as the shunt fraction or $\dot{Q}_s/\dot{Q}_t$. Note that the *actual* amount of venous blood that mixes with arterial blood differs from the *calculated* venous admixture. This difference occurs because the calculated amount assumes all of the blood mixing with arterial blood comes from this intrapulmonary shunt. In reality, other sources of poorly oxygenated blood, in addition to deoxygenated blood from the intrapulmonary shunt, account for the PaO_2 observed: blood from the bronchial and Thebesian (coronary) circulation; blood from any intracardiac shunting if present and; blood coming from 'low' areas of $\dot{V}/\dot{Q}$ mismatch ($\dot{V}/\dot{Q}$ ratios greater than zero but less than one) (Lumb & Pearl 2010). Thus, the calculated intrapulmonary shunt is usually a little higher than the true shunt, but is useful nonetheless, and provides a convenient, if slightly inaccurate, index.

1.3 Susceptibility of the horse to hypoxaemia

Why is the horse so much more susceptible to developing hypoxaemia than other domestic species? In 1865, the physiologist Claude Bernard said *certain animals offer favourable anatomical arrangements, or special susceptibility to certain influences*. In the standing horse, the majority of the lung is situated dorsally, on top of a long sloping diaphragm, over the abdominal viscera. This arrangement is favourable for athleticism, since the lung is very well ventilated in this anatomical position. Once the animal is anaesthetised and turned on its back, this favourable arrangement becomes detrimental to pulmonary function, as most of the lung is now prone to compression (Hedenstierna et al. 2005).

In the awake, standing horse, pulmonary blood flow increases from ventral to dorsal regions and is independent of gravity (Hlastala et al. 1996). Blood flows preferentially to the caudo-dorsal lung field in standing horses at rest and during exercise, and vessel reactivity in the dorsal lung fields of the horse demonstrate enhanced endothelial-mediated vasorelaxation, compared with vessels from ventral regions (Pelletier et al. 1998; Stack et al. 2014). This dorsal vasorelaxation favours improved perfusion in these well-ventilated regions. The pulmonary artery is stiffer in caudo-dorsal lung regions compared with caudo-ventral areas which may be a protective adaptation in response to intense exercise (Stack et al. 2014). This adaptation to exercise becomes unfavourable during

anaesthesia and recumbency, as blood will preferentially flow through regions of lung that are now atelectatic, which can lead to protracted periods of hypoxaemia, with its potential associated consequences. Thus horses, more than any other domestic species, are susceptible to developing significant impairment of pulmonary function during recumbency and anaesthesia, and a large right to left intrapulmonary shunt (Nyman et al. 2012).

Although arterial oxygenation is near ideal in conscious standing horses at rest, recumbency induces changes that lead to impaired oxygenation. In conscious ponies trained to lie in lateral recumbency, arterial oxygenation was impaired significantly after 10 minutes in this position (Rugh et al. 1984). There are no comparable data for conscious larger horses lying in lateral recumbency, or for conscious ponies and horses in dorsal recumbency. However, PaO_2 was significantly lower in laterally and dorsally recumbent horses sedated with glyceryl guaiacol ether, compared with standing horses (Schatzmann et al. 1982). In anaesthetised horses, a retrospective study found that dorsal recumbency, low pulse pressure, short procedures and emergent procedures, were strong predictors of low PaO_2 values (Whitehair & Willits 1999). The inclusion of short procedures as a predictor of low PaO_2 is somewhat counterintuitive, although the authors speculated that venous admixture is maximal, and inspired oxygen fraction is lowest, during the first hour of anaesthesia. This study also identified that male horses were more likely to become hypoxaemic but the cause of this was unknown, and that older and heavier horses were more likely to have PaO_2 values less than 250 mmHg (33.3 kPa). Body mass and body shape do have effects on oxygenation indices. The shape of the ventral abdominal contour of the horse influences gas exchange, with round-bellied horses having a greater $P(A\text{-}a)O_2$ than their flat-bellied counterparts, irrespective of body position (Moens et al. 1995). In further support of this relationship, Mansel & Clutton (2008) showed that tall, light horses had improved oxygenation when compared with shorter and stockier animals. However, despite all these potential associations, hypoxaemia may develop unpredictably in anaesthetised horses (Trim and Wan 1990).

Although multifactorial, the hypoxaemia observed is mainly a result of $\dot{V}/\dot{Q}$ mismatch (Nyman & Hedenstierna 1989; Moens et al. 1998; Nyman et al. 2012; Grubb et al. 2014). Radiographic studies have shown that diffuse radiopaque densities develop in the dependent lung in laterally recumbent horses, 20 minutes following induction to general anaesthesia (McDonnell et al. 1979). The radiographic densities which develop are regions of atelectatic lung on necropsy (Nyman et al. 1990). These areas can be eliminated by large volume inflations of the lung (Nyman et al. 1990), in a manner similar to humans (Rothen et al. 1993; Rothen et al. 1999). Despite the areas of atelectasis appearing to be relatively small when studied using CT, the atelectatic regions can comprise as much as 4 times

the lung tissue as aerated regions (Magnusson & Spahn 2003). There are 3 mechanisms of atelectasis described - compression atelectasis occurs when the transmural pressure that opens alveoli is reduced; absorption atelectasis occurs when the gas entering the alveolus is less than that being absorbed into the blood and; loss of surfactant, which influences alveolar distension, will also result in areas of atelectasis (Magnusson & Spahn 2003). In the horse however, due to the thoracic anatomical adaptation for exercise described above, compression atelectasis is the likeliest cause, possibly contributing up to 20% or 30% of total lung tissue in lateral and dorsal recumbency, respectively (Sorenson & Robinson 1980; Nyman & Hedenstierna 1989). This compression atelectasis is almost certainly responsible for the rapid fall in PaO_2. To compound matters further, as cranial displacement of the diaphragm occurs in anaesthetised horses (Benson et al. 1982), functional residual capacity (FRC) is reduced by up to 50% when compared with the animal standing (McDonnell et al. 1979, Sorenson & Robinson 1980). Functional residual capacity is defined as the lung volume at the end of a normal expiration i.e. the lung never empties completely, such that alveolar integrity is maintained (Lumb & Pearl 2010). This means that the closing capacity (the volume of the lung at which the small airways begin to close) of the lung may exceed FRC, leading to further airway collapse (Sorenson & Robinson 1980). The atelectatic regions are larger than those in other species, and this is mirrored by greater gas exchange impairment, as determined by calculating shunt fraction (Nyman et al. 1990). Indeed, it appeared from early work in the pony, that the magnitude of CT densities observed during general anaesthesia, correlated well with the degree of shunt (or venous admixture) (Nyman et al. 1990) as it does in humans (Reber et al. 1996). Once the lung becomes compressed and atelectatic, pulmonary blood flowing through this region is not oxygenated, and consequently, forms a right to left shunt once the blood enters the left atrium. Predominantly, it is these areas of shunt ($\dot{V}/\dot{Q}$ ratio of zero), together with areas of lung with 'low' $\dot{V}/\dot{Q}$ ratios ($\dot{V}/\dot{Q}$ ratio more than zero but less than one), which contribute most significantly to the development of the observed increased $P(A\text{-}a)O_2$ gradient. Areas of 'high' $\dot{V}/\dot{Q}$ ratios ($\dot{V}/\dot{Q}$ ratios greater than one), or alveolar dead space ($\dot{V}/\dot{Q}$ ratio of ∞), contribute little, since perfusion to these areas is poor.

1.4 Consequences of hypoxaemia

Is there evidence to demonstrate that the development of hypoxaemia during general anaesthesia is harmful? During halothane anaesthesia in spontaneously

breathing horses with reduced PaO_2, heart rate (HR) and cardiac output ($\dot{Q}t$) increased and total peripheral resistance, arterial blood pressure (ABP) and oxygen delivery (DO_2) decreased (Steffey et al. 1992). Cardiovascular function became worse under these same circumstances during mechanical ventilation (Steffey et al. 1992).

In desaturated (arterial haemoglobin saturation (SaO_2) of 70 – 75%) humans, coronary blood flow increases by up to 35% (Grubbström et al. 1993). Once this coronary reserve is overwhelmed, the myocardium produces increased levels of lactate and pH falls within the myocardium. This adversely affects contractility and $\dot{Q}t$ (Allen & Orchard 1987). Similar effects may occur within the equine heart during periods of acute hypoxaemia but evidence is lacking.

During periods of hypoxia, brain injury occurs because of: accumulation of lactic acid and calcium; the neurotoxic effects of neurotransmitters released during hypoxia; and the production of oxygen reactive species following re-oxygenation (Hopkins & Bigler 2001; Patel et al. 2015). Humans subjected to hypoxic environments experience blurred vision, incoordination, weakness, dizziness and the metabolic demand of the brain can increase by 15% during cognitive tasks (Turner et al. 2015). In horses, in situations where coordination is important, such as recovery from general anaesthesia, hypoxic brain injury may have an impact. In such circumstances, the quality of recovery may be affected, possibly leading to increased morbidity and mortality; this has not been documented or investigated to date.

Hypoxaemia can lead to altered surfactant production in the human lung (Levitsky 2013), and may be linked to the development of acute respiratory distress syndrome (ARDS). Hypoxia rapidly affects alveolar epithelial type II cells, with reduced surfactant production and decreased cell proliferation (Vaporidi et al. 2005). Loss of surfactant induced by hypoxaemia can compound $\dot{V}/\dot{Q}$ mismatch by worsening atelectasis (Magnusson & Spahn 2003). The effect of hypoxaemia on surfactant in the equine lung is not known.

The function of skeletal muscle depends upon the release of calcium from the sarcoplasmic reticulum and its reuptake. This is reduced during hypoxaemia and leads to reduced cross-bridge activation, possibly through lactate and hydrogen ion accumulation, or free radical production (Romer et al. 2006). In anaesthetised horses with experimentally induced hypoxaemia, oxygenation of muscle is reduced. (Portier et al. 2009), and this is worse if halothane is used as the anaesthetic (Whitehair et al. 1996). Again, this may have a significant impact upon the ability of a horse to stand during the recovery period.

In humans, wound healing and resistance to infection is dependent on adequate oxygen delivery to the wound (Gottrup 2004). Similar findings have been

described in horses undergoing abdominal surgery; PaO_2 values < 80 mmHg are implicated in the development of surgical site infection (Costa-Farré et al. 2014).

From the discussion above, it might be assumed that these deleterious effects of hypoxaemia on the cardiovascular system, the brain, the lung, muscle function and on wound healing and infection may be partly responsible for morbidity and mortality associated with anaesthesia in horses. Clearly though, further research is required to demonstrate that there are consequences of hypoxaemia during anaesthesia.

1.5 Treatment of hypoxaemia

1.5.1 Ventilatory strategies

In the horse, if MV is started from the outset of anaesthesia, this can lead to improved arterial oxygenation (Hall et al. 1968; Moens 2013). However, the detrimental effects of positive intra-thoracic pressure have also been documented (Hodgson et al. 1986; Mizuno et al. 1994; Raisis et al. 1995; Edner et al 2005; Wettstein 2006; Ambrósio et al. 2013), especially at high inflation pressures (Hopster et al. 2017). The distribution of pulmonary blood flow is critically dependent on both cardiac output and intrathoracic pressure (Hedenstierna 1987). If positive pressure is used to inflate alveoli, cardiac output is reduced as a result of a reduction in preload (due to compression of the vena cava), and impedance to cardiac filling (tamponade) (Shekerdemian & Bohn 1999). A fall in cardiac output reduces pulmonary perfusion and the positive pressure inside the alveoli also drives blood downwards, towards dependent areas of lung (West 2008), which may worsen any existing intrapulmonary shunt and PaO_2. These combined effects can reduce tissue oxygen delivery (DO_2). Mechanical ventilation in itself, can cause atelectasis as a result of alveolar damage, and consequent changes in pulmonary function (Lachmann 1992). The mechanisms for alveolar damage during MV are varied – damage to the alveolar wall through shear stresses, and surfactant molecules being squeezed out from small alveoli by rhythmic compression and decompression of the alveolar lining (Lachmann 1992).

Differential lung ventilation has been attempted in order to manage reduced PaO_2 in anaesthetised horses, but the clinical application of these techniques is limited (Nyman et al. 1987; Moens et al. 1994). Moens et al. (1992; 1994) developed a ‘tube-in-tube’ technique, to separate the two lungs; PaO_2 was improved and $\dot{Q}s/\dot{Q}t$ decreased. Nyman et al. (1987) also reported a technique whereby regions (rather than whole lung) of the lung could be separated. By applying positive pressure ventilation with positive end-expiratory pressure

(PEEP) to dependent lung regions, PaO_2 was significantly improved. This selective MV significantly reduced $\dot{Q}s/\dot{Q}t$ whereas conventional MV did not, indicating that areas of shunt due to atelectasis, were the cause of the increased $P(A\text{-}a)O_2$ gradient (Nyman & Hedenstierna 1989).

Recently, there has been renewed interest in a variety of MV strategies to treat hypoxaemia in anaesthetised horses, that can be used in a clinical setting. Largely, this has coincided with the advent of technologies, which enable the anaesthetist to easily provide PEEP, or continuous positive airway pressure (CPAP), to large animals such as the horse. Both PEEP and CPAP are used to support alveolar inflation by maintaining a degree of positive pressure within the lung (Lumb & Pearl 2010). The open lung concept (OLC) is used to treat hypoxaemia in humans, particularly in patients with ARDS. The basis of the OLC is to, *open up the whole lung (with an alveolar recruitment manoeuvre), and keep it totally open, with the least influence on the cardiocirculatory system* (Lachmann 1992). The technique should maintain a shunt fraction of less than 10%, at the minimum intra-thoracic pressure, to prevent adverse effects, such as alveolar trauma and cardiovascular depression (Papadakos & Lachmann 2007). Recruitment manoeuvres increase the amount of aerated lung tissue, and this is correlated to oxygenation (Hedenstierna & Lattuada 2002). Unfortunately, pressures required to recruit the human lung may be excessively high, and can be difficult to achieve in horses. It is challenging to generate intra-thoracic pressures of more than 40 cmH_2O in the horse, that are required to re-inflate collapsed alveoli. Moreover, it is difficult to maintain this pressure for the period of time necessary to have a significant effect on PaO_2.

Descriptions of ventilatory techniques that use modifications to the OLC in anaesthetised horses are numerous. These modifications consist of recruitment manoeuvres at lower inflation pressures, or recruitment achieved by the application of stepwise PEEP (a gradual increase in the amount of PEEP applied). Of note, MV with PEEP, but without an initial alveolar recruitment manoeuvre, does not improve pulmonary function in horses anaesthetised for colic surgery (Pauritsch 1997). Therefore, it is important that a recruitment manoeuvre is performed to first open the alveoli (Moens & Böhm 2011). Using a modified OLC technique improves PaO_2 significantly in healthy ponies (Wettstein et al. 2006) and in healthy and sick horses (Bringewatt et al. 2010, Hopster et al. 2011). However, in the study by Hopster et al. (2011), the recruitment had to be repeated, and the response from individual horses was unpredictable, with some not responding as anticipated. In this latter study, they also showed that improvements in oxygenation persisted into the recovery period, and recovery times were faster (but not better). Furthermore, MV and stepwise PEEP reduces gastrointestinal perfusion and oxygenation in anaesthetised horses by reducing DO_2 (Hopster et

al. 2017). Ambrósio et al. (2013), demonstrated that $\dot{Q}t$ was significantly lower in anaesthetised horses supported with MV and PEEP, although the reduction was relatively small. These horses had PEEP titrated upwards in increments of 5 cmH_2O, that may have alleviated some of the cardiovascular depression anticipated with pressure support techniques, and ephedrine was administered to maintain mean arterial pressures (MAP) between 60 and 80 mmHg. Edner et al. (2005) also showed that $\dot{Q}t$ and blood pressure were significantly lower in healthy anaesthetised horses being mechanically ventilated, compared with those that were spontaneously breathing. Muscle and skin perfusion were also adversely affected, which may have implications postoperatively. Wettstein et al. (2006), showed that a fall in MAP and $\dot{Q}t$ occurred during incremental stepwise PEEP, in ponies anaesthetised using total intravenous anaesthesia (TIVA). A single hyperinflation of 50 cmH_2O for 50 seconds has been investigated in anaesthetised horses (Santos et al. 2013). This technique appeared to provide only a small and transient benefit and significantly reduced MAP in some animals during the manoeuvre.

With the advent of technology that can provide CPAP to large animals, there have been preliminary investigations into its effect on arterial oxygenation. CPAP is a ventilatory modality, which maintains a positive airway pressure throughout the entire respiratory cycle during spontaneous breathing (SB) (Cairo 2012). This positive pressure helps to maintain FRC, prevents atelectasis and reduces $\dot{Q}s/\dot{Q}t$ (Cairo 2012). Intra-thoracic pressures are kept lower than with other MV techniques and cardiovascular function may be preserved. When CPAP is implemented during anaesthesia, horses had significantly higher PaO_2 values compared with horses breathing without CPAP, and there was no difference in blood pressure support required between groups (Mosing et al. 2013).

From the previous discussion, it might be concluded that ventilatory techniques which involve the use of recruitment, high inflation pressures and PEEP may not be the most suitable method to treat intraoperative hypoxaemia, particularly in horses where $\dot{Q}t$ is of concern (e.g. horses with colic). The negative effects of increasing intrathoracic pressure by applying MV are often overlooked, or at least tolerated by clinicians (Hedenstierna 1987). Although detrimental, changes may not occur in all horses, and it is impossible to predict those horses that may tolerate this type of mechanical ventilatory strategy. The implementation of PEEP may actually worsen the degree of shunt, presumably by forcing pulmonary blood down into dependent regions, therefore increasing the blood supply to the atelectatic region (Nyman et al. 1990; Swanson & Muir 1988). Indeed, in the past it has been suggested that the indiscriminate use of PEEP should be avoided, at least in humans (Hewlett et al. 1974). This further strengthens an argument that PEEP may not be a suitable technique to improve pulmonary function in the horse.

1.5.2 Changing inspired oxygen fraction

Manipulating the FIO_2 administered to horses is easily possible if the anaesthetic machine has an air supply in addition to oxygen. The administration of 100% oxygen (FIO_2 1.0), slightly less if carrying a volatile anaesthetic agent, can potentially contribute to $\dot{V}/\dot{Q}$ inequality, by exacerbating alveolar collapse and the degree of shunted blood (Marntell et al. 2005; Staffieri et al. 2009). Alveoli ventilated with air remain open for 8 – 9 hours, whereas those ventilated with 100% oxygen collapse within 8 minutes (Joyce et al. 1993). In human patients, alveoli expanded with a recruitment manoeuvre, de-recruited (became atelectatic again), after 5 minutes if 100% oxygen was administered, compared with 40 minutes if 40% oxygen in air (FIO_2 0.4) was administered (Rothen et al. 1995). If 100% oxygen was administered by facemask prior to induction of anaesthesia, the amount of atelectatic lung was greater immediately following induction, compared with those patients receiving 30% oxygen by facemask (Rothen at al. 1995). Cuvelliez et al. (1990) investigated the effects of 0.3 and > 0.85 FiO_2 in halothane anaesthetised horses, and demonstrated that horses breathing higher inspired oxygen concentrations hypoventilated more, and had greater $P(A\text{-}a)O_2$ gradients, suggestive of increased $\dot{V}/\dot{Q}$ mismatch. Similarly, healthy anaesthetised horses breathing an $FiO_2 > 0.95$, developed significant hypoventilation, presumably by reducing respiratory drive, and a greater $\dot{Q}s/\dot{Q}t$, than horses breathing an FIO_2 of 0.21 (Marntell et al. 2005). However, reducing FIO_2 from > 0.95 to 0.5 did not change $\dot{Q}s/\dot{Q}t$ in dorsally recumbent, mechanically ventilated horses (Hubbell et al. 2011). In humans, a normal arterial PaO_2 is achieved with an FIO_2 of approximately 0.3 – 0.35 (Nunn 1964). If normal arterial carbon dioxide partial pressure ($PaCO_2$), haemoglobin, and arterial-mixed venous oxygen content difference are assumed, the PaO_2 is largely determined by the FIO_2 together with venous admixture (Lumb & Pearl 2010). As the amount of venous admixture (or shunt fraction) increases, FIO_2 has very little influence on the PaO_2 (Benator et al. 1973). In some dorsally recumbent horses, PaO_2 values do not rise appreciably with increasing FIO_2, and we can assume that these animals have large areas of atelectatic lung, and therefore elevated venous admixture.

The provision of oxygen enriched gas mixtures may in itself, lead to pulmonary damage. Free radicals or reactive oxygen species (ROS) disrupt the activity of nitric oxide (NO), an endogenous pulmonary vasodilator, and enhance vasoconstrictive mediators. This leads to an increase in pulmonary vascular pressure (Mills & Higgins 1997). These ROS can also cause oxidative stress and inflammation when antioxidants are overwhelmed by pro-oxidants (Mills and Higgins 1997) Pulmonary epithelial cells are particularly susceptible to oxidant injury. ROS have also been shown to inhibit receptor-dependent production of NO in the canine coronary endothelium, which may have implications for the

anaesthetised horse (Seccombe et al. 1994). However, damage by ROS has not been proven during anaesthesia in the horse (Portier et al. 2009) and further investigations are warranted.

For the reasons outlined above, it is sometimes recommended that lower (< 1.0) FIO_2 are maintained when anaesthetising horses: horses may breathe more effectively, absorption atelectasis is minimised, and damage from ROS is prevented. However, as the atelectasis observed in anaesthetised horses occurs predominantly from compression, hypoxaemia may still occur, and increasing FIO_2 is often ineffective necessitating other treatment.

1.5.3 Manipulating pulmonary perfusion

β_2 adrenergic agonists

The administration of β_2 adrenergic agonist drugs have been administered to improve arterial oxygenation in anaesthetised horses. Intravenous administration of clenbuterol to anaesthetised horses did improve PaO_2 but was associated with an increased heart rate and profuse sweating (Keegan et al. 1991). In contrast, Lee et al. (1998) found no change in PaO_2 in clenbuterol treated ponies, minimal cardiovascular effects but muscle blood flow was significantly improved. Dodam et al. (1993) demonstrated that intravenous clenbuterol increased $\dot{Q}s/\dot{Q}t$, decreased PaO_2 and induced an increase in oxygen consumption. Due to the limited number of studies, and conflicting results, clenbuterol is not recommended for the treatment of hypoxaemia in anaesthetised horses, as the response appears to be unpredictable.

Salbutamol (albuterol) can be administered by inhalation to horses. Salbutamol administered by metered dose inhaler, significantly increased PaO_2 in treated horses. Cardiovascular factors were not thought to have played a role in the improvement, since no change in MAP was observed, but $\dot{Q}t$ was not measured (Robertson & Bailey 2002). In contrast, Patschova et al. (2010) demonstrated a significant increase in heart rate and $\dot{Q}t$ following the administration of inhaled salbutamol to anaesthetised horses. The PaO_2 values in treated horses increased although the degree of change varied between individuals. They speculated a change in pulmonary perfusion occurred following administration, through a β_2-mediated vasodilatory mechanism, i.e. there was an improvement in $\dot{V}/\dot{Q}$ matching. More recently, adverse cardiovascular events have been reported following the administration of inhaled salbutamol to anaesthetised horses, requiring interventional therapy (Casoni et al. 2014). Sinus and ventricular tachycardia and hypotension occurred in this case series of 5 horses and in some, no improvement in PaO_2 was observed following treatment. Currently however, in equine clinical practice, inhaled salbutamol continues to be administered

relatively commonly, in an effort to treat hypoxaemia in anaesthetised horses. Whilst clinical experience suggests that it is useful, further investigations are warranted to determine its actual mechanism of action, optimal dose, safety and repeatability.

Nitric oxide

Historically, NO was considered to be an environmental pollutant contained within vehicle exhaust fumes and exhaled cigarette smoke, with harmful effects on the ozone, and a key factor in the production of 'acid rain' (Zapol 2019). In 1984, an endothelium-derived relaxation factor, or EDRF, was discovered (Furchgott et al. 1984), and three years later this was identified to be endogenous NO (Palmer et al. 1987). This astounded physiologists at the time, who had only thought of NO as a toxic pollutant. Its biological and medical significance was later highlighted by the fact that the 3 pharmacologists central to its discovery – Robert F. Furchgott, Louis J. Ignarro and Ferid Murad - were awarded the Nobel Prize in Medicine in 1998.

Endogenous NO is produced by NO synthase (NOS) from L-arginine. There is enough L-arginine in the body to enable continuous production of NO. Three forms of NOS have been identified: neuronal (nNOS) and endothelial (eNOS) are considered to be constitutive, whereas inductive (iNOS) is associated with pro- and anti-inflammatory responses (Antosova et al. 2017). Alongside its vasodilatory effects, the functions of NO within the body also include neurotransmission and bactericidal activity (Bruckdorfer 2005). In terms of the vasodilatory effect of NO, once released into the lumen of the blood vessel, it readily diffuses into smooth muscle cells, where it reacts with the haem-containing enzyme, soluble guanylyl cyclase and forms cyclic guanosine monophosphate (cGMP) (Bruckdorfer 2005). The increase of cGMP leads to reduced calcium influx into the cell, hyperpolarization and inactivation of myosin chains, all of which induce smooth muscle relaxation. The cGMP is rapidly hydrolysed and deactivated in situ (Ichinose et al. 2004). Through its vasodilatory effects, NO is central to the regulation of blood pressure and blood flow in both the systemic and pulmonary vasculature (Bruckdorfer 2005).

Mills et al. (1996) demonstrated that endogenous nitric oxide is present in exhaled air from horses and that it increases during exercise. The source of this NO is uncertain, but may originate from the lower airways in the horse, in contrast to the upper airway and nose in man (Lundberg & Weitzberg 1999). This endogenous NO may counteract HPV to improve performance during exercise, and may also cause bronchodilation (Mills et al. 1996). Following on from these discoveries, the potential clinical application of exogenous NO was investigated.

A variety of vasodilators are known to have their therapeutic effect by causing the release of NO in order to mimic the action of endogenous NO (Frostell et al. 1991). However, systemic administration of these compounds causes generalised vasodilation, resulting in systemic hypotension, rather than selective pulmonary vasodilation (Frostell et al. 1991). The therapeutic use of NO gas administered by inhalation, results in selective pulmonary vasodilation (Gerlach et al. 1993; Nyman et al. 2012). Low doses of inhaled NO (iNO) have been shown to reduce pulmonary arterial pressure and improve $\dot{V}/\dot{Q}$ matching in neonatal pigs (Nelin et al. 1994). Nitric oxide is used in severe respiratory disease in humans, to selectively improve the perfusion of ventilated regions of lung, and reduce intrapulmonary shunting (Bigatello et al. 1994). In healthy human patients, the inhalation of NO during one lung ventilation diverts blood away from the hypoxic lung (Hambraeus-Jonzon et al. 1998). Pulmonary hypertension in pre-term and newborn human babies is treated with iNO, is approved for use, and is used similarly in adult humans (Ichinose et al. 2004). More recently, there are numerous reports of the use of iNO as rescue treatment for refractory hypoxaemia, in humans infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that is responsible for the COVID-19 pandemic (Bagate et al. 2020; Ferrari et al. 2020; Kobayashi et al. 2020). It is anticipated that iNO will add to the armamentarium for management of these patients.

The effects of iNO are restricted to the pulmonary circulation, because excess NO binds tightly to oxyhaemoglobin and is rapidly removed from the alveolus by the formation of methaemoglobin and nitrite (Wennmalm et al. 1993). However, high doses of iNO can lead to lethal methaemoglobinaemia and pulmonary oedema (Gerlach et al. 1993). Excess NO in the breathing circuit, or recirculation of exhaled NO, readily combines with oxygen to form nitrogen dioxide (NO_2). Pulmonary injury occurs in a dose-dependent manner when even low concentrations of NO_2 are inhaled, and may result in diffuse injury and oedema (Elsayed 1994). Major clinical toxicity from inhaled NO is only observed at levels of ≥ 80 ppm. Current dosing recommendations do not exceed 40 ppm (Weinberger et al. 2001), and the National Institute for Occupational Safety and Health (USA) has set a time-weighted average NO value of 25 ppm.

The administration of iNO in an attempt to treat hypoxaemia in anaesthetised horses was first described by Young et al. (1999). NO was delivered into the breathing circuit at 10 ppm, constantly, for 20 minutes during spontaneous breathing. Venous admixture did not change significantly and the authors speculated that ventilated alveoli were associated with maximally dilated pulmonary vessels. Previous work in humans with severe ARDS has demonstrated conflicting results, with some studies showing little effect at all (Rossaint et al. 1995; Brett et al. 1998), or even reductions in arterial oxygenation (Barberà et al.

1996). This may be explained by the administration of iNO to poorly or transiently ventilated alveoli in these patients. Improving the pulmonary blood supply to these areas would counteract hypoxic pulmonary vasoconstriction and increase $\dot{Q}_s/\dot{Q}_t$. Heinonen et al. (2000) compared the effects of constant delivery of iNO with pulsed delivery of iNO (PiNO) in pigs, during various phases of inspiration, ventilator settings and delivery modes. They demonstrated that PiNO was as effective as constantly inspired NO. Delivering the NO as a 'pulse' within the first part of the breath, targets well-ventilated alveoli and limits delivery to alveoli that open transiently and ventilate poorly. Heinonen et al. (2001) went on to use the horse as a model, as it is known that large areas of atelectasis develop during anaesthesia. Nitric oxide was delivered as a pulse during the first half of inspiration during both spontaneous and controlled ventilation. By using the multiple inert gas elimination technique (MIGET – see later), and measuring PaO_2 and calculating $\dot{Q}_s/\dot{Q}_t$, its beneficial effect on $\dot{V}/\dot{Q}$ matching was shown. This effect of PiNO was found to be transient, disappearing after 5 minutes following discontinuation. Timing of the pulse was shown to be critical: there was a significant reduction in $\dot{Q}_s/\dot{Q}_t$ and an increase in PaO_2 when horses received NO early in inspiration. However, when the delivery of NO was delayed and delivered during 50 – 80% of inspiration (i.e. the second half of inspiration), the effect was lost. Additionally, NO given early (in the first 30%) in inspiration was almost completely absorbed (92%) and did not appear to build up within the breathing circuit (Heinonen et al. 2002). It was clear that NO could be used to treat hypoxaemia in anaesthetised horses. Further work went on to demonstrate that the effect of PiNO did actually persist into the recovery period following 2.5 hours of treatment (Grubb et al. 2008), which was in contrast to earlier studies. This information is very important for 2 reasons: firstly, hypoxaemia has been identified in recumbent horses recovering from general anaesthesia (Mason et al. 1987; McMurphy & Cribb 1989), and therefore some persistence of the already demonstrated effect, once the PiNO administration is stopped is very useful; secondly, it was hypothesised that there may be a rebound effect from endogenously released endothelin-1 (ET-1), which acts as a pulmonary vasoconstrictor, that would cause a decrease in PaO_2 and an increase in pulmonary arterial pressure. Grubb (2008), Grubb, Edner et al. (2013), and Grubb, Frendin et al. (2013), could not find any significant change in measured ET-1 concentrations in horses receiving PiNO, which demonstrated a lack of rebound effect. Surprisingly, horses in the control group, which did not receive PiNO, had ET-1 concentrations, which were significantly higher than the treated group (Grubb, Edner et al. 2013). There appears to be a relationship between hypoxaemia and the secretion of ET-1 (Cargill et al. 1995). As plasma levels of ET-1 have been shown to increase in the presence of hypoxaemia, ET-1 concentrations may decline during PiNO treatment where PaO_2 is increased; this

may be the reason why ET-1 concentrations were not elevated. More experimental work demonstrated that if the NO was pulsed in the first 30 – 43% of inspiration, this resulted in the largest peak in PaO_2 (Nyman et al. 2012). This occurred because only well-ventilated but poorly perfused alveoli were targeted. Longer duration pulses deliver NO to boundary regions, which lie between the ventilated and atelectatic areas. NO delivered here may counteract hypoxic pulmonary vasoconstriction and may explain why poor results were obtained in initial experiments in which iNO was delivered continuously (Young et al. 1999). The PiNO technique ensures the smallest concentration of NO possible is delivered to the horse, that the NO is delivered to the correct alveoli, and that NO_2 does not build up in the rebreathing circuit.

In anaesthetised laterally recumbent horses it has been shown that PiNO is beneficial in terms of higher PaO_2 values and lower $\dot{Q}_s/\dot{Q}_t$ (Grubb, Edner et al. 2013). The same group continued to assess oxygenation and $\dot{Q}_s/\dot{Q}_t$ in the same horses but during the first 30 minutes of recovery (Grubb, Frendin et al. 2013). Again, the effects of PiNO persisted in the recovery period. More recently, the changes in PaO_2 and $\dot{Q}_s/\dot{Q}_t$ as a result of PiNO administration, have been shown to occur because of 'en masse' movement of blood against gravity (Grubb et al. 2014). Mapping of perfusion was performed with a scintigraphic technique using technetium Tc 99m-labelled micro-aggregated human albumin, and an automated nuclear medicine computer program. Despite the small number of animals, the evidence presented demonstrated that blood moves against gravity from dependent, presumably atelectatic lung, to non-dependent, ventilated lung. This change in perfusion was verified through simultaneous improvement of $\dot{V}/\dot{Q}$ matching measured using the MIGET, and a reduction in $\dot{Q}_s/\dot{Q}_t$ using blood gas analysis at the time of PiNO administration (Grubb et al. 2014).

The description of PiNO use in experimental equine studies has been presented in 2 theses (Heinonen 2002; Grubb 2012). Following on from these descriptions on efficacy and safety, tentative use of PiNO has been trialled in clinical cases. In horses anaesthetised for colic surgery and positioned in dorsal recumbency, the administration of PiNO significantly improved indices of oxygenation, compared with control horses (Wiklund et al. 2017). This latter study highlights the beneficial effect of PiNO in sick horses, that are especially prone to the development of $\dot{V}/\dot{Q}$ mismatch and significant $\dot{Q}_s/\dot{Q}_t$. These horses may also be more susceptible to the detrimental cardiovascular effects of MV strategies. However, further work is necessary to unravel the mechanism of action of PiNO in anaesthetised horses.

1.6 Measurement of regional pulmonary blood flow

Unlike the measurement of total blood flow in some organs (e.g. the brain), measurement of total blood flow to the lung is relatively straightforward, since it equals right ventricular output. However, it is the heterogeneity of pulmonary perfusion that has eluded physiologists in the past, and **regional** pulmonary blood flow or perfusion, rather than global flow that is important. Measurement of regional perfusion would facilitate in the diagnosis, and assessment of treatment, of a variety of lung disorders, in addition to the study of normal pulmonary physiology. In the anaesthetised horse, this would help us glean information on how best to manage oxygenation derangements that occur. Measurement of regional perfusion has been challenging, largely due to the fact that the lung is full of air and is somewhat problematic to image. Traditional approaches have involved estimation of ventilation and perfusion from whole lung gas exchange data, such as concentrations of respiratory or inert gases in expired gas or in blood, but these yield no topographical information (Vidal Melo et al. 2003). Functional lung imaging is a relatively recent concept. In 1960, West & Dollery used radioactive labelled CO_2 with ^{15}O to demonstrate the vertical gradient of pulmonary blood flow in upright humans. This led to the classical description of zones of the lung, whereby flow is determined by hydrostatic pressure effects on pulmonary arterial and venous blood flow, and their relationship with alveolar pressure (West et al. 1964). Other experimental techniques to measure regional pulmonary blood flow have been reported, some of which have been applied clinically, and will be summarised under the subheadings below.

1.6.1 Radioactive microspheres

The distribution of injected radioactive microspheres (typically macro-aggregated albumin or polystyrene), is similar to the distribution of $\dot{Q}t$ to a variety of organs (Buckberg et al. 1971). Historically, this has been the gold standard method to measure regional perfusion. The microsphere technique is essentially the same as for the indicator dilution technique, the microspheres acting as the ‘dye’. There are a variety of factors which have to be considered: microspheres must be mixed well and evenly once injected, so that microspheres reach all vascular branching at the same concentrations; distribution of the microspheres throughout a cross section of the vessel must be uniform: microsphere distribution should approximate RBC distribution; complete entrapment downstream from injection during the first pass and must remain entrapped until counted and; injection and entrapment should have no local tissue or circulatory effects. For albumin studies, either planar gamma scintigraphy or single-photon emission computed

tomography (SPECT) imaging is used for measurement of radioactivity. In animal studies, lungs are usually excised from the intact animal and sectioned before measurement (Dobson et al. 1985). Obviously, this necessitates sacrifice of experimental animals which is undesirable. The technique has been previously highly correlated to flow measured by electromagnetic flow transducers and indicator dilution techniques (Heymann et al. 1977). However, when using a radioactive microsphere technique in live horses, it was not possible to obtain detailed information of regional perfusion (Staddon & Weaver 1981).

1.6.2 Fluorescent – labelled microspheres

Fluorescent-labelled microspheres offer a non-radioactive alternative to radioactive microspheres, and have been validated for measuring perfusion in canine lung (Glenny et al. 1993). They must also fulfil several criteria: be trapped completely by the pulmonary microcirculation; have a distribution in the microcirculation similar to blood; must not affect local perfusion and; the number of microspheres must be large enough to produce a confident flow estimate in low-flow regions (Glenny et al. 1993; Walther et al. 1997). However, typically in animal studies, the subject is euthanased and perfusion measurements made form isolated organ preparations.

1.6.3 Multiple inert gas elimination technique

A technique to measure the distribution of $\dot{V}/\dot{Q}$ ratios within the lung was first described using the multiple inert gas elimination technique or MIGET (Wagner et al. 1974). MIGET involves the intravenous injection of a number of dissolved inert gases with differing solubilities. By comparing the concentrations of the gases in blood and in expired gas, a number of compartments, defined by their $\dot{V}/\dot{Q}$ ratios, can be determined, rather than by anatomical location as determined by microsphere studies. The magnitude of true intrapulmonary shunt (($\dot{V}/\dot{Q}$ ratio of zero) can be distinguished from regions of lung with low $\dot{V}/\dot{Q}$ ratios (Lumb & Pearl 2010). Whilst the development of MIGET was a major step forward in characterising $\dot{V}/\dot{Q}$ distribution, the technique is laborious, it lacks the more detailed spatial information offered by other methods, and is not used commonly outside of research laboratories.

1.6.4 Magnetic resonance imaging

Functional lung imaging using magnetic resonance imaging (MRI) is appealing, since there are no documented adverse effect reports, and no ‘dose’ limitations, meaning that physiologists can perform repeated scans safely (providing known

safety concerns, such as noise, are adequately addressed) (Hopkins & Prisk 2010). However, lung imaging using MRI results in signal to noise ratio (SNR) issues because the lung predominantly consists of air, and therefore, the MRI signal is inherently low (Hopkins & Prisk 2010). During MV this problem becomes even more apparent, because administered breaths are usually greater than resting tidal volumes. Some MRI sequences may be useful to image the lung, but due to their length, image capture during a single breath hold is difficult, subject to motion artefact and respiratory gating is necessary (Hopkins & Prisk 2010). Poor SNRs can be improved by the use of contrast agents, such as gadopentetate dimeglumine, and the application of indicator dilution techniques. However, the advantages of MRI described above are lost, since there is a dose limitation for contrast agents and a limitation to the number of measurements. Non-contrast MRI methods, collectively known as arterial spin labelling (ASL), offer the advantage that repeated measurements can be made during a sustained breath hold. These techniques are more difficult to perform than contrast-enhanced imaging.

1.6.5 Electrical impedance tomography

Electrical impedance tomography (EIT) uses a single ring of electrodes externally to image impedance changes within the body. Its advantages are that, it is radiation free, non-invasive, portable, and does not require contrast agent injection. However, it has very low spatial resolution, and is prone to noise artefacts and anatomical distortion (Nguyen et al. 2012). From a physiological viewpoint, measurement of regional pulmonary perfusion is as important as measurement of regional ventilation. Whilst EIT is able to concurrently measure impedance from both these factors, the low amplitude of perfusion impedance renders perfusion mapping very difficult (Nguyen et al. 2012). Hypertonic saline has been used as a contrast agent to measure pulmonary perfusion using EIT. Studies comparing this technique to SPECT showed good correlation. However, the use of hypertonic saline presents safety concerns when injected in large volumes. Although there are descriptions of EIT used experimentally, its application is rather more appropriate for clinical use, particularly for decision making on ventilation strategies in the intensive care unit (Nguyen et al. 2012).

1.6.6 Computed tomography angiography

Computed tomography (CT) is based upon the measurement of attenuation (reduction in intensity) of a modified x-ray beam, measured in Hounsfield units (HU). This attenuation differs between tissues and results in differing contrasts on the final image. CT offers advantages over conventional radiography in that it produces slice-like images over the area of interest, and thus a 3-dimensional

image can be generated (Bammer 2016). An intravenous contrast agent is necessary to highlight perfusion, and is termed perfusion CT or CT angiography (CTA). With the advent of multi-detector CT (MDCT) scanners, the lung can be scanned rapidly following injection, such that the peak of contrast appearance can be detected (Bammer 2016). These MDCT scanners can acquire 64 image 'slices' within a single rotation time of 0.33 seconds (i.e. three images per second). The potential use of CTA to measure regional perfusion is attractive because of increased resolution and reduction of motion artefacts, often associated with the long scanning times of other techniques (Wolfkiel & Rich 1992). Other advantages include the linear relationship between iodine concentration and tissue density, making mathematical modelling relatively simple, compared with other techniques (Kambadakone & Sahani 2009).

Tissue perfusion using CT is based upon examination of the relationships between arterial and tissue enhancement, following injection of a contrast bolus (Miles & Griffiths 2003). Perfusion is not measured *per* se; the actual measurement is a physiological variant of perfusion, but one that is useful nonetheless (Dawson 2006). The 'first pass' acquisition of a contrast-enhanced image can be evaluated to assess perfusion of a particular area of interest (Miles 2003). The same slice is scanned multiple times and an attenuation curve (time attenuation curve or TAC) is generated, which plots the HU number against time. This type of analysis considers the intravascular and extravascular spaces as single compartment, and hence the technique is known as the compartmental method, the slope method or the maximum slope model (MSM) (Chon et al. 2006; Dawson 2006; Zhao et al. 2020). Perfusion is calculated using the maximum slope of the TAC normalised to an arterial input function; usually the contrast enhancement in a main branch of the pulmonary artery is used (Miles 2003). This method makes various assumptions, the most important of which is that blood does not leave the intravascular space during image acquisition. Injection characteristics of the contrast bolus are extremely important, as peak arterial contrast concentration must occur prior to peak tissue enhancement, necessitating a short and sharp injection of contrast agent (Miles 2003; Kambadakone & Sahani 2009). There are a variety of techniques available for analysis of perfusion using CT data. The MSM has been validated in a wide variety of organs against a gold standard, such as radioactive labelled microsphere studies in animals, or positron emission tomography (PET) studies in humans (Miles 2003; Kambadakone & Sahani 2009). As the MSM only requires registration of the baseline image and images immediately prior to, and after, the time of maximal tissue enhancement, it is relatively straightforward compared with other methods. The faster the image acquisition, the more data points there are when generating the TAC, and therefore improved perfusion calculations. However, this needs to be balanced with the total

radiation dose given to the animal, particularly when multiple measurements are being made.

Whilst there have been numerous descriptions of the MSM technique in a number of species as described above, there are currently no studies that have assessed regional pulmonary perfusion in the normal equine lung. The technique would prove useful to determine the efficacy of interventional therapies used to manipulate pulmonary perfusion, to manage derangements in gas exchange during general anaesthesia (e.g. PiNO).

2. Aims

The overall aim of this project was to quantify, or measure, the effect of pulsed inhaled nitric oxide (PiNO), on pulmonary perfusion during general anaesthesia in the horse. Why does PiNO lead to such an immediate and dramatic improvement in oxygenation? To do this, a technique to image the lung that would offer good spatial resolution was needed, to show that improvements in oxygenation are a consequence of movement of blood, induced by the supposed regional effects of PiNO. It was also important to study the effects of PiNO in greater detail during periods of mechanical ventilation, since this ventilatory modality is used frequently in anaesthetised horses, and previous studies of the effects of PiNO have been performed during spontaneous breathing. The experiments provided further opportunity to document if any adverse effects were noted during PiNO delivery. The results were intended to prepare PiNO for widespread clinical use as an effective, predictable and safe treatment option for managing hypoxaemia in horses during general anaesthesia.

More specifically, the aims were to:

- develop a CT angiography method using the maximum slope model (MSM) for measurement of pulmonary perfusion, and to show that the measurements were reliable.
- describe the variability caused by repeated measurements by the same observer, and between observers, and the variability introduced by using computer software to automate calculations.
- demonstrate that improvements in oxygenation are a consequence of movement of blood, induced by regional effects of PiNO
- measure the effects of PiNO on pulmonary perfusion during spontaneous breathing and mechanical ventilation.
- measure the effects of PiNO on pulmonary perfusion during the two modes of ventilation but also during periods of hypotension and normotension.

3. Materials & Methods

3.1 Animals

The studies in this thesis were approved by the local Ethical Committee on Animal Experiments in Uppsala. In studies II, III and V, six adult, healthy ponies were used. As the ponies had to fit inside the CT gantry, an upper weight limit of approximately 250 kg was necessary. These six ponies were five Shetland ponies and one crossbreed pony (one mare, three geldings and two stallions) with a bodyweight range of 150-241 kg and age range 4-18 years. In study IV, 27 adult, healthy, Standardbred horses were used. These animals had a mean bodyweight of 375-610 kg and age range of 1-25 years.

3.1.1 Groups

In studies II and III, all ponies acted as their own controls, in that they were allowed to breathe spontaneously and were then mechanically ventilated, within the same experiment, with and without PiNO.

In study IV, all horses received PiNO and were allocated to 1 of 4 groups: spontaneous breathing with low blood pressure (SB-L, n=7); spontaneous breathing with normal blood pressure (SB-N, n=8); mechanical ventilation with low blood pressure (MV-L, n=6); and mechanical ventilation with normal blood pressure (MV-N, n=6).

In study V, ponies were anaesthetised on two separate occasions and were mechanically ventilated throughout. During both anaesthetics, hypotension (MAP < 70 mmHg) was allowed to develop initially, and PiNO was administered for 60 minutes and then discontinued. In the first anaesthetic (treatment group), six ponies were included and data were collected after 30 minutes of PiNO (T1). Dobutamine was then administered to achieve normotension (MAP 70 – 80

mmHg), and data collected after a further 30 minutes of PiNO (T2). PiNO was then discontinued and the six ponies split into two groups of three ponies. In the first group of three ponies, dobutamine was discontinued and hypotension allowed to develop with data collected after 45 minutes (T3a). In the second group of three ponies, dobutamine was continued and data collected at 45 minutes (T3b). Five ponies were included in the second anesthetic (control group), and hypotension (MAP < 70 mmHg) was not treated throughout. Data were collected after 30 and 60 minutes of PiNO and after 30 minutes following discontinuation of PiNO (timepoints C1, C2 and C3 respectively).

3.2 Anaesthesia

All animals in all experiments were administered a standardised anaesthetic protocol. Health status was confirmed by clinical examination and complete blood analysis. Food was withheld for 12 hours prior to anaesthesia and water provided *ad libitum* until premedication.

Initial premedication was achieved with intramuscular (IM) 0.03 mg kg^{-1} acepromazine (Plegicil vet, Pharmacia & Upjohn Animal Health, Sweden). Following intravenous (IV) catheterisation, 1.1 mg kg^{-1} xylazine (Rompun 2%, Bayer Animal Health, Sweden) and 0.025 mg kg^{-1} butorphanol (Butador, Vetoquinol, Sweden) were administered IV. General anaesthesia was induced with 2.2 mg kg^{-1} ketamine (Ketaminol, Intervet, Sweden) mixed with 0.05 mg kg^{-1} diazepam (Diazepam, Ratiopharm, Germany) and administered IV. Once unconscious, the trachea was intubated with an appropriately sized endotracheal tube (internal diameter 20 mm for studies II, III and V and 26 mm for study IV). The tube was connected to an anaesthetic machine (Smith, Denmark for studies II, III and V; Tafonius, Vetronic Services, UK for study IV). Anaesthesia was maintained with isoflurane (IsoFlo, Orion Pharma Animal Health, Sweden), vaporised in oxygen, to achieve an FIO_2 of approximately 0.9. All animals were positioned on a padded table in dorsal recumbency. Intravenous fluid therapy (Ringer-acetat, Fresenius Kabi, Sweden) was provided at a rate of 5 ml $kg^{-1}hr^{-1}$. Where blood pressure support was needed, dobutamine (Dobutamin Carino, Carinopharm GmbH, Germany) was administered as a variable rate IV infusion (0.5 – 5.0 μg $kg^{-1}min^{-1}$). For studies II, III, IV and V, analgesia was administered at the end of anaesthesia using 1.1 mg kg^{-1} flunixin meglumine (Flunixin N-vet, N-vet AB, Sweden) IV, and 0.1 mg kg^{-1} morphine (Morfin Meda, Meda AB, Sweden) IM. Some horses in study IV were euthanased (barbiturate overdose) at the end of the procedure if this was pre-planned for cadaveric teaching.

3.3 Ventilation

For studies II and III, mechanical ventilation was instituted after a period of spontaneous breathing. For study IV, 2 groups of horses were allowed to breathe spontaneously and 2 groups were mechanically ventilated. For study V, mechanical ventilation was instituted in all ponies throughout the experiment. For studies II, III and V, the ventilator used was Smith, Denmark. For study IV, the ventilator used was Tafonius, Vetronic Services, UK. For all ventilated animals, tidal volume (TV) and rate were adjusted to maintain $PaCO_2$ between 6.0 – 9.0 kPa. Peak inspiratory pressure (PIP) was maintained below 30 cmH_2O.

3.4 Instrumentation

Following premedication with acepromazine, hair was clipped over the right and left jugular veins and the skin prepared aseptically. A 14 gauge x 9 cm catheter (Mila International Inc, Kentucky, US) was placed in the left jugular vein, and two 8.5 Fr sheath introducers (Argon Exacta, Argon Medical Devices, Netherlands) placed into the right jugular vein. The skin was infiltrated with lidocaine (Xylocain, AstraZeneca, Sweden) prior to placement. The left jugular catheter was used for drug administration. Following induction of anaesthesia and positioning, the facial artery was catheterised for blood pressure measurement and arterial blood sampling (Insyte-W, Becton-Dickinson, Sweden). A 7 Fr thermodilution pulmonary artery catheter (Swan Ganz Criticath SP5507H TD catheter, Argon Medical Devices, Netherlands) was inserted into the pulmonary artery via the most distal pre-placed jugular introducer, under pressure waveform guidance. This catheter was used to measure cardiac output ($\dot{Q}t$) by thermodilution, sampling of mixed venous blood and measurement of mean pulmonary arterial pressure (MPAP) and pulmonary capillary wedge pressure (PCWP) The other more proximal introducer was used to place a pigtail, multi-hole catheter (Cook Europe A7S, Denmark) inserted into the right ventricle and then retracted into the right atrium also using pressure waveform guidance. This catheter was used for injection of ice-cold saline for $\dot{Q}t$ measurement and for injection of iodinated contrast in studies II, III and V.

Each horse or pony was connected to a multiparameter monitor (Datex Ohmeda AS/3, Finland), for measurement of the electrocardiogram (ECG), heart rate (HR), arterial haemoglobin oxygen saturation (SpO_2), end tidal carbon dioxide concentration ($ETCO_2$), FIO_2, end tidal isoflurane vapour concentration, respiratory rate (*f*R), TV, PIP, systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressure, MPAP, PCWP and $\dot{Q}t$. Cardiac output was measured using

thermodilution. The same person injected 20 ml of ice-cold saline by hand during the expiratory phase, into the pigtail catheter in the right atrium. The pulmonary artery catheter was connected to the multiparameter monitor and measured the change in temperature over time. A minimum of three injections were made at each data collection point and the average recorded.

3.5 Blood gas analysis

At each data point, arterial and mixed venous blood samples were taken simultaneously from the facial arterial and pulmonary artery catheters, respectively. Blood was placed into heparinized syringes and analysed immediately, using a standard electrode technique and spectrophotometry (ABL 90 flex, Radiometer, Denmark). Measured values were arterial pH (pHa) mixed venous pH ($pH\bar{v}$), partial pressure of oxygen in arterial (PaO_2) and mixed venous ($P\bar{v}O_2$) blood, partial pressure of carbon dioxide in arterial blood ($PaCO_2$), haemoglobin oxygen saturation in arterial (SaO_2) and mixed venous ($S\bar{v}O_2$) blood, haemoglobin concentration [Hb] and lactate concentration. Measurements were corrected for atmospheric pressure but not body temperature.

3.6 Nitric oxide delivery

Nitric oxide was delivered as a pulse (PiNO) using an airway triggered device developed at the Datex-Ohmeda Research Unit, Helsinki, Finland (Heinonen et al. 2000). Timepoints for delivery are illustrated for each study below. Nitric oxide was supplied in a cylinder containing 2000 ppm medical grade NO in N_2 (AGA, AB, Sweden) The device was either triggered by negative pressure during spontaneous breathing, or positive pressure during mechanical ventilation. Each pulse was delivered for the first 30-45% of inspiration giving a dose of 2.5 – 5.0 μM NO each time.

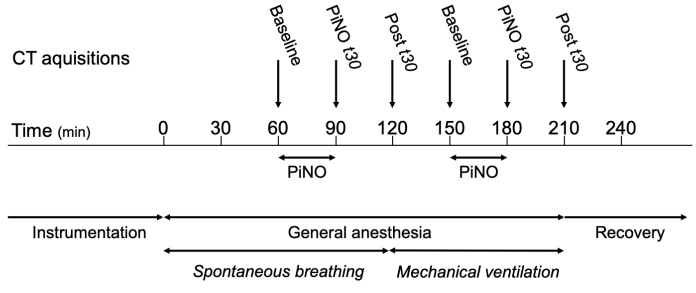
3.7 Experimental timelines and data collection

3.7.1 Timeline for studies II and III – Figure 1

All ponies were allowed to breathe spontaneously at first. During a period of equilibration, the pigtail, pulmonary artery and facial artery catheters were placed. Blood pressure was supported to maintain MAP 70 - 80 mmHg using dobutamine. Data were collected after 60 minutes of anaesthesia (SB baseline). PiNO was administered for 30 minutes and data collected (SB PiNO *t30*). PiNO was stopped

and data collected 30 minutes later (SB Post *t30*). Mechanical ventilation was started as in section 3.3. After 30 minutes, data were collected (MV baseline). PiNO was started and after 30 minutes, data collected (MV PiNO *t30*). PiNO was stopped and data collected again, 30 minutes later (MV Post *t30*).

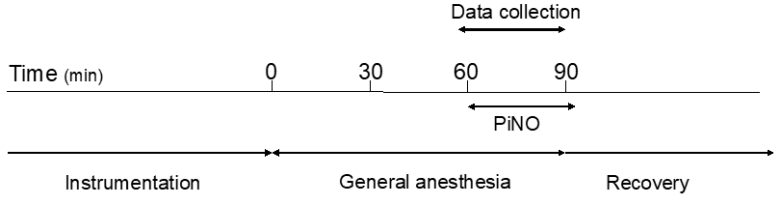
Figure 1 - Experimental timeline of events showing time in minutes and interventions during the procedure for studies II and III. Contrast injection and CT acquisitions are shown as downward arrows.



3.7.2 Timeline for study IV – Figure 2

Horses were randomly allocated to 1 of 4 groups (see 3.1.1) Baseline data was taken following a period of equilibration, PiNO was then delivered for 30 minutes and data collected at 15 and 30 minutes during PiNO delivery.

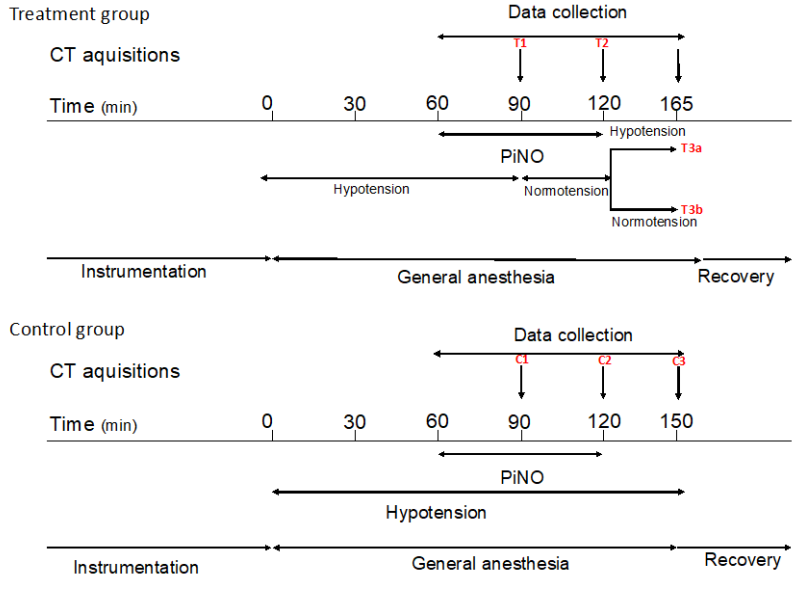
Figure 2 - Experimental timeline of events showing time in minutes and interventions during the procedure for study IV



3.7.3 Timeline for study V – Figure 3

Ponies were anaesthetised on two separate occasions and mechanically ventilated (see 3.1.1 for detailed explanation of groups). Following 60 minutes of hypotension (MAP < 70 mmHg), PiNO was delivered for 60 minutes and data were collected after 30 minutes of PiNO. In the first anaesthetic (treatment group), blood pressure was then supported with dobutamine to achieve a MAP 70 - 80 mmHg. In the second anaesthetic (control group), blood pressure was not supported. Data were collected after a further 30 minutes of PiNO. PiNO was stopped and in the first anaesthetic, the six ponies were split in to two further subgroups: dobutamine was discontinued in one subgroup and continued in the other subgroup and data collected after 45 minutes. In the second anaesthetic data were collected after 30 minutes of PiNO discontinuation.

Figure 3 - Experimental timeline of events showing time in minutes and interventions during the procedure for study V during 2 separate anaesthetics. Contrast injection and CT acquisitions are shown as downward arrows. Time points are highlighted in red (see 3.1.1)



In all studies, data collected were: HR, SAP, MAP DAP, SpO_2, $ETCO_2$, FIO_2, *f*R, TV, PIP, MPAP, PCWP and $\dot{Q}t$. All measurements from blood gas analysis as detailed in section 3.5 above, were recorded. From this data, a number of calculations were made as follows:
Oxygen content of end capillary ($Cc'O_2$), arterial (CaO_2) and mixed venous ($C\bar{v}O_2$) blood were calculated as $CxO_2 = (1.36 \times [Hb] \times SxO_2) + (0.003 \times PxO_2)$, where *x* is c', a or $\bar{v}$; alveolar partial pressure of oxygen (PAO_2) was used to calculate $Cc'O_2$.
PAO_2 was calculated using the alveolar gas equation:
$PAO_2 = FIO_2$ (barometric pressure – water vapor pressure) - $PACO_2/RQ$. Where: $PACO_2$ is substituted with $PaCO_2$ and RQ is the respiratory quotient taken as 0.8 (West 2008).
The alveolar-arterial oxygen partial pressure difference $P(A\text{-}a)O_2 = PAO_2 – PaO_2$.
Oxygen delivery (DO_2) was calculated as $CaO_2 \times \dot{Q}t$.
Venous admixture ($\dot{Q}s/\dot{Q}t$) was calculated using the Berggren shunt formula:
$\dot{Q}s/\dot{Q}t = (Cc'O_2 – CaO_2)/(Cc'O_2 – C\bar{v}O_2)$(Berggren 1942)
Cardiac index (CI) was calculated as $\dot{Q}t$/body weight in kg.
Minute volume (V_E) was calculated as *f*R x TV.
Alveolar dead space (Vd/Vt) was estimated as:
$Vd/Vt = (PaCO_2 - ETCO_2)/PaCO_2$.
The oxygen extraction ratio (OER) was calculated as:
$OER = C(a – \bar{v})O_2 / CaO_2$.
Pulmonary vascular resistance (PVR) was calculated as:
PVR = MPAP-PCWP/ $\dot{Q}t \times 80$

In studies II, III and V, contrast-enhanced CT images were acquired at data collection points as detailed in the next section.

3.8 CT protocol (studies II, III and V)

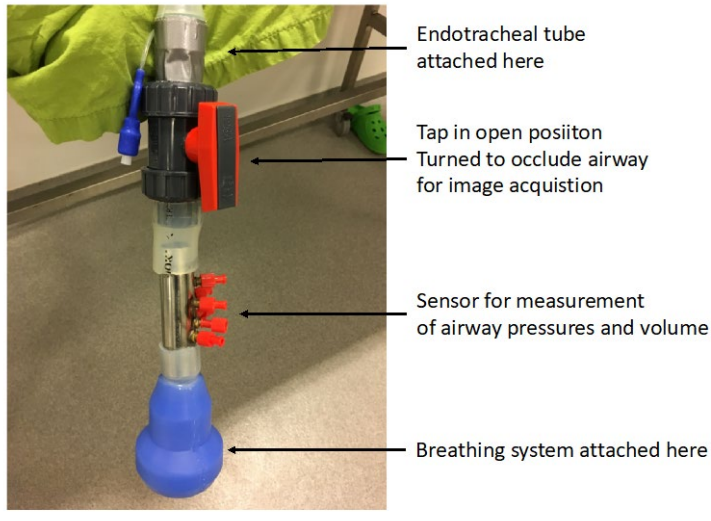
In studies II, III and V, the same CT protocol was used to image the thorax. Each pony was positioned in dorsal recumbency on the CT bed with the head facing the gantry and supported with padding. The limbs were allowed to fall into a natural position and were supported where necessary. The CT scanner (Somatom Definition AS, Siemens Medical Systems, Erlängen, Germany) was a third generation, 64-slice, MDCT scanner. Exposures were 70 mAs and 120 kV, slice thickness 15.0 mm and rotation time 0.33 sec. A topogram was used to identify correct anatomical positioning and for selection of a suitable thoracic slice, caudal to the heart. The slice chosen ensured that the different lung regions were of

sufficient size for analysis later. In pilot studies, we used two different iodinated contrast agents to assess if one was superior to the other. Contrast agents used were Omnipaque (300 mg ml^{-1}, GE Healthcare, UK) and Ultravist (370 mg ml^{-1}, Bayer, USA). There was little difference between the two agents, and we opted to use the lower concentration contrast agent for all further imaging. The pilot studies also aimed to modify the injection of contrast in order to achieve rapid injection and appropriate enhancement of the CT image. By removing a spiral extension set, using alternative tubing that was wider and shorter, using a larger volume of contrast agent, warming the contrast before injection to approximately 37°C, and injecting into the right atrium, we were able to achieve an injection rate of 10 ml sec^{-1} (vs 8 ml sec^{-1}). The volume of contrast injected was 60 ml followed by a 30 ml chaser of saline using an automated injector (Medrad Stellant dual syringe CT injection system, Bayer AG, Germany) directly into the right atrium using the pigtail catheter. There was a 4 second delay from the start of scanning to the start of injection so that baseline HU could be measured. Each scan sequence lasted approximately 50 seconds.

As respiratory motion is a major cause of artefact during CT scanning, we needed to arrest breathing during imaging. In pilot studies, we attempted to clamp the endotracheal tube at peak inspiration and end expiration. However, this proved extremely problematic, with leakage of gas from the lung, and the pony able to breathe through the partially clamped tube. Our solution was adaptation of a simple plumbing ball valve with a tap, that was inserted between the endotracheal tube and the breathing system of the anaesthetic machine (Figure 4). Simply by turning the tap, we successfully managed to arrest breathing, and this was repeatable. During MV, the ventilator was turned off at the same time.

In studies II and III, we performed CT scans during contrast injection at end expiration when ponies were spontaneously breathing and at end expiration and peak inspiration when ponies were mechanically ventilated. In study V, we only performed CT scans at peak inspiration, as these images were much easier to analyse.

Figure 4 – adaptation of a simple plumbing ball valve used for occlusion of the airway during image acquisition.



3.9 CT image analysis (studies II, III and V)

In studies II and III, after multiple training sessions, two blinded observers (AA and PL), analysed each CT image sequence twice, with a period of at least seven days between each repeated analysis, in order to prevent recall bias. In study V, only one observer analysed each CT image. Digital Imaging and Communications in Medicine (DICOM) files as a series of images at 3 per second were exported to Osirix (an image processing application, v.5.8.5 64-bit, Switzerland). ImageJ (an open source image processing program, v1.49, National Institutes of Health, USA) was used to delineate lung regions using HU thresholds. In studies II and III, thresholds used were +100 to -100 HU for atelectatic lung; -100 to -350 HU for poorly aerated lung and -350 to -1000 HU for well ventilated lung. In study V, thresholds used were +100 to -100 HU for atelectatic lung; -100 to -500 HU for poorly aerated lung and -500 to -1000 HU for well ventilated lung (Gattinoni et al. 1988, Vieira et al. 1998). A region of interest (ROI) was drawn within the pulmonary artery and used as the arterial input function (AIF) in calculations of perfusion (see below). Further ROIs were drawn within the margins of the lung regions delineated by the thresholds, avoiding large vessels and airways by scrolling through the image sequence. Any respiratory motion was noted at this time. From the ROIs, time attenuation curves (TACs) were generated and the baseline peak and tail identified prior to further analysis.

3.9.1 Manual CT calculations

Manual CT calculations were made in study II. From the ROI within the pulmonary artery, net attenuation used as the AIF was calculated by subtracting its baseline HU from its peak HU. The maximum slopes of TACs from ROIs drawn within lung regions were measured using the *x* and *y* coordinates of 2 points on the observed linear slope. As cardiac pulsation affected some of the TACs from the aerated lung ROIs, the peaks of these oscillations were used to measure maximum slope. Despite attempts to stop breathing during image acquisition, there was still some leakage or absorption of gas from the aerated lung in some image sequences and resulting movement of the chest wall and diaphragm was detectable. This was evident on TACs generated from aerated lung ROIs as a gradual increase of HU over time, independent of the contrast bolus rise. If this occurred during the upslope, determined by scrolling through the frames, we measured the slope of this gas loss and subtracted it from the maximum slope of the TAC to calculate net rate of change (net maximum slope). Perfusion was calculated by dividing the maximum slope of the TAC, by the maximum arterial enhancement (AIF from the pulmonary artery) using Equation 1 (Miles & Griffiths 2003):

Equation 1

$$Perfusion = \frac{\frac{\mathrm{d}}{\mathrm{d}t}[c(t)]_{max}}{a\,(t)_{max}}$$

c = tissue concentration of contrast agent
a = arterial concentration of contrast agent
t = time

Pulmonary perfusion was normalized to tissue mass to account for spatial heterogeneity by incorporating CT attenuation values into the equation (Zhao et al. 2020):

Equation 2

$$Normalised\ Perfusion(\text{ml min}^{-1}\text{gram}^{-1}) = \frac{Perfusion}{\left(\frac{HU_x - HU_{air}}{HU_{tissue} - HU_{air}}\right)}$$

HU_x = HU of ROI in non-contrast enhanced image
HU_{air} = -1000
HU_{tissue} = 50

Perfusion values are presented in ml min^{-1}gram $tissue^{-1}$. All calculations were made in Excel spreadsheet format (Microsoft Excel for Mac, version 16.16.27).

3.9.2 Semi-automated CT calculations (smoothed curve method)

As oscillations from cardiac pulsation were frequently observed within TACs generated from ROIs drawn within aerated lung, computer software was used to smooth the curve and measure the slope. This was devised as a way to speed up the analysis process for future studies, and is hereafter called the *smoothed curve method*. The ROI (*x* and *y*) data from one observer (AA) was imported in excel spreadsheet format into a specially coded Matlab program (The MathWorks, Inc., MA, USA). The program calculated the maximum slope between selected points and subtracted the effect of gas loss, if present, to calculate net maximum slope. Noise removal and smoothing of the signal was performed using cubic spline (de Boor 1978) and a smoothing factor of 0.15. In the graphical user interface, the user marked the following epochs in the signal: 1. before the contrast entered the field of view; 2. the upslope and downslope in the signal resulting from passage of contrast; 3. after the contrast had left the field of view. Data points of epochs 1 and 3 were fitted using a second order polynomial (using a least squared approach) that represented the drift in the baseline of the signal. The fitted polynomial was then removed from the signal. The point of maximum positive slope was then identified automatically and used for further calculations. The smoothed curve perfusion calculations were assessed for agreement in study II and used for analyses in studies III and V.

3.9.3 Perfusion correction for cardiac output (study III)

Since pulmonary perfusion is directly related to $\dot{Q}t$, and $\dot{Q}t$ differed between ponies and over time, perfusion values were corrected for this change.

$\dot{Q}t$ corrected perfusion at each time point during SB within each individual pony was calculated using the equation: perfusion/$\dot{Q}t$.

The per cent change in $\dot{Q}t$ corrected perfusion within each individual pony from baseline to PiNO *t30* was calculated by: $\dot{Q}t$ corrected perfusion baseline/$\dot{Q}t$ corrected perfusion PiNO*t30* – 1.

The per cent change in $\dot{Q}t$ corrected perfusion within each individual pony from PiNO *t30 to* Post *t30* was calculated by: $\dot{Q}t$ corrected perfusion PiNO *t30* /$\dot{Q}t$ corrected perfusion Post *t30* – 1.

This was repeated for measurements made during MV.

3.9.4 Accumulation of contrast over time

As each pony received numerous injections of contrast agent, and the assumptions underlying the model include that the contrast agent should not leave the compartment during image acquisition, baseline HU over time were plotted for each pony.

3.10 External validation of perfusion measurements

There was no gold standard to compare our perfusion measurements against. The gold standard used in animals is the radioactive microsphere technique (see 1.6.1). However, this method necessitates euthanasia and extraction of the lung from the animal, preventing repeated measurements that were essential to measure the effect of PiNO on pulmonary perfusion. In order to validate the CTA method described in this thesis, the results were compared with a previous microsphere study in excised equine lungs (Dobson et al. 1985). In that study, horses were anaesthetised and placed in dorsal recumbency. Microspheres were injected and the lungs excised and divided into 4 sections. From their data, mean perfusion values were calculated and compared CTA perfusion measurements in atelectatic lung with their outer dorsal lung, and CTA perfusion measurements in aerated lung with their non-dependent lung sections.

3.11 Statistical analysis

Commercially available statistical software was used for analysis in all studies (MedCalc, v17.1 64-bit, Ostend, Belgium; SAS version 9.4, SAS Institute Inc, NC, USA, GraphPad Prism, version 5, GraphPad Software Inc., CA, USA).

Study II: Measurements of ROI area, maximum slope and perfusion calculations were compared for repeated measurements by the same observer (intraobserver), and paired measurements from each observer were averaged and then compared between the observers (interobserver). Measurements made by a single observer were compared with the smoothed curve method. Correlations between the two observers and between a single observer and the smoothed curve method were testing using Pearson's correlation coefficient (R). Intraobserver and interobserver agreement was assessed using Bland Altman analysis. The repeatability of these measurements was also tested using the intraclass correlation coefficient (ICC). The source of any variation in the measurements was determined using a mixed model statistical analysis of variance (Littell 2006), with the pony as a random effect and intervention (contrast injection and mode of ventilation), measurement, observer and their interactions as fixed effects. Where the effect of the intervention

was significant, multiple comparisons at separate time points were performed. Using the mixed model, covariance parameter estimates were used to calculate the coefficient of variation (CV) between the two observers, and between a single observer and the smooth curve method.

Study III: The mixed model of SAS software was used. Assumptions underlying the models were checked with diagnostic plots. Post-hoc comparisons were adjusted for multiplicity using Tukey's method. As there were several observations for each pony, a mixed linear model was used. The fixed part of the model included the intervention (experimental time point), ventilation mode and the interaction between them, with pony as the random factor.

Study IV: Normality was tested using the Shapiro-Wilk test. Mann-Whitney tests were used to compare differences between spontaneous breathing and mechanically ventilated horses during hypotension and normotension. Friedman ANOVA and Dunn multiple comparisons post hoc tests were used to compare differences over time within each group.

Study V: Assumptions were checked using diagnostic plots. During each anesthetic, data were collected at six time points, denoted 1, 2 and 3. Thus, there were three measurements per pony: C1, C2, C3 and T1, T2, T3. Dobutamine was only given at measurement T2. Since there were several observations per pony, data were analysed using the mixed procedure of the SAS/STAT® software (2018 SAS Institute Inc.) (Littell et al. 2006). The fixed part of the model included anaesthetic (C or T), time, and the anaesthetic*time interaction. Pony was set as a random factor. In a post-hoc comparison, the average for measurements C1, C2 and T1 was compared with the T2 value, i.e. the observation with active treatment was compared with the average of the untreated observations using linear contrasts. In the treatment group, the six ponies were split into two further groups, hence there were now only three ponies in each of those subgroups. Due to the small numbers of ponies in each group, data at time points C3 and T3 are presented as descriptive data only.

4. Results

4.1 CT methodology (Study II)

4.1.1 General points

Tissue enhancement did not change appreciably between the two types of contrast agent tested in the pilot studies. For all CT acquisitions used within the main studies (II, III and V), 300 mg ml^{-1} iodinated contrast was used.

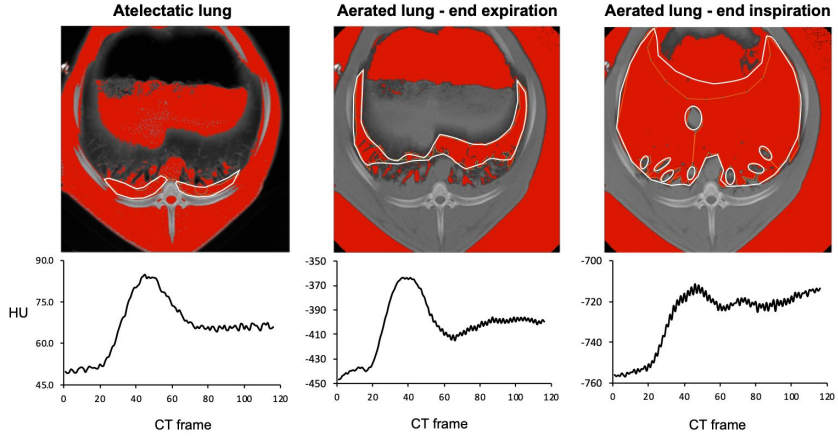
Images during the first pass of contrast, suitable for analysis were acquired in all ponies, at all experimental time points.

As perfusion did not change in ROIs drawn in poorly aerated lung regions (threshold-100 to-350 HU), it was not included in statistical analysis (also study III).

Cardiac pulsation interfered with TACs drawn from ROIs of aerated lung regions (Figure 5).

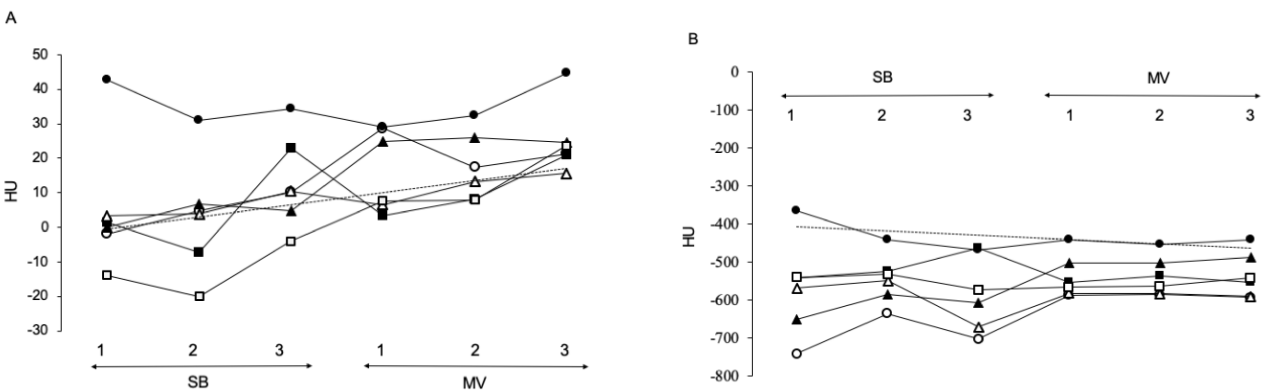
There was slow leakage or absorption of gas in 55% of image sequences in aerated lung regions held at peak inspiration in mechanically ventilated ponies. This gas loss was evident as a slow increase in tissue density over time, independent of the maximum slope of the TAC. This gas loss was not detected in images acquired from spontaneously breathing ponies as the lung was imaged at end expiration (Figure 5).

Figure 5 – CT images with lung regions delineated using thresholds in atelectatic lung, aerated lung at end expiration and at end inspiration. Regions of interest (ROI) drawn are shown as a white line and corresponding time attenuation curves (TACs) generated from the ROIs are presented below each image. Note the cardiac pulsation in the aerated curves and the slow increase in attenuation over time, independent of the initial slope of the first pass of contrast, due to slow absorption or leakage of gas. The slope of this gas loss was measured and removed from the maximum slope.



Line plots of baseline HU over time for each pony are presented in Figure 6; there was no significant accumulation of contrast.

Figure 6 - Change in baseline HU over time in atelectatic (A) and aerated (B) lung regions in 6 anesthetized ponies during spontaneous breathing (SB) and mechanical ventilation (MV). The numbers 1, 2 and 3 along the abscissa represent the time course of the experiment which involved the injection of several contrast boluses. Each pony is represented by a different data symbol



4.1.2 Intraobserver and interobserver correlation and agreement

Correlation between observers was high and values are presented in Table 1.

The mean difference (bias), 95% limits of agreement (LOA) and coefficient of repeatability from Bland Altman analysis within and between observers for ROI area, slope and perfusion calculations are presented in Tables 2 and 3. For intraobserver agreement, zero was included within the confidence interval of the mean difference showing that there was no systematic bias. For interobserver agreement, there was some evidence of systematic bias for perfusion in atelectatic lung regions i.e. one observer tended to measure higher values than the other. The LOA between observers were wider than within the same observer, indicative of greater variability between measurements, although approximately 95% of data points were within the LOA. Bland and Altman plots for interobserver agreement for TAC slopes and perfusion calculations from atelectatic lung regions are shown in Figures 7A and 7C.

Table 1 - Correlations of region of interest (ROI) area, slope of time attenuation curve (TAC) and calculated perfusion between two observers (AA and PL) and Matlab (ML) processed data. Pearson correlation coefficient (R^2), intraclass correlation coefficient (ICC), coefficient of variation (CV) and contribution of pony, experimental stage and the observer on the variability between measurements.
Observers: 1=AA-PL (n=54) 2=AA-ML (n=54)
* There is no comparison of ROI area between AA and ML since these values are identical

Variable		**Obse-rvers**	**R^2**	**ICC**	**CV (%)**	**Contribution to variability (%)**			
						Pony	**Stage of experiment**	**Observer**	**Total**
Atelectatic ***lung region***	***ROI Area***	1*	0.88	0.86	8.6	85	11	4	100
	Slope	1	0.95	0.91	9.0	70	28	2	100
		2	0.99	0.99	4.7	62	37	1	100
	Perfusion	1	0.97	0.93	8.5	36	62	2	100
		2	0.98	0.99	5.6	32	68	0	100
Aerated ***lung region***	***ROI Area***	1*	0.98	0.97	5.8	58	41	1	100
	Slope	1	0.96	0.96	11.0	55	44	1	100
		2	0.93	0.94	12.3	60	40	0	100
	Perfusion	1	0.94	0.94	12.4	31	68	1	100
		2	0.93	0.93	13.1	29	71	0	100

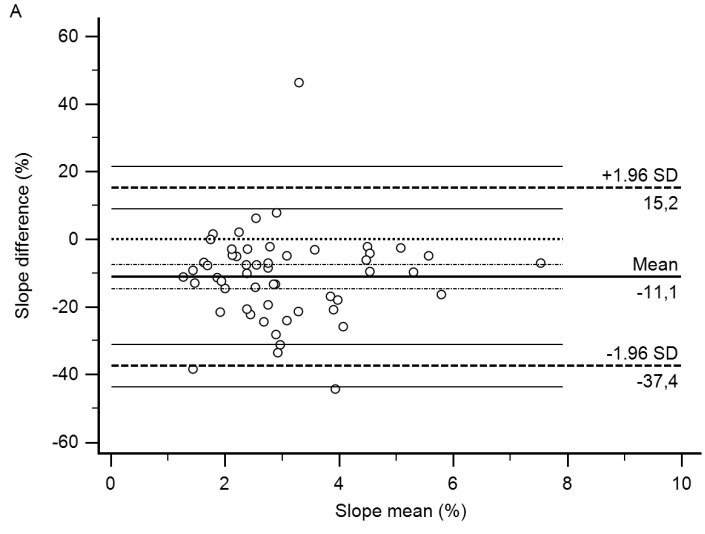
Table 2 - Bland & Altman analysis for intraobserver agreement between paired measurements. Bias (mean difference) and 95% confidence interval (CI) with limits of agreement (LOA) are presented. The coefficient of repeatability (COR) represents the maximum likely difference between measurements. Regions of interest (ROI) areas and time attenuation curve maximum slopes are presented as percentage difference.
Observers:1=AA (n=108) 2=PL (n=108)

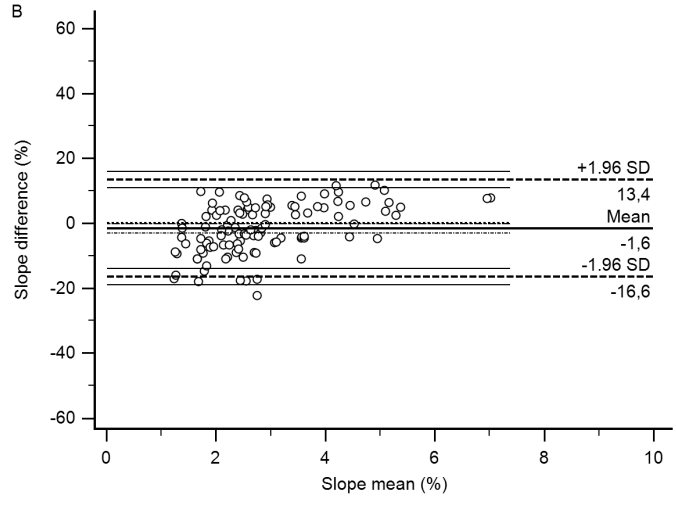
Intra observer		**Obser -ver**	**Bias**	**95% CI**	**LOA**	**COR**
Atelectatic *lung region*	***ROI Area (%)***	1 2	-0.4 -0.6	-3.0; 2.1 -2.4;1.3	18.8;17.9 14.0;12.8	848.6 mm^2 727.1 mm^2
	Slope (%)	1 2	0.3 -0.5	-2.0;2.6 -3.2;2.1	-16.3;16.9 19.7;18.6	0.4 0.6
	Perfusion (ml/min/gram)	1 2	-0.01 -0.04	-0.1;0.1 -0.1;0.05	-0.6;0.6 -0.7;0.6	0.6 0.7
Aerated *lung region*	***ROI Area (%)***	1 2	1.0 -0.3	0.0;2.0 -1. 9;1.2	-6.5;8.5 -11.5;10.9	2300.6 mm^2 3497.7 mm^2
	Slope (%)	1 2	1.6 -2.2	-1.5;4.7 -5.7;1.4	-20.6;23.9 -27.6;23.2	0.2 0.57
	Perfusion (ml/min/gram)	1 2	-0.03 -0.2	-0.1;0.1 -0.4;0.02	0.9;0.8 1.8;1.4	0.9 1.6

Table 3 - Bland & Altman analysis for interobserver agreement between paired measurements from two observers (AA and PL) and Matlab (ML) (smoothed curve) processed data. Bias (mean difference) and 95% confidence interval (CI) with limits of agreement (LOA) are presented. The coefficient of repeatability (COR) represents the maximum likely difference between measurements. Regions of interest (ROI) areas, time attenuation curve maximum slopes, calculations of perfusion and gas loss slopes are presented as percentage difference. Observers:1=AA-PL (n=54) 2=AA-ML (n=54) gas loss slope (n=52)

Interobserver		**Observer**	**Bias**	**95% CI**	**LOA**	**COR**
Atelectatic	***ROI Area (%)***	1	-6.6	-11.0 ; -2.2	-38.1; 25.0	1559 mm^2
lung	***Slope (%)***	1	-11.1	-14.8 ; -7.5	-37.4 ; 15.2	1.01
region		2	-1.6	-3.0 ; -0.1	-16.6 ; 13.4	0.4
	Perfusion	1	-0.4	-0.5 ; -0.3	-1.1 ; 0.4	1.0
	(ml/min/gram)	2	-0.02	-0.07 ; 0.04	-0.4 ; 0.4	0.4
Aerated	***ROI Area (%)***	1	1.5	-2.3 ; 5.3	-25.7 ; 28.8	12821 mm^2
lung	***Slope (%)***	1	-0.55	-6.0 ; 4.9	-39.6 ; 38.5	0.7
region		2	-1.5	-4.5 ; 1.6	-32.8 ; 29.8	0.8
	Perfusion	1	-0.1	-0.4 ; 0.2	-2.3 ; 2.1	2.2
	(ml/min/gram)	2	-0.2	-0.5 ; 0.1	-2.4 ; 2.1	2.2
	Gas loss slope (%)	2	-22.2	-39.8 ; -4.6	-146.3 ; 101.9	

Figure 7A and 7B - Bland Altman plots for atelectatic lung regions: (A) interobserver time attenuation curve (TAC) slopes; (B) Matlab processed data (smoothed curve method) vs single observer TAC slopes. The mean of paired measurements from each observer was used and then compared (n=54), and data are presented as percentage differences. The bold solid line represents the mean difference (bias), with dot-dashed lines the 95% confidence interval of the mean difference, the dashed lines the upper and lower 95% limits of agreement with solid lines the 95% confidence intervals of the limits of agreement.





Results from the mixed model analysis for ICC and CV are presented in Table 1. Most variability within the measurements was related to individual ponies or differences between measurements at each experimental stage. Very little variation between measurements could be attributed to differences between observers. Generally, the ICC was greater than 0.9 between observers, indicative of high correlation.

4.1.3 Smoothed curve method correlation and agreement

Correlation between the single observer and measurements of slope and perfusion using the smoothed curve method are presented in Table 1. All measurements were positively correlated. The mean difference (bias), 95% limits of agreement (LOA) and coefficient of repeatability from Bland Altman analysis between the single observer and the smoothed curve method for slope and perfusion calculations are presented in Table 3. ROI area was not compared since this was identical. There was some evidence of systematic bias in that the after smoothing, the program tended to calculate measurements higher than the human observer in atelectatic lung regions. However, the LOA were narrower than between the two human observers and 95% of the data points were within those LOA. Bland and Altman plots for agreement between a single observer and the smoothed curve method for TAC slopes and perfusion calculations from atelectatic lung regions are shown in Figures 7B and 7D.

From the mixed model analysis, the ICC was greater than the interobserver ICC suggestive of superior correlation. Similar to the interobserver variability, the mixed model showed that most variation was due to individual pony differences, or differences at each stage of the experiment. There was little variation attributed between the smoothed curve method and the single observer. These results are presented in Table 1.

As we had to remove the effect of gas loss from the TAC slopes of aerated lung regions imaged at peak inspiration, measurements of the slope of this gas loss was compared between the single observer and the smoothed curve method. These results are shown in Table 3. There was evidence of systematic bias, in that the smoothed curve method tended to measure a higher gas loss slope than the single observer and the LOA are wide.

4.1.4 External validation

Comparisons between a previous radioactive microsphere study in excised equine lungs (Dobson et al. 1985), and our perfusion measurements were made. Mean perfusion values compared well between the two studies and results are shown in Table 4.

Figure 7C and 7D - Bland Altman plots for atelectatic lung regions: (C) interobserver perfusion calculations; (D) Matlab processed data (smoothed curve method) vs single observer perfusion calculations. The mean of paired measurements from each observer was used and then compared (n=54), and data is presented as percentage differences. The bold solid line represents the mean difference (bias), with dot-dashed lines the 95% confidence interval of the mean difference, the dashed lines the upper and lower 95% limits of agreement with solid lines the 95% confidence intervals of the limits of agreement.

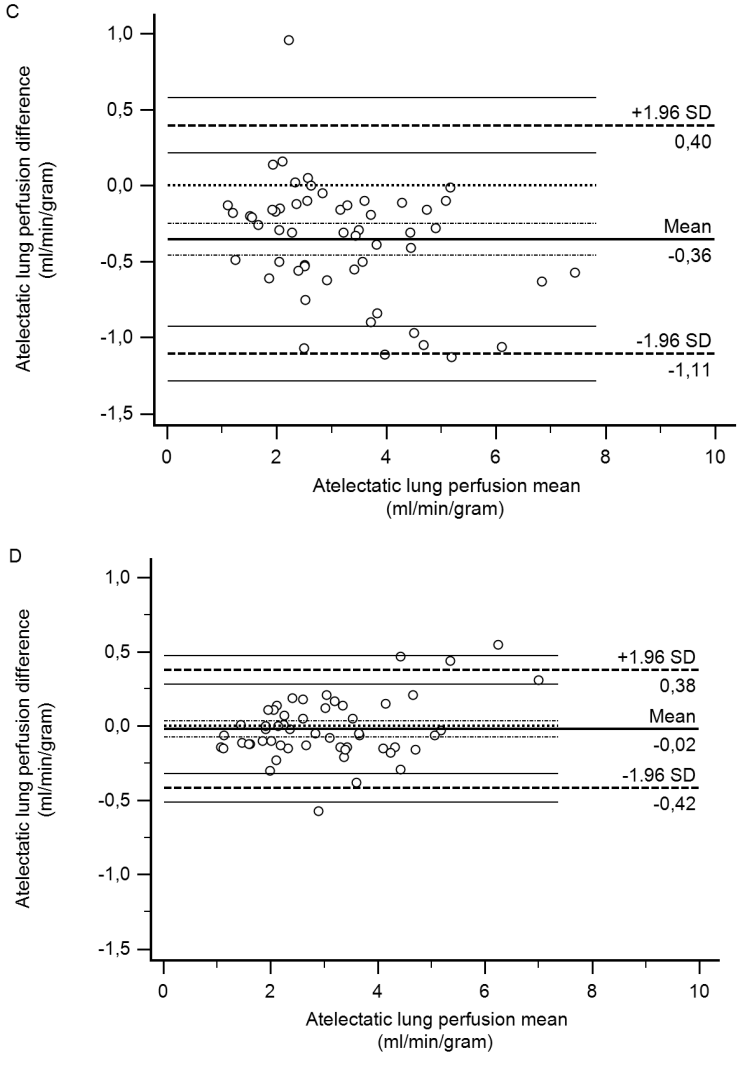


Table 4 - Distribution of pulmonary perfusion in aerated and atelectatic lung regions according to the microsphere technique in anaesthetised horses (Dobson et al. 1985), and the maximum slope model (MSM) in anaesthetised ponies, during spontaneous breathing (SB), and mechanical ventilation (MV)
Insp = perfusion measured at end inspiration; Exp = perfusion measured at end expiration. Results are presented as mean value ± SD.

	Distribution of perfusion according to microsphere technique (ml/min/gram)		**Distribution of perfusion according to MSM (ml/min/gram)**	
	Aerated lung	Atelectatic lung	Aerated lung	Atelectatic lung
SB	2.3 +/- 1.0	3.9 +/-0.4	4.0 ± 1.9	5.0 ±1.2
MV	1.8 +/- 1.1	3.9 +/- 0.6	Insp 4.1 ± 0.5	Insp 2.7 ± 0.6
			Exp 4.6 ± 1.2	Exp 2.7 ± 0.7

4.2 The effect of PiNO on the distribution of pulmonary perfusion during spontaneous breathing and mechanical ventilation (study III)

Only changes in perfusion in atelectatic (+100 to -100 HU) and well ventilated (-350 to -1000 HU) lung are presented. Perfusion to poorly ventilated lung regions (-100 to -350 HU) did not change, and was not included in further analysis.

During spontaneous breathing (SB), perfusion results from CT images acquired at end expiration are presented. During mechanical ventilation (MV) perfusion results from images acquired at end expiration and peak inspiration are presented (Table 5).

Table 5 - Mean value ± SD of perfusion in dorsally recumbent anaesthetised ponies, (Baseline, 30 minutes of PiNO (PiNO *t30*), after termination of PiNO (Post *t30*)) in aerated and atelectatic lung regions during spontaneous breathing (SB) or mechanical ventilation (MV). The effect of interventions during anaesthesia (Baseline, PiNO *t30*, Post *t30*), ventilation mode, and the interaction between these were compared using a mixed linear model. Post-hoc comparisons were adjusted for multiplicity using Tukey's method.
insp = perfusion measured at end inspiration; exp = perfusion measured at end expiration.
*, ** Significant effect of the interventions during anesthesia ($p<0.05$, $p<0.01$, respectively)
†, †† Significantly different from Baseline (($p<0.05$, $p<0.01$, respectively)
§ Significantly different from PiNO ($p<0.05$)
*** MV significantly different from SB ($p<0.001$)

	Distribution of Perfusion (ml blood/min/gram tissue)		
Aerated lung **	**Baseline**	**PiNO *t30*** ††	**Post *t30*** **§**
SB exp	4.0 ± 1.9	7.2 ± 3.0	4.9 ± 1.6
MV exp	4.6 ± 1.2	7.5 ± 3.1	5.2 ± 1.7
MV insp	4.1 ± 0.5	5.2 ± 0.6	4.5 ± 0.9
Atelectatic lung *	**Baseline**	**PiNO *t30*** †	**Post *t30***
SB exp	5.0 ± 1.2	3.6 ± 1.3	4.0 ± 0.5
MV exp ***	2.7 ± 0.7	2.2 ± 0.9	2.3 ± 1.1
MV insp ***	2.7 ± 0.6	2.3 ± 1.2	2.5 ± 1.0

The mixed linear modelling showed that perfusion was affected by both ventilation mode and PiNO. During both SB and MV, perfusion to aerated lung regions increased during PiNO administration. Conversely, perfusion to atelectatic lung regions decreased when PiNO was administered during both ventilation modes. However, this effect was significantly greater during SB compared with MV (Figure 8). When we considered changes in $\dot{Q}t$, the increase in perfusion to aerated lung regions and decrease in perfusion to atelectatic lung regions during PiNO administration were still significant. During SB, there was a greater decrease in perfusion to atelectatic lung compared with MV (Figure 9).

Figure 8 - Changes in perfusion (ml blood/min/gram tissue) in aerated and atelectatic lung regions during spontaneous breathing (SB), and mechanical ventilation at baseline, following 30 minutes of PiNO (PiNO *t30*) and 30 minutes after termination of PiNO administration (Post *t30*). The effect of PiNO, ventilation mode and the interaction between these were compared using a mixed linear model. Post-hoc comparisons were adjusted for multiplicity using Tukey's method.
Data are presented as mean values.
*** Significantly different from SB ($p<0.001$)
†, †† Significantly different from Baseline (($p<0.05$, $p<0.01$, respectively)
§ Significantly different from PiNO *t30* ($p<0.05$)

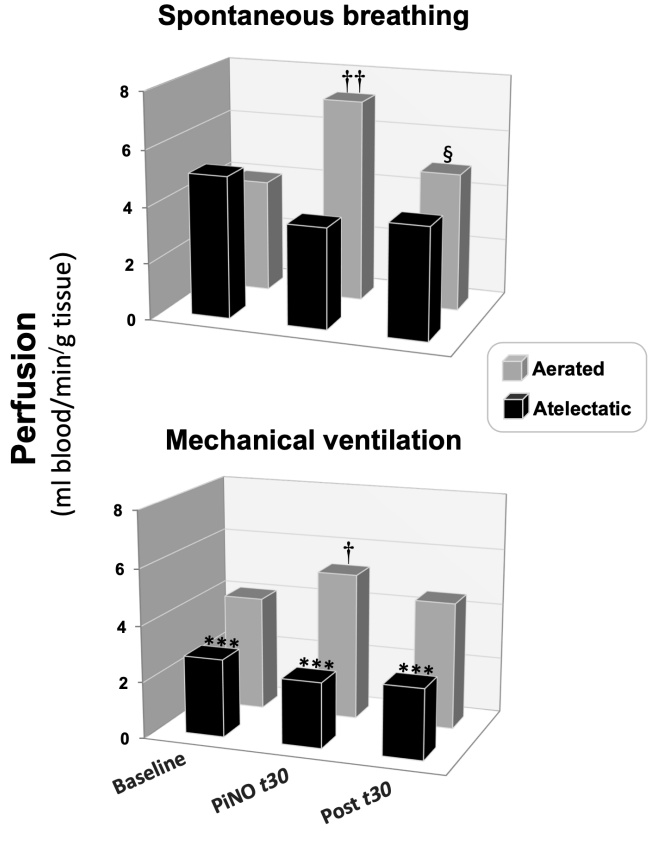
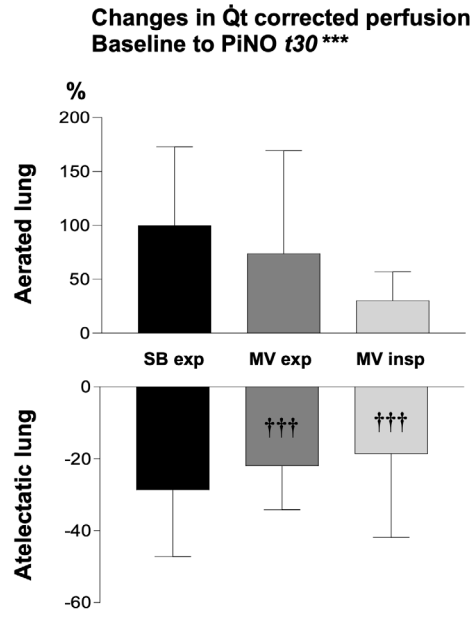


Figure 9 - Change (%) in Q̇t corrected perfusion in aerated and atelectatic lung regions, between baseline and following 30 minutes of PiNO (BL-PiNO *t30*), during spontaneous breathing (SB) ,or mechanical ventilation (MV).
insp = perfusion measured at end inspiration; exp = perfusion measured at end expiration. Results are presented as mean (± SD).
*** Significantly different from baseline to PiNO *t30* ($p < 0.001$)
††† Significantly different from SB ($p<0.001$)



4.3 The effect of PiNO on physiological variables during spontaneous breathing and mechanical ventilation (study III)

PaO_2 was higher and $P(A\text{-}a)O_2$ was lower during PiNO administration compared to baseline during both SB and MV. $P\bar{v}O_2$ was higher during PiNO administration but lower during MV compared with SB. SaO_2 and CaO_2 were both higher during MV compared with SB at all time points Q̇s/Q̇t was significantly lower during MV compared with SB and lower during PiNO administration in both ventilation modes.

MPAP and PVR decreased when PiNO was administered compared to baseline during both SB and MV. These results are presented in Table 6.
Other results not included in Table 6 are as follows: There were no significant differences in MAP, CI or lactate concentrations and no significant differences in dobutamine requirement between the ponies or between the interventions within the experiment. HR and [Hb] were higher during MV compared with SB at all time points. FIO_2 was lower during PiNO administration during both SB and MV. End tidal isoflurane concentration was not different between ventilation modes or during PiNO administration. V_E, *f*R, PIP and pH were higher during MV compared with SB. $PaCO_2$ was lower during MV compared with SB and there was an interaction over time between the ventilation modes. Estimated alveolar dead space was lower during PiNO administration.

Table 6 - Mean value ± SD at each measured time point during anesthesia (Baseline, 30 minutes of PiNO (PiNO *t30*), 30 minutes after termination of PiNO (Post *t30*)). The effect of PiNO, ventilation mode and the interaction between these were compared using a mixed linear model. Post-hoc comparisons were adjusted for multiplicity using Tukey's method
*, ** significant difference between treatments over time (interaction) ($p<0.05$, $p<0.001$, respectively)
†, †† Significantly different from SB ($p<0.05$, $p<0.001$, respectively).

Parameter	Ventilation	Baseline	PiNO *t30*	Post *t30*
PaO_2 ** kPa	SB	17.1 ± 6.4	30.1 ± 15.2	24.8 ± 10.1
	MV††	35.5 ± 12.5	46.0 ± 11.5	38.3 ± 7.3
$P(A\text{-}a)O_2$ ** kPa	SB	52.8 ± 7.5	36.5 ± 15.1	43.5 ± 10.7
	MV††	36.5 ± 12.0	25.6 ± 10.9	34.1 ± 5.3
$P\bar{v}O_2$ * kPa	SB	7.0 ± 1.0	8.0 ± 1.1	7.9 ± 1.0
	MV†	6.9 ± 0.8	7.3 ± 1.2	7.1 ± 1.0
SaO_2 %	SB	93.8 ± 2.9	95.9 ± 1.1	95.3 ± 1.5
	MV††	96.4 ± 0.6	96.8 ± 0.3	96.7 ± 0.1
CaO_2 mL/L	SB	117 ± 16	131 ± 14	135 ± 18
	MV††	146 ± 25	150 ± 30	150 ± 33
$\dot{Q}s/\dot{Q}t$ ** %	SB	49 ±9	37 ±10	43 ±8
	MV ††	34 ±7	29 ±9	35 ±6
PAP * mmHg	SB	20 ±6	16 ±4	18 ±7
	MV	18 ±5	16 ±4	20 ±8
PVR * dynes/sec/cm^{-5}	SB	19.7 ±5.9	14.9 ±4.0	17.6 ±6.8
	MV	17.1 ±4.9	15.1 ±4.1	16.6 ±3.6

4.4 Effects of ventilation mode and blood flow on physiological variables during PiNO administration (study IV)

4.4.1 General results

Age and body weight did not differ between horses within the 4 groups (study IV)

4.4.2 Physiological results (Table 7, Figures 10 and 11)

When horses had physiologically normal MAP (> 70 mmHg) (groups SB-N and MV-N), HR, MAP and CI were higher compared with horses with low MAP (groups SB-L and MV-L). When PiNO was administered, PaO_2, SaO_2 and CaO_2 were higher in all groups except in group MV-L, and $P(A\text{-}a)O_2$ was lower in all groups except MV-L. PaO_2, SaO_2 and CaO_2 were significantly lower at all time points in MV-L compared with MV-N, but there was no difference between the SB groups. SaO_2 was significantly lower at all time points in MV-L compared with SB-L. PaO_2 changed over time in SB-L, SB-N and MV-N but not in MV-L. DO_2 increased during PiNO in all groups except MV-L, and was significantly higher at all time points in SB-N and MV-N than for SB-L and MV-L respectively. $S\bar{v}O_2$ was significantly lower and OER significantly higher at all time points in MV-L compared with MV-N, with no other differences between groups. $\dot{Q}s/\dot{Q}t$ was significantly lower in all groups when PiNO was administered but did not differ between the groups. These results are presented in Table 7 and in Figures 10 and 11.

Other results not included in Table 7 are as follows: MAP in SB-L was significantly higher than MV-L at 15 and 30 minutes. CI was not different between SB-N and MV-N or between SB-L and MV-L. MPAP did not change between groups. Minute ventilation and fR were higher in the MV groups compared with SB but no differences between MV-L and MV-N or between SB-L and SB-N and no differences over time in any of the groups. $PaCO_2$ was lower at 15 and 30 min in the MV groups compared with SB groups, but again, no difference between MV-L and MV-N or between SB-L and SB-N. In the SB-L group the $PaCO_2$ was higher at 15 and 30 minutes compared with baseline. The Vd/Vt was higher in MV-L compared with MV-N at 15 and 30 minutes. Other less important changes were that [Hb] was higher in SB-N and MV-N compared with SB.-L and MV-L; lactate concentrations were higher in MV-L compared with MV-N.

Table 7 - Mean value ± SD at each measured time point during anaesthesia (Baseline, PiNO 15 minutes, PiNO 30 minutes).
SB-L (spontaneous breathing and mean arterial pressure (MAP) < 70 mmHg)
SB-N (spontaneous breathing and MAP > 70 mmHg)
MV-L (mechanical ventilation and MAP < 70 mmHg)
MV-N (mechanical ventilation and MAP > 70 mmHg)
The effect over time within each group was compared using Friedman ANOVA with Dunn's Multiple Comparison Test.
***** significant difference from baseline, † significantly different from N within each ventilation mode (SB or MV) at corresponding time point and ‡significant difference from SB-L or SB-N, respectively, $p<0.05$, ns non-significant. See over page for remainder of Table 7.

Parameter	Group	Baseline	PiNO 15 min	PiNO 30 min	*p* value	*n*
HR beats min^{-1}	SB-L	38 ± 7	36 ± 5	35 ± 6†	ns	7
	SB-N	48 ± 15	45 ± 14	42 ± 12	ns	8
	MV-L	35 ± 3†	33 ±3†	32 ± 3*†	0.0017	6
	MV-N	45 ± 10	49 ± 11	50 ± 12	ns	6
MAP mmHg	SB-L	53 ± 10†	66 ± 7†	68 ± 6*†	0.0036	7
	SB-N	72 ± 5	80 ± 7	80 ± 8	0.0179	8
	MV-L	53 ± 9†	55 ± 8†‡	56 ± 9†‡	ns	6
	MV-N	77 ± 11	82 ± 4	81 ± 3	ns	6
CI mL kg^{-1} min^{-1}	SB-L	45 ± 9†	50 ± 10†	50 ± 11†	0.0272	7
	SB-N	84 ± 30	81 ± 23	79 ± 22	ns	8
	MV-L	45 ± 6†	43 ± 7†	46 ± 5†	ns	6
	MV-N	72 ± 10	80 ± 8	81 ± 10	ns	6
PaO_2 kPa	SB-L	13.0 ± 3.8†	25.2 ± 7.7*	26.6 ± 7.9*	0.0003	7
	SB-N	20.4 ± 16.1	31.6 ± 16.3*	33.4 ± 13.0*	0.0003	8
	MV-L	9.2 ± 7.4†	10.1 ± 6.2†‡	10.1 ± 5.4†‡	ns	6
	MV-N	29.0 ± 19.4	39.1 ± 20.2*	40.2 ± 19.0*	0.0017	6
$P(A\text{-}a)O_2$ kPa	SB-L	66.0 ± 5.7	52.1 ± 9.3*	50.3 ± 8.1*	0.0027	7
	SB-N	53.6 ± 17.3	42.5 ± 15.0	40.8 ± 13.3*	0.0375	8
	MV-L	63.2 ± 6.1	62.0 ± 6.5†	62.2 ± 6.1†‡	ns	6
	MV-N	46.6 ± 19.0	35.2 ± 18.6	33.8 ± 17.8*	0.0055	6
SaO_2 %	SB-L	95.7 ± 2.2	98.6 ± 1.3	98.8 ± 1.2*	0.0003	7
	SB-N	94.7 ± 2.7	97.9 ± 1.1*	97.8 ± 1.7*	<0.0001	8
	MV-L	85.0 ± 7.4†‡	89.3 ± 6.2†‡	89.8 ± 7.1†‡	ns	6
	MV-N	95.5 ± 1.2	96.3 ± 0.7	96.7 ± 0.4*	<0.0001	6
CaO_2 mL L^{-1}	SB-L	130.6 ± 6.3	136.9 ± 4.8*	137.5 ± 4.9**	0.0003	7
	SB-N	146.3 ± 24.5	153.2 ± 21.4**	153.5 ± 20.0**	0.0003	8
	MV-L	120.6 ± 14.9†	126.8 ± 15.4†	127.0 ± 16.7†	ns	6
	MV-N	169.4 ± 15.6	176.2 ± 16.3*	178.8 ± 20.9*	0.0017	6
DO_2 mL kg^{-1} min^{-1}	SB-L	6.0 ± 1.2†	6.9 ± 1.5*†	7.0 ± 1.6*†	<0.0001	7
	SB-N	12.8 ± 6.4	12.6 ± 4.9*	12.3 ± 4.3	ns	8
	MV-L	5.5 ± 0.8†	5.2 ± 1.0†	5.6 ± 1.0†	ns	6

	MV-N	12.2 ± 2.8	14.5 ± 2.1	15.1 ± 3.9*	0.0081	6
$P\bar{v}O_2$ kPa	SB-L	5.3 ± 1.0	5.5 ± 0.7	5.7 ± 0.7†	ns	7
	SB-N	6.9 ± 1.6	7.0 ± 1.3	7.2 ± 1.7	ns	8
	MV-L	4.2 ± 0.5†‡	4.2 ± 0.5†‡	4.1 ± 0.5†‡	ns	6
	MV-N	6.8 ± 1.2	7.6 ± 1.0*	7.6 ± 1.4	0.0120	6
$S\bar{v}O_2$ %	SB-L	67 ± 12	73 ± 14*	73 ± 13	0.0036	7
	SB-N	74 ± 10	77 ± 11	78 ± 9	ns	8
	MV-L	60 ± 11†	60 ± 11†	58 ± 10†	ns	6
	MV-N	82 ± 6	86 ± 4	85 ± 5	0.0289	6
Tissue O_2 extraction %	SB-L	31 ± 12	32 ± 8	29 ± 13	ns	6
	SB-N	23 ± 9	24± 11	23 ± 10	ns	6
	MV-L	31 ± 7†	34 ± 7†	36 ± 7†	ns	7
	MV-N	17 ± 5	15 ± 4	16 ± 4	ns	8
$\dot{Q}s/\dot{Q}t$	SB-L	0.35 ± 0.10	0.29 ± 0.12	0.28 ± 0.12*	0.0003	7
	SB-N	0.38 ± 0.10	0.28 ± 0.09*	0.28 ± 0.11	0.0048	8
	MV-L	0.46 ± 0.07	0.37 ± 0.09	0.36 ± 0.09*	0.0001	6
	MV-N	0.39 ± 0.10	0.36 ± 0.10	0.32 ± 0.07*	0.0001	6

Figure 10 - Arterial oxygen partial pressure (PaO_2, kPa, filled diamonds), mean cardiac output ($\dot{Q}t$, L min^{-1}, filled bars), and minute ventilation (V_E, L min^{-1}, open bars), at baseline, and at 15 and 30 minutes of PiNO in four groups of horses:
SB-L (spontaneous breathing and mean arterial pressure (MAP) < 70 mmHg)
SB-N (spontaneous breathing and MAP > 70 mmHg)
MV-L (mechanical ventilation and MAP < 70 mmHg)
MV-N (mechanical ventilation and MAP > 70 mmHg)

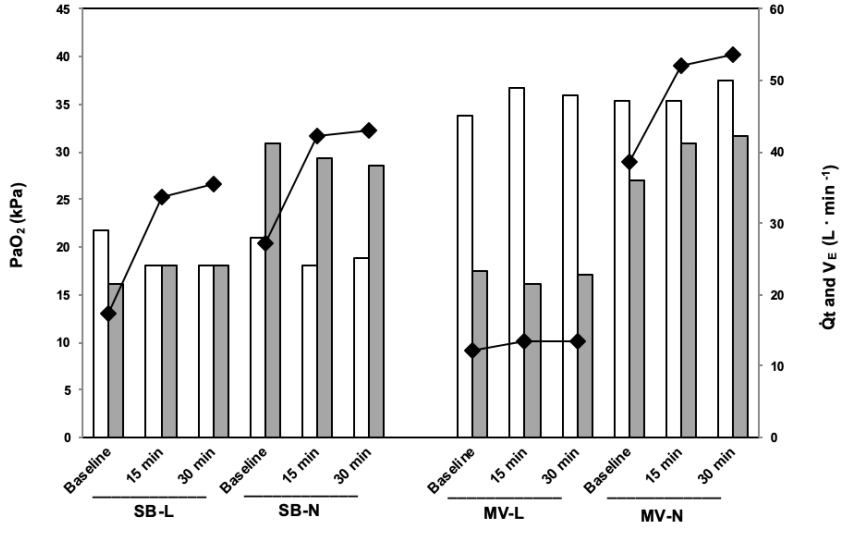
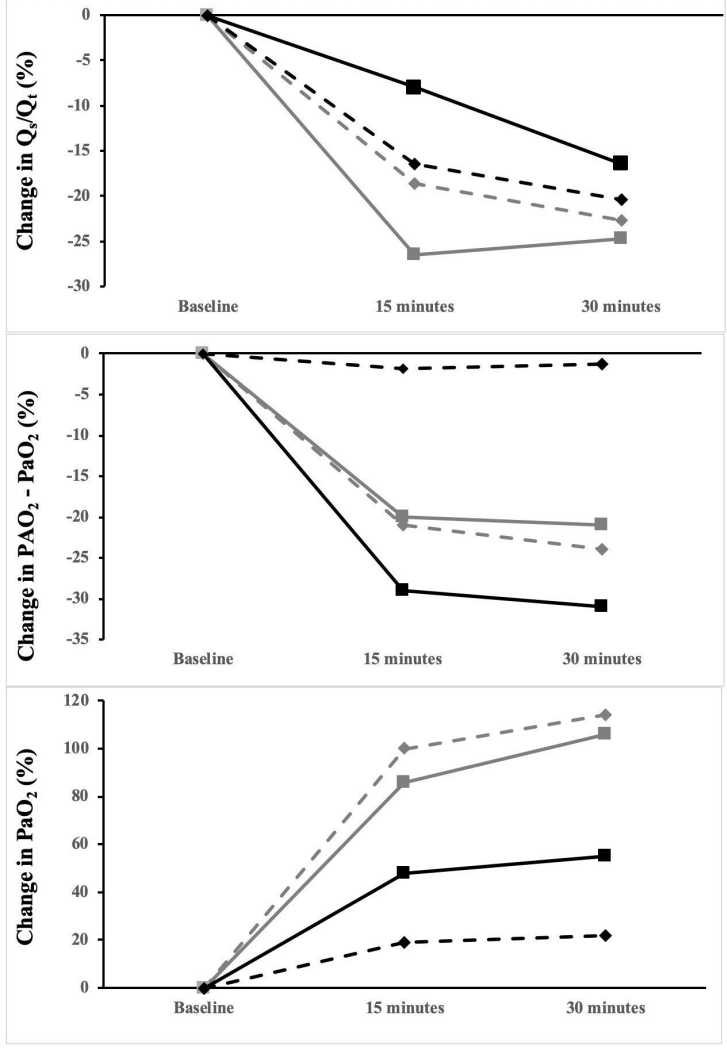


Figure 11 - Percentage (%) change in: (A) venous admixture ($\dot{Q}s/\dot{Q}t$); (B) alveolar-arterial oxygen partial pressure gradient ($P(A\text{-}a)O_2$); (C) arterial oxygen partial pressure PaO_2, over time from baseline, and at 15 and 30 minutes of PiNO
SB-L (spontaneous breathing and mean arterial pressure (MAP) < 70 mmHg) grey diamonds and dashed line
SB-N (spontaneous breathing and MAP > 70 mmHg) grey squares and solid line
MV-L (mechanical ventilation and MAP < 70 mmHg) black diamonds and dashed line
MV-N (mechanical ventilation and MAP > 70 mmHg) black squares and solid line



4.5 Effect of cardiac output and blood pressure on the distribution of pulmonary perfusion during PiNO administration (study V)

4.5.1 Perfusion results (Table 8)

When ponies were hypotensive (low $\dot{Q}t$) and were mechanically ventilated (control experiment), regional pulmonary perfusion did not change significantly during PiNO delivery. When blood pressure was supported during the treatment experiment, perfusion to well-ventilated lung (-1000 to -500 HU) increased significantly. Whilst perfusion to poorly ventilated lung also increased during dobutamine administration, the change did not reach statistical significance.

During the treatment experiment, the six ponies were divided into two further subgroups once PiNO was discontinued. In the first subgroup in which hypotension developed again, perfusion to atelectatic lung (+100 to -100 HU) decreased. In the second subgroup in which normotension was maintained, perfusion to atelectatic lung did not change (Figure 12). These results were not tested statistically due to the small number of ponies within each subgroup.

4.5.2 Physiological results (Tables 9 and 10)

In the control group, no significant changes occurred in any of the physiological data collected over the entire experimental timeline. During dobutamine treatment, there was a significant interaction with PiNO administration: ABP, PAP, $\dot{Q}t$, HR, DO_2 and [Hb] increased and Vd/Vt and $P(A\text{-}a)O_2$ decreased. There were also significant differences between time points T1 and T2: PAP, $\dot{Q}t$, HR, DO_2, $\dot{Q}s/\dot{Q}t$ and [Hb] increased and $P(A\text{-}a)O_2$ decreased. PaO_2 also increased during dobutamine and PiNO treatment, but did not reach statistical significance.

During the treatment experiment, the six ponies were divided into two further subgroups once PiNO was discontinued. In the second subgroup in which dobutamine administration continued and normotension was maintained, $\dot{Q}s/\dot{Q}t$ and Vd/Vt increased compared to the previous measurement. This correlated with a fall in PaO_2. Again, these results were not tested statistically due to the small number of ponies within each subgroup.

Table 8 - Distribution of perfusion in different lung regions, in mechanically ventilated anesthetized ponies, during two different anesthetics. Lung regions were delineated using threshold windows: for atelectatic lung +100 to -100 Hounsfield Units (HU); for poorly ventilated lung -100 to -500 HU; and for normally ventilated lung -500 to -1000 HU.
Control experiment time points: C1 – hypotension and PiNO delivery for 30 minutes; C2 – hypotension and PiNO delivery for 60 minutes; C3 – hypotension and PiNO discontinued for 30 minutes
Treatment experiment time points: T1 – hypotension and PiNO delivery for 30 minutes; T2 – normotension (dobutamine administration) and PiNO delivery for 60 minutes; T3a – hypotension and PiNO discontinued for 45 minutes; T3b – normotension (dobutamine administration) and PiNO discontinued for 45 minutes.
Statistical comparisons were performed for T1 and T2 and C1 and C2. A significant interaction was seen between the distribution of perfusion to lung regions and the effect of dobutamine during concurrent pulsed inhaled nitric oxide, * $p<0.001$. Difference in perfusion compared to the previous measurement, † $p<0.021$.

	Distribution of perfusion (ml blood/min/gram tissue)					
Lung region based on HU	**C1**	**C2**	**C3**	**T1**	**T2 ***	**T3a** ***T3b***
-500 to -1000	3.9 ± 1.2	3.7 ± 0.9	2.9 ± 0.4	4.1 ± 0.8	7.0 ± 3.3 †	3.6 ± 1.3 *6.1 ± 0.4*
-100 to -500	4.5 ± 1.1	4.4 ± 0.9	3.0 ±0.4	4.3 ± 1.0	6.7 ± 2.1	3.3 ± 0.4 *4.7 ± 0.5*
100 to -100	3.8 ± 1.4	2.5 ± 0.9	2.0 ± 0.5	1.7 ± 1.4	2.7 ± 0.7	1.5 ± 0.3 *2.7 ± 0.2*

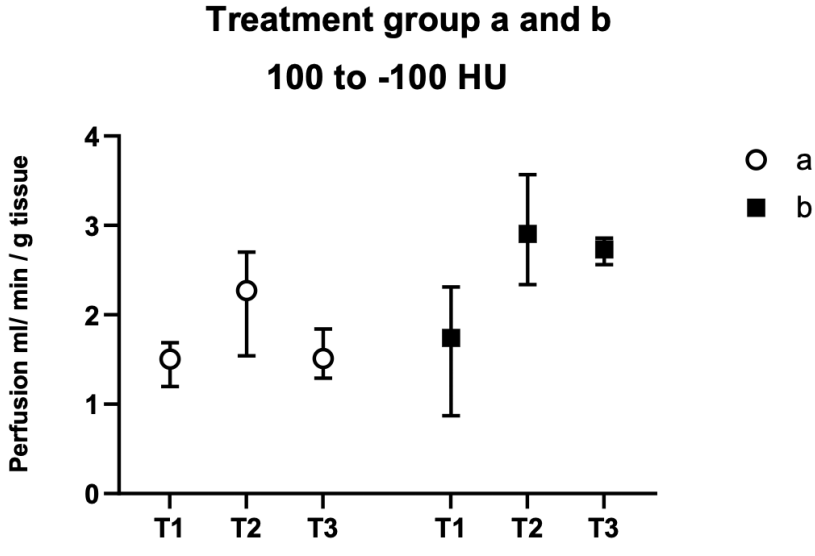
Table 9 - Physiological parameters during the control experiment, in mechanically ventilated anesthetized ponies.
Control experiment time points: C1 – hypotension and PiNO delivery for 30 minutes; C2 – hypotension and PiNO delivery for 60 minutes; C3 – hypotension and PiNO discontinued for 30 minutes

Anaesthesia 1 – Control Group			
	C1 n = 5	**C2 n = 5**	**C3 n = 5**
ABP mmHg	56 ± 6	54 ± 3	54 ± 1
PAP mmHg	15.6 ± 3.5	15.6 ± 2.5	14.7 ± 1.5
$\dot{Q}t$ L/min	10.5 ± 3.3	8.9 ± 0.7	8.2 ± 0.9
HR beats/min	42 ± 6	40 ± 4	39 ± 2.1
V_E L/min	20.3 ± 1.6	20.3 ± 4.1	19.8 ± 2.0
$PaCO_2$ kPa	7.1 ± 1.3	7.2 ± 1.5	7.8 ± 1.8
V_D/V_T %	19 ± 3	22 ± 4	25 ± 6
$\dot{Q}s/\dot{Q}t$ %	28 ± 4	28 ± 4	37 ± 5
$P(A\text{-}a)O_2$ kPa	36.0 ± 8.0	38.0 ± 8.8	44.8 ± 14.8
PaO_2 kPa	40.0 ± 10.1	39.1 ± 10.1	36.6 ± 17.0
SaO_2 %	96.5 ± 0.4	96.5 ± 0.4	96.1 ± 1.0
DO_2 L/min	1.09 ± 0.28	0.96 ± 0.20	0.85 ± 0.20
[Hb] g/L	74.0 ± 14.1	75.0 ± 16.7	73.0 ± 14.1

Table 10 - Physiological parameters during the treatment experiment, in mechanically ventilated anesthetized ponies.
Time points: T1 – hypotension and PiNO delivery for 30 minutes; T2 – normotension (dobutamine administration) and PiNO delivery for 60 minutes; T3a – hypotension and PiNO discontinued for 45 minutes; T3b – normotension (dobutamine administration) and PiNO discontinued for 45 minutes.
Statistical comparisons were performed for T1 and T2. A significant interaction was seen between the effect of dobutamine and concurrent pulsed inhaled nitric oxide, * $p<0.001$. Significantly different compared to the previous measurement, ‡ $p<0.05$.

Anaesthesia 2 – Treatment Group			
	T1 n = 6	**T2 n = 6**	**T3a n = 3** ***T3b n = 3***
ABP * **mmHg**	54 ± 2	76 ± 2	46.7 ± 1.5 *72.7 ± 1.5*
PAP * **mmHg**	8.2 ± 2.8	13.7 ± 3.9 ‡	9.3 ± 4.2 *18.7 ± 0.6*
$\dot{Q}t$ * **L/min**	9.1 ± 2.2	17.2 ± 4.5 ‡	7.7 ± 0.8 *20.4 ± 0.6*
HR * **beats/min**	41 ± 4	53 ± 9 ‡	39 ± 6 *68 ± 15*
V_E **L/min**	19.0 ± 4.3	19.1 ± 4.1	16.4 ± 2.2 *22.1 ± 3.9*
$PaCO_2$ **kPa**	7.4 ± 1.1	7.8 ± 1.0	6.7 ± 1.5 *8.7 ± 0.5*
V_D/V_T * **%**	27 ± 4	25 ± 2	27 ± 1 *33 ± 3*
$\dot{Q}s/\dot{Q}t$ **%**	26 ± 7	33 ± 6 ‡	25 ± 9 *43 ± 4*
$P(A\text{-}a)O_2$ **kPa**	35.7 ± 9.7	21.2 ± 5.8 ‡	30.1 ± 19.3 *32.9 ± 9.6*
PaO_2 **kPa**	38.9 ± 10.2	52.9 ± 5.6	47.6 ± 21.6 *41.2 ± 0.9*
SaO_2 **%**	96.7 ± 0.3	96.8 ± 0.2	96.7 ± 0.3 *96.6 ± 0.0*
DO_2 * **L/min**	0.93 ± 0.21	2.8 ± 0.9 ‡	0.91 ± 0.17 *3.2 ± 0.17*
[Hb] * **g/L**	81.3 ± 8.4	114.5 ± 8.4 ‡	81.0 ± 5.3 *112.3 ± 7.2*

Figure 12 - Changes in perfusion (ml blood/min/gram tissue) in the atelectatic lung region (+100 to -100 HU) in mechanically ventilated, anaesthetised ponies:
T1 - 30 minutes of PiNO during hypotension (MAP < 70 mmHg).
T2 – 60 minutes of PiNO following treatment with dobutamine to achieve normotension (MAP 70-80 mmHg).
T3a – PiNO and dobutamine discontinued for 45 minutes (hypotensive).
T3b – PiNO discontinued for 45 minutes with continued dobutamine treatment (normotensive).
Data are presented as mean perfusion vales with range.



5. Discussion

5.1 CT angiography and the maximum slope model methodology

In the research presented in this thesis, the aim was to measure the distribution of pulmonary perfusion in anaesthetised ponies, using CT angiography and the maximum slope model (MSM). Furthermore, by using an imaging technique that provides good spatial resolution, it was investigated how the distribution of pulmonary perfusion changes when PiNO is delivered to the lung, to try and show why indices of oxygenation consistently and dramatically improve. As hypoventilation and hypotension are frequently encountered in conjunction with hypoxaemia during general anaesthesia in horses, it was also important to compare how PiNO affects perfusion within the lung during spontaneous breathing and mechanical ventilation, and also to compare the response during periods of hypotension with normotension. The information contained within this thesis, adds to existing evidence that PiNO is suitable for clinical use, but that certain conditions must be met in order to achieve maximum efficacy of treatment.

5.1.1 Variability in perfusion measurements

The results from study II show that the distribution of perfusion within the lung can be measured using CTA and application of a simple formula – the MSM. However, as the technique involves the manual drawing of ROIs within CT images, the method is subject to some variability. A period of training and instruction improves between-observer variation when measuring perfusion within the brain, and in solid lung tumours (Sanelli et al. 2007; Sauter et al. 2012). Compared with these latter two studies, there was greater interobserver variability in our measurements of perfusion. However, measurement of perfusion of normal lung is inherently more difficult, since it is full of gas, and is also subject to motion

artefact despite best efforts to prevent this. This explains why the variation in our study was greater than in the Sanelli et al. (2007) and Sauter et al. (2012) studies. Furthermore, automated software was used in both these latter studies, making comparisons difficult. Whilst automated software would be advantageous for measuring perfusion of the equine lung, currently there is no such software available for this particular application.

As manual ROI drawing introduces subjectivity, a threshold function within the imaging software was used. The HU thresholds chosen were based upon earlier work (Gattinoni et al. 1988; Vieira et al. 1998). By using HU thresholds, well aerated and atelectatic regions of the lung were better delineated, reducing some of the potential variation. Additionally, a mixed model analytical approach was applied, to identify where the source of variation was. The majority of variation was due to differences between individual ponies, and differences at each stage of the experiment, which are difficult to control. Very little of the variation could be attributed to using two different observers. However, as intraobserver variation was less, it is recommended that a single observer is used to delineate ROIs in future studies.

The process of ROI drawing and TAC interpretation is time-consuming, laborious, and subject to error if done manually. As the MSM CTA methodology will be used in future studies, designing software to speed up the process was desirable. Moreover, as cardiac pulsation interfered with manual measurement of the maximum slope, using software to smooth the slope without introducing error was also necessary. The specially coded Matlab software that was written for study II, sped up the analysis of images considerably. Whilst this was advantageous, it was necessary for the results from this smoothed curve method to be tested for variability, when compared to manually measured unsmoothed slopes. Although there is some variation, it is less than between 2 human observers and is suitable for use in the future.

5.1.2 External validation

In studies II, III and V, the change in distribution of perfusion was measured at different time points within the experiment. Generally, a microsphere method is considered as the gold standard to measure regional perfusion of the lung, as trapping of microspheres correlates well to regional flow of red blood cells (Beck 1987). The microsphere technique necessitates euthanasia of the test subject, and excision and sectioning of lung, and so contemporaneous measurements of perfusion using microspheres was not possible in our studies. Ensuring that a technique accurately measures what it purports to measure is challenging (Hopkins et al. 2007). Numerous alternative methods of validation have been used, including

cross-validation between studies (Hopkins 2007). In study II, perfusion in excised equine lung from a previously reported microsphere study (Dobson et al. 1985), were compared with our results. Both the microsphere technique and the MSM quantify perfusion in ml min^{-1} gram of $tissue^{-1}$, and are therefore essentially measuring the same thing, but by a different method. This is important when using cross-validation to validate a new technique. Perfusion of atelectatic lung in our study compared well with outer dorsal lung in the Dobson et al. (1985) study. For aerated lung regions there was a little more variability in the compared measurements. This is not surprising since, in these present studies, perfusion within the entire aerated lung region was measured, whereas the microsphere study measured perfusion in only 3 distinct sections of lung. Additionally, Dobson et al. (1985) used larger horses than we were able to study using CTA, and this may also introduce some variation in measurements.

There is also the question of 'do the results make physiological sense'? For example, whilst the MIGET is considered to be a gold standard to measure $\dot{V}/\dot{Q}$ heterogeneity, it has never been directly validated. Over time, it has been gradually accepted, as results from MIGET studies make physiological sense (Hopkins et al. 2007). In study III, the changes in the distribution of pulmonary perfusion make sense, when interpreted in the context of corresponding changes in physiological variables such as oxygenation indices. As perfusion to aerated lung increases and to atelectatic lung decreases, PaO_2 increases and this is the response one would expect from PiNO delivery. The changes in pulmonary perfusion distribution in response to PiNO, also agree with a scintigraphic method used in anaesthetised horses (Grubb et al. 2014).

5.1.3 Sources of error and limitations

In images acquired at peak inspiration, a slow leakage or absorption of gas from the lung over time was documented. This was evident when scrolling through image stacks. This slow deflation of the lung resulted in an increase in CT attenuation. This was evident on the TACs from aerated lung regions, as a second rising slope, independent of the maximum slope, and was not possible to rectify. Since the breathhold was maintained for 50 seconds during scanning, it is likely that this was indeed absorption of gas from the lung. To account for this, the magnitude of this slow increase in HU was calculated by measuring the second slope. The slope of this gas loss was then removed from the maximum slope. As two slopes were now being measured and the slope of the gas loss was somewhat subjective to measure, it is possible that this introduced error. The gas loss slope was also measured by the Matlab software and agreement between this and the manually measured gas loss slope was assessed. Again, as with comparisons

between the maximum slopes measured, there was some variation between the methods but this was not great enough to adversely affect the final results. It is clear though, in future studies, one technique should be used to measure the TAC slopes.

Motion artefact, and in particular respiratory motion, during image acquisition is a major problem in CT imaging of the lung (Kambadakone & Sahani 2009). As the subjects used in these experiments were animals, it was necessary to find a way to arrest breathing for the 50 second scan time. Peripheral muscle relaxants can be administered in order to paralyse the respiratory muscles for a period of time, and this was considered. However, succinylcholine administered to anaesthetised humans, increased lung density area observed with CT, compared with density prior to administration (Tokics et al. 1987). In this latter study, they speculated that altered diaphragmatic function may have been the cause. Moreover, in the studies described here, it was necessary that ponies were mechanically ventilated and breathed spontaneously within the same experiment. As a consequence of these considerations, it was decided not to use muscle relaxants to arrest breathing. To overcome the problem, a simple plumbing ball valve, or stopcock, was adapted for use within the airway. This device was inserted between the endotracheal tube and the breathing system. By turning the tap on the valve, breathing could be arrested at end inspiration during mechanical ventilation and prevented the pony taking a breath at end expiration during spontaneous breathing. This was 100% repeatable throughout studies II, III and V. However, respiratory hold manoeuvres can affect pulmonary perfusion distribution: perfusion measured by MRI was lower during an inspiratory hold, than an expiratory hold, presumably due to the effects on flow characteristics following lung inflation (Fink et al. 2005). Whilst this will introduce an error into the method, by performing the breath hold at peak or end inspiration every time, the effect at each time point will be similar. As the maximum slope of the TAC appears relatively quickly following contrast injection – within the first 10 seconds – the risk of the pony attempting to take another breath, with a consequent effect of negative pressure on perfusion, was minimised. For spontaneously breathing ponies, the natural respiratory frequency was generally quite slow during the experiments, again, minimising the risk of an attempted breath interfering with measurements. For ponies that were being mechanically ventilated, a spontaneous breath during image acquisition was again unlikely, partly due to the fact that $PaCO_2$ was more controlled (i.e. the stimulus for breathing was removed).

It was necessary to test our images to ensure that contrast did not accumulate during the course of each experiment; and that the contrast bolus remained within the ROI undergoing analysis. Accumulation would result in overestimation of perfusion (Miles 2003). By assessing baseline HU over time (i.e.

HU before the injection of contrast during the 4 second delay at each imaging time point), it was possible to rule out this potential error.

Injection characteristics of the contrast bolus are extremely important to consider when using the MSM (Hallett & Fleischmann 2006). Peak arterial contrast concentration must occur prior to peak tissue enhancement (Miles 2003) necessitating a short and sharp injection. We began with published recommendations for adult humans (American College of Radiology Committee Manual on Contrast Media v2020). Initially, during pilot studies, tissue enhancement was barely sufficient due to a combination of potential problems: the rate of injection; the injection site; the volume of the bolus; the concentration of iodine within the solution; and the viscosity of the contrast agent. The mechanical injector limited the rate of injection of contrast agent, and the pressure limit programmed into the device was reached. The final refinement used a larger volume (60 ml vs 40 ml), a faster rate of injection (10 ml sec^{-1} vs 8 ml sec^{-1}) and the solution was heated prior to injection to approximately 37°C. Numerous investigators have demonstrated that extrinsic warming of iodinated contrast agents improves flow kinetics (reduction in injection pressure and increased speed of injection) (Hughes & Bissett 1991; Roth et al. 1991; Schwab et al. 2009). Extrinsic warming enabled a faster injection rate to be attained, facilitating a faster time to peak enhancement. Furthermore, a faster injection speed was achieved by removing a spiral extension set, and using alternative injection tubing, which was wider and shorter. This injection was made directly into the right atrium through the pigtail catheter.

5.1.4 Harm from iodinated contrast agents

There are specific risks associated with the injection (and particularly repeated injection) of iodinated contrast media. There is some debate whether or not true allergic reactions to contrast agents occur, and it is more likely that reactions are due to direct release of histamine. The risk of these reactions occurring is low, and may be independent of dose and concentration above a certain threshold (American College of Radiology Committee Manual on Contrast Media v2020). In the ponies used in this study, demonstrable evidence of allergic or allergic-like reactions, immediately or delayed, was not observed. The safety and efficacy of central venous contrast media injection has been confirmed in humans (Herts et al. 2001). Furthermore, there was no evidence of extravasation of contrast agent; presumably this risk was negated due to the length and position of the administration catheter in the right atrium. Post-contrast acute kidney injury (PC-AKI) due to contrast-induced nephropathy (CIN), has been investigated in

humans. However, clear guidelines have not been established due to a paucity of suitable studies. There appear to be multiple risk factors associated with CIN – particularly the presence of a pre-existing renal insufficiency (Goldfarb et al. 2009; American College of Radiology Committee Manual on Contrast Media v2020). The ponies used in this study did not demonstrate any clinical signs, or changes in serum biochemistry, associated with a pre-existing renal condition. One risk factor for the development of CIN is multiple iodinated contrast medium doses in a short (< 24 hours) time interval (American College of Radiology Committee Manual on Contrast Media v2020), to which the ponies in this study were exposed. However, this risk has not been robustly confirmed. In some of the experiments in this study, especially in those where parts were repeated due to methodological problems inherent in any new technique, large amounts of contrast were administered to some individual ponies (up to 1 g kg^{-1}). Intravenous fluid therapy was provided throughout each anaesthetic period and blood pressure was supported where necessary. Despite the potential risk, no problems were encountered with any of the ponies.

5.2 Distribution of pulmonary perfusion during PiNO delivery in spontaneously breathing or mechanically ventilated ponies

Using the CTA method and the MSM described above, it was possible to measure how pulmonary perfusion changes during PiNO delivery. In earlier work, it was postulated that by delivering iNO as a pulse in the first part of inspiration, rather than continuously, this would target well ventilated regions of lung (Heinonen et al. 2002). As NO diffuses rapidly into its target cell (i.e. vascular smooth muscle) (Bruckdorfer 2005), the local vasodilatory effects cause a redistribution of blood from dependent lung, to those regions of lung where the PiNO is being delivered (Grubb et al. 2014). This improves $\dot{V}/\dot{Q}$ matching and consequently improves oxygenation indices. This was confirmed using the MIGET and scintigraphy in spontaneously breathing horses, but lacked the resolution required to definitively confirm the effect of PiNO (Grubb et al. 2014). Using our CTA methodology, we can confirm that PiNO increases perfusion to non-dependent, well-ventilated regions of lung by diverting blood away from dependent, atelectatic lung.

As mechanical ventilation is used frequently in anaesthetised horses to manage hypoventilation, it was essential that the effect of PiNO on the distribution of pulmonary perfusion during different modes of ventilation was investigated. In study III, oxygenation was better during MV, even before PiNO was delivered and this agrees with earlier work (Edner et al. 2005). It is likely that functional residual

capacity was improved during MV with a consequent improvement in gas exchange. However, despite different starting points in terms of oxygenation during SB and MV, PiNO administration still led to a similar redistribution of perfusion from dependent to non-dependent lung regions. The effect appeared to more pronounced during SB, possibly augmented by normal negative intrathoracic pressure.

5.3 Physiological effects of PiNO in spontaneously breathing or mechanically ventilated ponies

In addition to documenting the effects of PiNO on the distribution of pulmonary perfusion, the physiological effects of PiNO on the cardiovascular and respiratory systems during both modes of ventilation was also assessed. By changing perfusion distribution with PiNO as described above, indices of oxygenation consistently improved. Conventional treatment strategies to manage $\dot{V}/\dot{Q}$ mismatch involve the use of ventilatory techniques, and positive intrathoracic pressure during MV may squeeze blood downwards into atelectatic lung regions, effectively worsening $\dot{Q}s/\dot{Q}t$ (Cairo 2012). By causing local vasodilation, PiNO may be able to counteract this effect and we did not find that $\dot{Q}s/\dot{Q}t$ was worse during MV. In fact, $\dot{Q}s/\dot{Q}t$ improved significantly during PiNO delivery in both ventilation modes.

The effect of PiNO on pulmonary vascular resistance (PVR) has not been investigated to date. The results from these studies demonstrated that PVR did decrease, but was of a magnitude that was not clinically significant, and suggests a local effect. Furthermore, there was no effect on systemic blood pressure, strengthening the assumption that PiNO has a local selective action on the pulmonary circulation. However, as cardiac output was supported with dobutamine, this may have masked the vasodilatory effect of PiNO, but there was no increased requirement for dobutamine during PiNO delivery. This is important to highlight, since horses commonly develop systemic hypotension during general anaesthesia, and using a therapy that exacerbates this would be undesirable.

Pulmonary perfusion is directly correlated to $\dot{Q}t$, since all blood leaving the right ventricle must pass through the lung. Cardiac output differed between ponies and over time and so perfusion measurements were corrected for those differences, to investigate if this changed our results. After correcting for $\dot{Q}t$, PiNO was more effective during SB in that aerated lung regions were better perfused. Therefore, MV may indeed have a negative effect on the distribution of perfusion in the lung; this superior effect of PiNO during SB has been shown in previous work (Auckburally et al. 2019). However, PaO_2 values during MV were significantly

higher at baseline in study III and so the effect of PiNO may appear somewhat diminished as a result.

One limitation in interpreting the physiological effects of PiNO in study III, was that no pony was hypoxaemic from the start. This may reflect the size of ponies that were investigated, since they had to be small enough to fit into the CT gantry. It is known that the magnitude of blood gas derangements is related to thoracic dimension (Mansell & Clutton 2008). However, the aim of study III was to investigate how pulmonary perfusion was redistributed during PiNO delivery during SB and MV, regardless of the baseline PaO_2.

5.4 The effect of blood flow on the response to PiNO during 2 modes of ventilation

An unexpected finding during the experiments presented in this thesis, was that PiNO was ineffective if a horse was mechanically ventilated and simultaneously hypotensive (MAP < 70 mmHg). This was a fortunate discovery, since anaesthetised horses are frequently hypotensive and hypoventilate at the same time, necessitating mechanical ventilation. Thus, it became important to document how to optimize the effect of PiNO during mechanical ventilation.

Mechanical ventilation can have deleterious effects on $\dot{V}/\dot{Q}$ matching for a number of reasons. Firstly, $\dot{Q}t$ is reduced as positive intrathoracic pressure reduces cardiac filling pressures (Cournand et al. 1948). Secondly, the positive pressure induced by MV, over-ventilates normally ventilated alveoli. Both factors effectively reduce alveolar perfusion and increase Vd/Vt (Moens 1989; Day et al. 1995). This was shown in study IV, as group MV-L (horses that were MV with low blood pressure), had higher Vd/Vt. Of more importance is that, positive intrathoracic pressure can drive blood towards dependent atelectatic lung, worsening $\dot{Q}s/\dot{Q}t$ as described above (Bindslev 1981; Nyman & Hedenstierna 1989; Cairo 2012). Conventional MV cannot re-inflate compressed atelectatic lung in the same way as a recruitment manoeuvre, and so dependent lung remains collapsed. Further, PVR increases during MV, again, adversely affecting alveolar perfusion (Cairo 2012). These combined effects worsen $\dot{V}/\dot{Q}$ mismatch leading to derangements in gas exchange, and are almost certainly compounded by pre-existing hypotension induced by anaesthesia and recumbency. In the MV horses that had blood pressure supported with dobutamine, PiNO was effective at improving oxygenation, presumably through a combined effect to improve perfusion of aerated lung regions.

In study IV, it was clear that the effect of PiNO was optimal during SB, regardless of whether hypotension was present or not. The favourable respiratory

mechanics of SB are well documented: there is better ventilation in juxtadiaphragmatic regions of lung (Neumann et al. 2005; Wrigge et al. 2005) and alveolar cyclical collapse in dependent lung regions is prevented (Wrigge et al. 2005). Moreover, cardiovascular function is also augmented by negative intrathoracic pressure, and hypercapnia induced increases in systemic vascular resistance (Mosing & Senior 2018). The combined overall effects likely helped to maintain better $\dot{V}/\dot{Q}$ matching in SB horses in study IV. As the effects of PiNO in SB horses was the same, irrespective of blood pressure, it was clear that it was the mode of ventilation that was important, and not overall total blood flow.

As we did not include a dobutamine only group within study IV, it is reasonable to think that in group MV-N, the dobutamine itself may have been responsible for the improvement in oxygenation during MV, rather than it being an effect of PiNO *per se*. Increased pulmonary perfusion in conjunction with increased $\dot{Q}t$ leads to improvements in oxygenation (Kelman et al. 1967). In horses, dobutamine increases $\dot{Q}t$, systemic vascular resistance and PVR (Swanson et al. 1985; Gasthuys et al. 1991; Mizuno et al. 1995; Young et al. 1998; De Vries et al. 2009). It follows then, that dobutamine may lead to improvements in oxygenation. However, in humans it has been shown that dobutamine can also worsen $\dot{V}/\dot{Q}$ mismatch through recruitment of intrapulmonary shunt vessels as $\dot{Q}t$ increases, and an increase in shunt fraction (Molloy et al. 1986; Renotte et al. 1989; Stickland et al. 2006; Bryan et al. 2012). A correlation between increased $\dot{Q}t$ and increased $\dot{Q}s/\dot{Q}t$ has also been shown in other species (Smith et al. 1974; Lynch et al. 1979; Russell & James 2004) with intrapulmonary shunt vessels identified in dogs (Stickland et al. 2007) and cats and rabbits (Prinzmetal et al. 1948). In horses, there are conflicting reports regarding the presence of intrapulmonary shunt vessels (Nyman et al. 1995; Manohar & Goetz 2005; La Gerche et al. 2013). Nevertheless, administration of dobutamine to anaesthetised horses does not improve pulmonary gas exchange, possibly as a consequence of increased blood flow to atelectatic lung or effects on hypoxic pulmonary vasoconstriction (Swanson & Muir 1986). In humans with pre-existing pulmonary hypertension, administering dobutamine alone worsens intrapulmonary shunting, but a combination of dobutamine with iNO enhances pulmonary perfusion (Vizza et al. 2001). From our results the improvement in oxygenation in MV horses supported with dobutamine may have resulted from selective pulmonary vasodilation by NO in conjunction with increased cardiac output, so increasing perfusion in well-ventilated lung.

5.5 Distribution of pulmonary perfusion when PiNO is administered during low and normal blood flow in mechanically ventilated anaesthetised ponies

Following on from study IV, the final study (study V) aimed to measure the effect of PiNO on the distribution of pulmonary perfusion when ponies had low blood pressure (MAP < 70 mmHg) or when they were supported with dobutamine (MAP 70-80 mmHg), using CTA and the MSM. We confirmed our speculative findings from study IV and showed that a combination of dobutamine to support $\dot{Q}t$ and PiNO to promote selective pulmonary vasodilation, promoted better gas exchange during MV.

The reasons for this response, or lack of, to PiNO are multiple. As described above, MV can affect oxygenation in a number of ways. Global $\dot{Q}t$ is reduced through increases in intrathoracic pressure, reducing cardiac filling pressures and impeding venous return (Cournand et al. 1948; Day et al. 1995). This reduction in $\dot{Q}t$ leads to a fall in $S\bar{v}O_2$ as more oxygen becomes extracted at the level of the tissues (Day et al. 1995). Positive intrathoracic pressure can also disturb $\dot{V}/\dot{Q}$ matching by forcing pulmonary blood downwards to dependent lung and increasing $\dot{Q}s/\dot{Q}t$ (Nyman & Hedenstierna 1989; Cairo 2012), over-ventilating normally ventilated lung regions and reducing blood supply to these areas by increasing PVR (Marshall & Grange 1966; Tyberg et al. 2000). This is compounded by a general fall in $\dot{Q}t$ induced by anaesthesia and recumbency, reducing overall lung perfusion and increasing dead space (Tyler 1983). Delivery of PiNO can counteract these deleterious effects of MV on $\dot{V}/\dot{Q}$ matching, but only if blood pressure is adequate. In this study, PiNO was able to elicit a redistribution of pulmonary perfusion to well-ventilated regions of lung during dobutamine infusion, which correlated with a decrease in $P(A\text{-}a)O_2$. In the face of hypotension, the effect of PiNO is lost.

As PVR increases with MV, delivery of PiNO may be able to offset this through its vasodilatory effect. Furthermore, the administration of dobutamine also reduces PVR whilst augmenting $\dot{Q}t$ (Bradford et al. 2000). The additive effects of PiNO and dobutamine on PVR may explain the beneficial effects of PiNO when dobutamine is administered concurrently, and this has been reported in humans with pre-existing pulmonary hypertension (Vizza et al. 2001).

It is plausible that hypotension in itself can disturb pulmonary gas exchange. This has been demonstrated in humans during nitroprusside administration (Wildsmith et al. 1975; Casthely et al. 1982). However, if hypotension is induced with isoflurane, there is little effect on gas exchange (Nicholas & Lam 1984). Therefore, it seems unlikely that the hypotension *per se* was responsible for the poor response to PiNO in this study.

In the treatment experiment in this study, the effect of dobutamine alone when PiNO was discontinued was assessed. This was to determine if dobutamine administration itself, led to improvements in regional pulmonary perfusion, to add to suppositions from study IV. Previously, it has been shown that dobutamine administration does not improve pulmonary gas exchange in anaesthetised horses (Swanson & Muir 1986). In study V, perfusion to dependent lung regions increased during dobutamine administration, and remained at this magnitude once PiNO was discontinued, leading to an increase in $\dot{Q}s/\dot{Q}t$ and Vd/Vt. The $\dot{Q}s/\dot{Q}t$ decreased in those ponies where PiNO and dobutamine were discontinued together. This agrees with findings in humans, pigs, dogs, cats and rabbits (Prinzmetal et al. 1948; Smith et al. 1974; Lynch et al. 1979; Molloy et al. 1986; Rennotte et al. 1989; Russell & James 2004; Stickland et al. 2006; Stickland et al. 2007), that when $\dot{Q}t$ increases, $\dot{Q}s/\dot{Q}t$ also increases.

Similar limitations to study III were associated with study V. Due to the size of the CT gantry, we were limited to using ponies in our experiments. It is known that gas exchange disturbances are related to body mass and thoracic dimensions (Mansel & Clutton 2008). Furthermore, no pony developed significant hypoxaemia during the experiments, which may explain why increases in PaO_2 during PiNO and dobutamine administration did not reach statistical significance. Finally, in the treatment experiment, we split the group into two subgroups, meaning that there were only three ponies in each subgroup.

In summary, ponies that were hypotensive during PiNO delivery, did not demonstrate improvements in indices of oxygenation, as regional pulmonary perfusion did not change. This finding was presumably due to the negative effects of mechanical ventilation. If blood pressure and $\dot{Q}t$ were supported with dobutamine, perfusion to well-ventilated regions of lung improved when PiNO was delivered. This led to improvements in indices of oxygenation by counteracting the effects of mechanical ventilation.

6. Conclusions

One of the hurdles to overcome, before PiNO could realistically be recommended for general clinical use, was being able to show without question, its true mechanism of action, but also to further illustrate its efficacy during mechanical ventilation. Whilst it was assumed that regional pulmonary vasodilation effectively caused a redistribution of pulmonary perfusion to optimise $\dot{V}/\dot{Q}$ matching, this is the first time this has been documented using CTA and the MSM in anaesthetised ponies. In summary:

- By developing a CT angiography method using the MSM, this research and thesis has built on previous work using scintigraphy, in that it was possible to prove that this redistribution of blood from atelectatic lung regions to better ventilated lung regions, was significant.
- These measurements of perfusion compared well with a previously reported microsphere technique in anaesthetised horses.
- The variability introduced when using different observers to analyse CT images was described, and also how incorporating computer software could reliably speed up analysis and calculations without introducing further variability.
- Administration of PiNO results in favourable regional redistribution of blood with significant improvements in oxygenation indices and a reduction in intrapulmonary shunt.
- These improvements occur when PiNO is delivered if the pony is breathing spontaneously, but also during periods of mechanical ventilation.

One surprising finding during the course of these experiments, was that blood pressure and $\dot{Q}t$, had a significant impact upon the efficacy of PiNO during mechanical ventilation, but not if the horse was spontaneously breathing. If a horse has low blood pressure during anaesthesia and is being mechanically ventilated, the effect of PiNO is lost, presumably due to the negative effects of positive

intrathoracic pressure on pulmonary perfusion, compounded by the reduction in global $\dot{Q}t$. To manage hypoxaemia using PiNO, blood pressure and $\dot{Q}t$ must be supported if the horse is being ventilated. These findings add to existing evidence that PiNO is an effective treatment option for hypoxaemia encountered in anaesthetised horses, by manipulating perfusion rather than ventilation to enhance gas exchange. As hypotension, hypercapnia and hypoxaemia can all occur together, guidelines can now be provided for the use of PiNO in clinical settings. By being able to more effectively treat hypoxaemia, PiNO may have a positive impact on mortality rates associated with general anaesthesia in the horse.

7. Future work

There is more work to come…..

Firstly, it is essential that a partner within industry is found, to build a device to deliver PiNO that can be marketed commercially. Devices available for management of pulmonary hypertension in humans may be adaptable for use in the horse. This is the next step in preparing PiNO for clinical use.

Secondly, now we have been able to measure regional pulmonary perfusion during anaesthesia in ponies, we need to investigate how other drugs affect perfusion within the lung, and how they impact the response to PiNO. Alpha 2 adrenergic agonist drugs are used frequently during anaesthesia, particularly by infusion. These drugs have profound effects on blood vessels, and on $\dot{Q}t$, and are likely to affect $\dot{V}/\dot{Q}$ matching within the lung. Combining the use of these drugs with PiNO may change the response of the pulmonary vasculature, and it is crucial that we explore that.

Finally, following on from questions raised within the introduction of this thesis, we need to document if the use of PiNO has an impact on recovery from anaesthesia, and in particular, does it reduce mortality rates in anaesthetised horses.

Currently, work at SLU is finding solutions to all of these problems and questions, and the research group remains very active in delving more into this tiny molecule of gas.

8. References

Allen DG & Orchard CH (1987) Myocardial contractile function during ischemia and hypoxia. *Circ Res*. 60, 153–168.

Ambrósio AM, Ida KK, Souto MTMR, Oshiro AH, Fantoni DT (2013) Effects of positive end-expiratory pressure titration on gas exchange, respiratory mechanics and hemodynamics in anesthetized horses. *Vet Anaesth Analg*. 40, 564–572.

American College of Radiology Committee on Drugs and Contrast Media. ACR Manual on Contrast Media (Version 2020). American College of Radiology.https://www.acr.org/-/media/ACR/Files/Clinical-Resources/Contrast_Media.pdf accessed January 2021.

Antosova M, Mokra D, Pepucha L (2017) Physiology of nitric oxide in the respiratory system. *Physiol Res*. 66, S159-S172.

Auckburally A, Grubb TL, Wiklund M, Nyman G (2019) Effects of ventilation mode and blood flow on arterial oxygenation during pulse-delivered inhaled nitric oxide in anesthetized horses. *Am J Vet Res*. 80, 275-283.

Bagate F, Tuffet S, Masi P, Perier F, Razazi K, de Prost N, Carteaux G, Payen D, Mekontso Dessap A (2020) Rescue therapy with inhaled nitric oxide and almitrine in COVID-19 patients with severe acute respiratory distress syndrome. *Annals Int Care*. 10, article number 51.

Bammer R. (2016) Acquisition schemes for computed tomography perfusion. In: Bammer R, ed. *MR and CT Perfusion and Pharmacokinetic Imaging.* 1st ed. Wolters Kluwer, USA, 114-144.

Barberà JA, Roger N, Roca J, Rodriguez-Roisin R, Rovira I, Higenbottam TW (1996) Worsening of pulmonary gas exchange with nitric oxide inhalation in chronic obstructive pulmonary disease. *Lancet*. 347, 436– 440.

Beck KC (1987) Regional trapping of microspheres in the lung compares well with regional blood flow. *J Appl Physiol*. 63, 883–889.

Benator SR, Hewlett AM, Nunn JF (1973) The use of iso-shunt lines for control of oxygen therapy. *Br J Anaesth*. 45, 711–718.

Benson GJ, Kneller SK, Manohar M, Thurmon JC (1982) Radiographic characterization of diaphragmatic excursion in halothane-anesthetized ponies: Effect of mode of ventilation and muscle paralysis. *Equine Vet Sci*. 2, 90–94.

Berggren SM (1942) Oxygen deficit of arterial blood caused by non-ventilating parts of the lung. *Acta Physiol Scand*. 4 (Suppl II), 7–92.

Bernard C [1865] (1957) Introduction á l'étude de la médecine expérimentale (An Introduction to the Study of Experimental Medicine). Reprint: Greene HC, Dover, New York.

Bidwell LA, Bramlage LR, Rood WA (2007) Equine perioperative fatalities associated with general anaesthesia at a private practice – a retrospective case series. *Vet Anaesth Analg*. 34 (1), 23–30.

Bigatello LM, Hurford WE, Kacmarek RM (1994) Prolonged inhalation of low concentrations of nitric oxide in patients with severe adult respiratory distress syndrome: effects on pulmonary hemodynamics and oxygenation. *Anesthesiol*. 80, 761–770.

Bindslev L, Hedenstierna G, Santesson J, Gottlieb I, Carvallhas A (1981) Ventilation-perfusion distribution during inhalation anaesthesia. *Acta Anaesth Scand*. 25, 360–371.

Bradford KK, Bhaskar D, Pearl RG (2000) Combination therapy with inhaled nitric oxide and intravenous dobutamine during pulmonary hypertension in the rabbit. *J Cardiovasc Pharm*. 36 (2), 146–151.

Brett SJ, Hansell DM, Evans TW (1998) Clinical correlates in acute lung injury response to inhaled nitric oxide. *Chest.* 114, 1397–1404.

Bringewatt T, Hopster K, Kästner SBR, Rohn K, Ohnesorge B (2010) Influence of modified open lung concept ventilation on the cardiovascular and pulmonary function of horses during total intravenous anaesthesia. *Vet Rec*. 167, 1002–1006.

Bruckdorfer R (2005) The basics about nitric oxide. *Molec Aspects Med*. 26, 3-31.

Bryan TL, van Diepen S, Bhutani M, Shanks M, Welsh RC, Stickland MK (2012) The effects of dobutamine and dopamine on intrapulmonary shunt and gas exchange in healthy humans. *J Appl Physiol*. 113, 541–548.

Buckberg GD, Luck JC, Payne B, Hoffman JIE, Archie JP, Fixler DE (1971) Some sources of error in measuring regional blood flow with radioactive microspheres. *J Appl Physiol.* 31, 598-604.

Cairo JM (2012) Effects of positive-pressure ventilation on the pulmonary system. In: Cairo JM, ed. *Pilbeam's Mechanical Ventilation – Physiological Concepts and Clinical Applications.* 5th ed. Elsevier, Missouri, USA, 2012:327–352.

Cargill RI, Kiely DG, Clark RA, Lipworth BJ (1995) Hypoxaemia and release of endothelin-1. *Thorax*. 50, 1308–1310.

Casoni D, Spadavecchia C, Adami C (2014) Cardiovascular changes after administration of aerosolized salbutamol in horses: five cases. *Acta Vet Scand*. 56, article number 49.

Casthely PA, Lear S, Cottrell JE, Lear E (1982) Intrapulmonary shunting during induced hypotension. *Anesth Analg*, 61, 231-235.

Chon D, Beck KC, Larsen RL, Shikata H, Hoffman EA (2006) Regional pulmonary blood flow in dogs by 4D-X-ray CT. *J Appl Physio* 10, 1451–1465.

Costa-Farré C, Prades M, Ribera T, Valero O, Taurà P (2014) Does intraoperative low arterial partial pressure of oxygen increase the risk of surgical site infection

following emergency exploratory laparotomy in horses? *Vet J*. 200 (1), 175–180.
Cournand A, Motley HL, Werko L, Richards DW (1948) Physiologic studies on the effects of intermittent positive pressure breathing on cardiac output in man. *Am J Physiol*. 52, 162-174.
Cuvelliez SG, Eicker SW, McLauchlan C, Brunson DB (1990) Cardiovascular and respiratory effects of inspired oxygen fraction in halothane-anesthetized horses. *Am J Vet Res*. 51 (8), 1226–1231.
Dawson P (2006) Functional imaging in CT. *Eur J Radiol*. 60, 331–340.
Day TK, Gaynor JS, Muir WW, Bednarski RM, Mason DE (1995) Blood gas values during intermittent positive pressure ventilation and spontaneous ventilation in 160 anesthetized horses positioned in lateral or dorsal recumbency. *Vet Surg*. 24, 266-276.
de Boor CR (1978) A practical guide to splines. *Appl Math Sci*. 27.
De Vries A, Brearly JC, Taylor PM (2009) Effects of dobutamine on cardiac index and arterial blood pressure in isoflurane-anaesthetized horses under clinical conditions. *J Vet Pharmacol Ther*. 32 (4), 353–358.
Dobson A, Gleed RD, Meyer RE (1985) Changes in blood flow distribution in equine lungs induced by anaesthesia. *Quart J Exp Physiol*. 70, 283-297.
Dodam JR, Moon RE, Olson NC, Exposito AJ, Fawcett TA, Huang YC, Theil DR, Camporesi E, Swanson CR (1993) Effects of clenbuterol hydrochloride on pulmonary gas exchange and hemodynamics in anesthetized horses. *Am J Vet Res*. 54 (5), 776–782.
Dugdale AH, Obhrai J, Cripps PJ (2016) Twenty years later: a single-centre, repeat retrospective analysis of equine perioperative mortality and investigation of recovery quality. *Vet Anaesth Analg*. 43, 171–178.
Edner A, Nyman G, Essén-Gustavsson B (2005) The effects of spontaneous and mechanical ventilation on central cardiovascular function and peripheral perfusion during isoflurane anaesthesia in horses. *Vet Anaesth Analg*. 32, 136–146.
Elsayed NM (1994) Toxicity of nitrogen dioxide: an introduction. *Toxicol.* 89, 161–174.
Ferrari M, Santini A, Protti A et al. (2020) Inhaled nitric oxide in mechanically ventilated patients with COVID-19. *J Crit Care*. 60, 59-160.
Fink C, Ley, S, Risse F, Eichinger M, Zaporozhan J, Buhmann R, Puderbach M, Plathow C, Kauczor H (2005) Effect of inspiratory and expiratory breathhold on pulmonary perfusion: assessment by pulmonary perfusion magnetic resonance imaging. *Invest Radiol*. 40, 72–79.
Frostell C, Fratacci M, Wain JC, Jones R, Zapol WM (1991) A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation*. 83, 2038–2047.
Furchgott RF, Cherry PD, Zawadzki JV (1984) Endothelial cells as mediators of vasodilation of arteries. *J Cardiovasc Pharmacol*. 6, S36-S343.
Gasthuys F, de Moor A, Parmentier D (1991) Influence of dopamine and dobutamine on the cardiovascular depression during a standard halothane anaesthesia in dorsally recumbent ventilated ponies. *J Vet Med Assoc*. 38, 494-500.
Gattinoni L, Pesenti A, Bombino S, Baglioni S, Rivolta M, Rossi F, Rossi G, Fumagalli

R, Marcolin R, Mascheroni D, Torresin A (1988) Relationships between lung computed tomographic density, gas exchange, and PEEP in acute respiratory failure. *Anesthesiol*. 69, 824–832.

Gerlach H, Pappert D, Lewandowski K, Rossaint R, Falke KJ (1993) Long-term inhalation with evaluated low doses of nitric oxide for selective improvement of oxygenation in patients with acute respiratory distress syndrome. *Int Care Med*. 19, 443–449.

Gleed RD & Dobson A (1988) Improvement in arterial oxygen tension with change in posture in anaesthetized horses. *Res Vet Sci*. 44, 255–259.

Glenny RW, Bernard S, Brinkley M (1993) Validation of fluorescent-labeled microspheres for measurement of regional organ perfusion. *J Appl Physiol.* 74, 2585-2597.

Goldfarb S, McCullough PA, McDermott J, Gay SB (2009) Contrast-induced acute kidney injury: specialty-specific protocols for interventional radiology, diagnostic computed tomography radiology and interventional cardiology. *Mayo Clin Proc.* 84, 170-179.

Gottrup F (2004) Oxygen in wound healing and infection. *World J Surg*. 28, 312–315.

Grubb T (2012) *Evaluation of efficacy and safety of pulsed inhaled nitric oxide in the anesthetized horse: preparing for clinical use.* Doctoral thesis, Swedish University of Agricultural Sciences, Skara.

Grubb T, Edner A, Frendin JHM, Funkquist P, Rýden A, Nyman G (2013) Oxygenation and plasma endothelin-1 concentrations in healthy horses recovering from isoflurane anaesthesia administered with or without pulse-delivered inhaled nitric oxide. *Vet Anaesth Analg*. 40, e9–e18.

Grubb T, Frendin JHM, Edner A, Funkquist P, Hedenstierna G, Nyman G (2013) The effects of pulse-delivered inhaled nitric oxide on arterial oxygenation, ventilation-perfusion distribution and plasma endothelin-1 concentration in laterally recumbent isoflurane-anaesthetized horses. *Vet Anaesth Analg*. 40, e19–e30.

Grubb TL, Högman M, Edner A, Frendin JHM, Heinonen E, Malavasi LM, Frostell CG, Rýden A, Alving K, Nyman G (2008) Physiologic responses and plasma endothelin-1 concentrations associated with abrupt cessation of nitric oxide inhalation in isoflurane-anesthetized horses. *Am J Vet Res*. 69 (3), 423–430.

Grubb TL, Lord PF, Berger M, Larsson C, Rýden A, Frendin J, Funkquist P, Edner A, Nyman G (2014) Effects of pulse-delivered inhaled nitric oxide administration on pulmonary perfusion and arterial oxygenation in dorsally recumbent isoflurane-anesthetized horses. *Am J Vet Res*. 75 (11), 949 – 955.

Grubbström J, Berglund B, Kaijser L (1993) Myocardial oxygen supply and lactate metabolism during marked arterial hypoxaemia. *Acta Physiol Scand*. 149, 303–310.

Hall LW, Gillespie JR, Tyler WS (1968) Alveolar - arterial oxygen tension differences in anaesthetised horses. *Br J Anaesth*. 40, 560–568.

Hallett RL & Fleischmann D (2006). Tools of the trade for CTA: MDCT scanners and contrast medium injection protocols. *Tech Vasc Interventional Radiol*. 9, 134–142.

Hambraeus-Jonzon K, Bindslev L, Frostell C, Hedenstierna G (1998) Individual lung blood flow during unilateral hypoxia: effects of inhaled nitric oxide. *Eur Respir J*. 11, 565–570.

Hedenstierna G (1987) Pulmonary perfusion: effects of changes in cardiac output and intrathoracic pressure. In: *Cardiopulmonary interactions in acute respiratory failure*. Vincent JL & Suter PM, (eds) Springer-Verlag, Berlin, Germany. 49-60.

Hedenstierna G & Lattuada M (2002) Gas exchange in the ventilated patient. *Curr Opin Crit Care*. 8, 39–44.

Hedenstierna G, Nyman G, Frostell C (2005) Animal models of lung physiology during anesthesia. In: *Handbook of Laboratory Animal Science – Volume III: Animal Models* (2nd ed). Hau J & Van Hoosier Jr GL (eds), CRC Press, Florida, US, 263–288.

Heinonen E (2002) *Synchronized delivery of inspired nitric oxide. Effects on oxygenation and pulmonary tension during artificial ventilation.* Doctoral thesis, Uppsala University, Uppsala.

Heinonen E, Hedenstierna G, Meriläinen P, Högman M, Nyman G (2001) Pulsed delivery of nitric oxide counteracts hypoxaemia in the anaesthetised horse. *Vet Anaesth Analg*. 28, 3–11.

Heinonen E, Högman M, Meriläinen P (2000) Theoretical and experimental comparison of constant inspired concentration and pulsed delivery in NO therapy. *Int Care Med*. 26, 1116–1123.

Heinonen E, Nyman G, Meriläinen P, Högman M (2002) Effect of different pulses of nitric oxide on venous admixture in the anaesthetized horse. *Br J Anaesth.* 88 (3), 394–398.

Herts BR, O'Malley CM, Wirth SL, Lieber ML, Pohlman B (2001) Power injection of contrast media using central venous catheters: feasibility, safety and efficacy. *Am J Radiol*. 176, 447–454.

Hewlett AM, Hulands GH, Nunn JF, Milledge JS (1974) Functional residual capacity during anaesthesia III: Artificial ventilation. *Br J Anaesth*. 46, 495-503.

Heymann MA, Payne BD, Hoffman JIE, Rudolph AM (1977) Blood flow measurements with radionuclide-labeled particles. *Progress Cardiovasc Disease*. 20, 55-79.

Hlastala MP, Bernard SL, Erickson HH, Fedde MR, Gaughan EM, McMurphy R, Emery MJ, Polissar N, Glenny RW (1996) Pulmonary blood flow distribution in standing horses is not dominated by gravity. *J Appl Physiol*. 81, 1051-1061.

Hodgson DS, Steffey EP, Grandy JL, Woliner MJ (1986) Effects of spontaneous, assisted and controlled ventilatory modes in halothane-anesthetized geldings. *Am J Vet Res*. 47 (5), 992-996.

Hopkins RO & Bigler ED (2001) Pulmonary Disorders. In: *Medical Neuropsychology* (2nd ed.) Tarter RE, Butters M, Beers SR (eds.). Kluwer Academic, New York, US. 25 – 50.

Hopkins SR, Levin DL, Emami K, Kadlecek S, Yu J, Ishii M, Rizi RR (2007) Advances in magnetic resonance imaging of lung physiology. *J Appl Physiol.* 102, 1244-1254.

Hopkins SR & Prisk GK (2010) Lung perfusion measured using magnetic resonance imaging: new tools for physiological insights into the pulmonary circulation. *J*

Mag Reson Imag. 32, 1287–1301.
Hopster K, Kästner SBR, Rohn K, Ohnesorge B (2011) Intermittent positive pressure ventilation with constant positive end-expiratory pressure and alveolar recruitment manoeuvre during inhalation anaesthesia in horses undergoing surgery for colic, and its influence on the early recovery period. *Vet Anaesth Analg*. 38, 169–177.
Hopster K, Wogatzki A, Geburek F, Conze P, Kästner SBR (2017) Effects of positive end-expiratory pressure titration on intestinal oxygenation and perfusion in isoflurane anaesthetised horses. *Equine Vet J*. 49, 250–256.
Hubbell JAE, Aarnes TK, Bednarski RM, Lerche P, Muir WW (2011) Effect of 50% and maximal inspired oxygen concentrations on respiratory variables in isoflurane-anesthetized horses. *BMC Veterinary Research*. 7 (23), doi:10.1186/1746-6148-7-23.
Hubbell JAE & Muir WW (2015) Oxygenation, oxygen delivery and anaesthesia in the horse. *Eq Vet J*. 47, 25–35.
Hughes PM & Bisset R (1991) Non-ionic contrast media: a comparison of iodine delivery rates during manual injection angiography. *Brit J Radiol*. 64, 417–419.
Ichinose F, Roberts JD, Zapol WM (2004) Inhaled nitric oxide. *Circulation.* 109, 3106-3111.
Johnston GM, Eastment JK, Wood JLN, Taylor PM (2002) The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of Phases 1 and 2. *Vet Anaesth Analg*. 29, 159-170.
Joyce CJ, Baker AB, Kennedy FR (1993) Gas uptake from unventilated areas of lung: computer model of absorption atelectasis. *J Appl Physiol*. 74, 1107–1116.
Kambadakone AR & Sahani DV (2009) Body perfusion CT: technique, clinical applications, and advances. *Radiol Clin N Am*. 47, 161–178.
Keegan D, Gleed RD, Sanders EA, Seaman GC, Wertz EM, Short CE (1991) Treatment of low arterial oxygen tension in anesthetized horses with clenbuterol. *Vet Surg*. 20 (2), 148–152.
Kelman GR, Nunn JF, Prys-Roberts C, Greenbaum R (1967) The influence of cardiac output on arterial oxygenation: a theoretical study. *Br J Anaesth*. 39, 450–458.
Kobayashi J & Murata I (2020) Nitric oxide inhalation as an interventional rescue therapy for COVID-19-induced acute respiratory distress syndrome. *Annal Int Care*. 10, Article number 61.
Lachmann B (1992) Open up the lung and keep it open. *Intensive Care Med*. 18, 319–321.
La Gerche A, Daffy JR, Mooney DJ, Forbes G, Davie AJ (2013) Transit of micro-bubbles through the pulmonary circulation of Thoroughbred horses during exercise. *Res Vet Sci*. 95, 644-647.
Lee YHL, Clarke KW, Alibhai HLK (1998) The cardiopulmonary effects of clenbuterol when administered to dorsally recumbent halothane-anaesthetised ponies – failure to increase arterial oxygenation. *Res Vet Sci*. 65, 227–232.
Levitsky (2013) Mechanics of Breathing. In: *Pulmonary Physiology* (8th ed), McGraw-Hill Medical, New York, US, 12–57.

Littell RC, Milliken GA, Stroup WW et al (2006). In: *SAS for mixed models*, 2nd ed. SAS Institute Inc, NC, USA.

Lumb AB & Pearl RG (2010) Distribution of pulmonary ventilation and perfusion. In: *Nunn's Applied Respiratory Physiology* (7th ed.). Elsevier, Oxford, UK, 119 – 144.

Lundberg JON & Weitzberg E (1999) Nasal nitric oxide in man. *Thorax*. 54, 947–952.

Lynch JP, Mhyre JG, Dantzker DR (1979) Influence of cardiac output on intrapulmonary shunt. *J Appl Physiol*. 46, 315-321.

Magnusson L & Spahn DR (2003) New concepts of atelectasis during general anaesthesia. *Br J Anaesth*. 91 (1), 61–72.

Manohar M & Goetz TE (1998) Regional distribution of blood flow in the brain of horses at rest and during exercise. *Am J Vet Res*. 59 (7), 893–897.

Manohar M & Goetz TE (2005) Intrapulmonary arteriovenous shunts of > 15μm in diameter probably do not contribute to arterial hypoxaemia in maximally exercising Thoroughbred horses. *J Appl Physiol*. 99 (1), 224–229.

Mansell JC & Clutton RE (2008) The influence of body mass and thoracic dimensions on arterial oxygenation in anaesthetized horses and ponies. *Vet Anaesth Analg*. 35, 392–399.

Marntell S, Nyman G, Hedenstierna G (2005) High inspired oxygen concentrations increase intrapulmonary shunt in anaesthetised horses. *Vet Anaesth Analg*. 32, 338–347.

Marshall BE & Grange RA (1966) Changes in respiratory physiology during ether/air anaesthesia. *Brit J Anaesth*, 38, 329-338.

Mason DE, Muir WW, Wade A (1987) Arterial blood gas tensions in the horse during recovery from general anesthesia. *J Am Vet Med Assoc*. 190 (8), 989-994.

McDonnell WN, Hall LW, Jeffcott LB (1979) Radiographic evidence of impaired pulmonary function in laterally recumbent anaesthetized horses. *Equine Vet J.* 11, 24–32.

McMurphy RM & Cribb PH (1989) Alleviation of postanesthetic hypoxemia in the horse. *Can Vet J*. 30, 37–41.

Miles KA (2003) Perfusion CT for the assessment of tumour vascularity: which protocol? *Br J Radiol*. 76, S36–S42.

Miles KA & Griffiths MR (2003) Perfusion CT: a worthwhile enhancement? *Br J Radiol*. 76, 220–231.

Mills PC & Higgins AJ (1997) Oxidant injury, nitric oxide and pulmonary vascular function: Implications for the exercising horse. *Vet J*. 153, 125–148.

Mills PC, Marlin DJ, Demoncheaux E, Scott C, Casas I, Smith NC, Higenbottam T (1996) Nitric oxide and exercise in the horse. *J Physiol*, 495 (3), 863–874.

Mitchell B & Littlejohn A (1974) The effect of anaesthesia and posture on the exchange of respiratory gases and on the heart rate. *Equine Vet J.* 6 (4), 177–178.

Mizuno Y, Aida H, Fujinaga T (1995) Effects of dobutamine infusion in dorsally recumbent isoflurane-anesthetized horses. *J Eq Sci.* 5 (3), 87-94.

Mizuno Y, Aida H, Hara H, Fujinaga T (1994) Cardiovascular effects of intermittent positive pressure ventilation in the anesthetized horse. *J Vet Med Sci*. 56 (1), 39 -44.

Moens Y (1989) Arterial-alveolar carbon dioxide tension difference and alveolar dead space in halothane-anaesthetised horses. *Equine Vet J*. 21, 282–284.
Moens Y (2013) Mechanical ventilation and respiratory mechanics during equine anesthesia. *Vet Clin Equine*. 29, 51–67.
Moens Y & Böhm S (2011) Ventilating horses: moving away from old paradigms. *Vet Anaesth Analg*. 38, 165–168.
Moens Y, Gootjes P, Lagerweij E (1992) A tracheal tube-in-tube technique for functional separation of the lungs in the horse. *Eq Vet J*. 24 (2), 103–106.
Moens Y, Lagerweij E, Gootjes P, Poortman J (1994) Differential artificial ventilation in anesthetized horses positioned in lateral recumbency. *Am J Vet Res.* 55 (9), 1319–1326.
Moens Y, Lagerweij E, Gootjes P, Poortman J (1995) Distribution of inspired gas to each lung in the anaesthetised horse and the influence of body shape. *Equine Vet J*. 27 (2), 110–116.
Moens Y, Lagerweij E, Gootjes P, Poortman J (1998) Influence of tidal volume and positive end-expiratory pressure on inspiratory gas distribution and gas exchange during mechanical ventilation in horses positioned in lateral recumbency. *Am J Vet Res*. 59 (3), 307–312.
Molloy DW, Ducas J, Dobson K, Girling L, Prewitt RM (1986) Hemodynamic management in clinical acute hypoxemic respiratory failure. Dopamine vs dobutamine. *Chest*. 89, 636-640.
Mosing M, Rysnik M, Bardell D, Cripps PJ, MacFarlane P (2013) Use of continuous positive airway pressure (CPAP) to optimise oxygenation in anaesthetised horses – a clinical study. *Eq Vet J*. 45, 414–418.
Mosing M & Senior JM (2017) Maintenance of equine anaesthesia over the last 50 years: controlled inhalation of volatile anaesthetics and pulmonary ventilation. *Eq Vet J*. 50, 282–291.
Nelin LD, Moshin J, Thomas CJ, Sasidharan P, Dawson CA (1994) The effect of inhaled nitric oxide on the pulmonary circulation of the neonatal pig. *Pediat Res*. 35, 20–24.
Neumann P, Wrigge H, Zinserling J, Hinz J, Maripuu E, Andersson LG, Putensen C, Hedenstierna G (2005). Spontaneous breathing affects the spatial ventilation and perfusion distribution during mechanical ventilatory support. *Crit Care Med*. 33 (5), 1090-1095.
Nguyen DT, Jin C, Thiagalingam A, McEwan AL (2012) A review on electrical impedance tomography for pulmonary perfusion imaging. *Physiol Meas.* 33, 695-706.
Nicholas JF & Lam AM (1984) Isoflurane-induced hypotension does not casue impairment in pulmonary gas exchange. *Can Anaesth Soc J*, 31, 352-358.
Nitric oxide (1994) Centers for disease control and prevention, U.S. Department of Health & Human Services. Available at: http://www.cdc.gov/niosh/idlh/10102439.html [Accessed: 9th March 2016]
Nunn JF (1964) Factors influencing the arterial oxygen tension during halothane anaesthesia with spontaneous respiration. *Br J Anaesth*. 36, 327–341.

Nyman G, Björk M, Funkquist P, Persson GB, Wagner PD (1995) Ventilation-perfusion relationships during graded exercise in the Standardbred trotter. *Eq Vet J Suppl*. 18, 63–69.

Nyman G, Frostell C, Hedenstierna G, Funkquist B, Kvart C, Blomqvist H (1987) Selective mechanical ventilation of dependent lung regions in the anaesthetized horse in dorsal recumbency. *Br J Anaesth*. 59, 1027–1034.

Nyman G, Funkquist B, Kvart C (1988) Postural effects on blood gas tension, blood pressure, heart rate, ECG and respiratory rate during prolonged anesthesia in the horse. *J Vet Med Assoc*. 35, 54–62.

Nyman G, Funkquist B, Kvart C, Frostell C, Tokics L, Strandberg Å, Lundquist H, Lundh B, Brismar B, Hedenstierna G (1990) Atelectasis causes gas exchange impairment in the anaesthetised horse. *Eq Vet J*. 22 (5), 317– 324.

Nyman G, Grubb TL, Heinonen E, Frendin J, Edner A, Malavasi LM, Frostell C, Högman M (2012) Pulsed delivery of inhaled nitric oxide counteracts hypoxaemia during 2.5 hours of inhalation anaesthesia in dorsally recumbent horses. *Vet Anaesth Analg*. 39, 480–487.

Nyman G & Hedenstierna G (1989) Ventilation-perfusion relationships in the anaesthetised horse. *Equine Vet J*. 21 (4), 274–281.

Palmer RMJ, Ferrige AG, Moncada SA (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 327, 524–526.

Papadakos PJ & Lachmann B (2007) The open lung concept of mechanical ventilation: The role of recruitment and stabilization. *Critical Care Clinics*. 23 (2), 241–250.

Pascoe PJ, McDonell, WN, Trim CM, Van Gorder J (1983) Mortality rates and associated factors in equine colic operations – a retrospective study of 341 operations. *Can Vet J*. 24, 76–85.

Patel PM, Drummond JC, Lemkuil BP (2015) Cerebral physiology and the effects of anesthetic drugs. In: *Miller's Anesthesia* (8th ed), Cohen NH, Eriksson LI, Fleisher LA, Winer-Kronish JP, Young WL (eds) Elsevier Saunders, Philadelphia, US. 387 – 422.

Patschova M, Kabes R, Krisova S (2010) The effects of inhalation salbutamol administration on systemic and pulmonary hemodynamic, pulmonary mechanics and oxygen balance during general anaesthesia in the horse. *Vet Med*. 55, 445–456.

Pauritsch K (1997) *Positive end-expiratory pressure ventilation in combination with end-inspiratory breath-holding during anaesthesia in colic hor*ses. DVM dissertation, Equine Clinic, University of Hannover.

Pelletier N, Robinson NE, Kaiser L, Derksen FJ, (1998) Regional differences in endothelial function in horse lungs: possible role in blood flow distribution. *J Appl Physiol*. 85 (2), 537–542.

Portier K, Crouzier D, Guichardant M, Prost M, Debouzy J, Kirschvink N, Fellmann N, Lekeux P, Coudert J (2009) Effects of high and low inspired fractions of oxygen on horse erythrocyte membrane properties, blood viscosity and muscle oxygenation during anaesthesia. *Vet Anaesth Analg*. 36 (4), 287–298.

Prinzmetal M, Ornitz EM, Simkin B, Bergman HC (1948) Arterio-venous anastomoses in liver, spleen and lungs. *Am J Physiol*. 152, 48-52.

Raisis AL, Blissitt KJ, Henley W, Rogers K, Adams V, Young LE (1995) The effects of halothane and isoflurane on cardiovascular function in laterally recumbent horses. *Br J Anaesth*. 95 (3), 317–325.

Reber A, Engberg G, Sporre B, Kviele L, Rother H-U, Wegenius G, Nylind U, Hedenstierna G (1996) Volumetric analysis of aeration in the lungs during general anaesthesia. *Br J Anaesth*. 76, 760–766.

Rennotte MT, Reynaert M, Clerbaux T, Willems E, Roeseleer J, Verlter C, Rodenstein D, Frans A (1989) Effects of two inotropic drugs, dopamine and dobutamine, on pulmonary gas exchange in artificially ventilated patients. *Int Care Med*.15 (3), 160-165.

Robertson SA & Bailey JE (2002) Aerosolized salbutamol (albuterol) improves PaO_2 in hypoxaemic anaesthetized horses – a prospective clinical trial in 81 horses. *Vet Anaes Analg*. 29, 212–218.

Romer LM, Dempsey JA, Lovering A, Eldridge M (2006) Exercise-induced arterial hypoxemia: Consequences for locomotor muscle fatigue. *Advances in Exp Med Biol*. 588, 47–55.

Rossaint R, Gerlach H, Schmidt-Ruhnke H, Pappert D, Lewandowski K, Steudel W, Falke K (1995) Efficacy of inhaled nitric oxide in patients with severe ARDS. *Chest*. 107, 1107–1115.

Roth R, Akin M, Deligonul U, Kern MJ (1991) Influence of radiographic contrast media viscosity to flow through coronary angiographic catheters. *Cathet Cardiovasc Diagn*. 22, 290–294.

Rothen HU, Neumann P, Berglund JE, Valtysson J, Magnusson A, Hedenstierna G (1999) Dynamics of re-expansion of atelectasis during general anaesthesia. *Br J Anaesth*. 82 (4), 551–556.

Rothen HU, Sporre B, Engberg G, Wegenius G, Hedenstierna G (1993) Re-expansion of atelectasis during general anaesthesia: a computed tomography study. *Br J Anaesth*. 71, 788–795.

Rothen HU, Sporre B, Engberg G, Wegenius G, Reber A, Hedenstierna G (1995) Prevention of atelectasis during general anaesthesia. *Lancet*. 345, 1387–1391.

Rugh KS, Garner HE, Hatfield DG, Herrold D (1984) Arterial oxygen and carbon dioxide tensions in conscious laterally recumbent ponies. *Eq Vet J*. 16 (3), 185–188.

Russell WJ & James MF (2004) The effects on arterial haemoglobin saturation and on shunt of increasing cardiac output with dopamine or dobutamine during one-lung ventilation. *Anaesth Int Care*. 32, 644-648.

Sanelli PC, Nicola G, Johnson R, Tsiouris AJ, Ougorets I, Knight C, Frommer B, Veronelli S, Zimmerman RD (2007) Effect of training and experience on qualitative and quantitative CT perfusion data. *Am J Neuroradiol*. 28, 428–432.

Santos M, Ibancovichi JA, Lopez-Sanroman FJ, Tendillo FJ (2013) Effects of single hyperinflation using a sustained high pressure manoeuvre during inhalation anaesthesia in horses. *Vet J.* 197, 892–895.

Sauter AW, Merkle A, Schulze M, Spira D, Hetzel J, Claussen C, Horger MS (2012) Intraobserver and interobserver agreement of volume perfusion CT (VPCT) measurements in patients with lung lesions. *Eur J Radiol*. 81:2853–2859.

Schatzmann U, Koehli M, Dudan F, Rohr W, Jones RS (1982) Effect of postural changes on certain circulatory and respiratory values in the horse. *Am J Vet Res*. 43, 1003–1005.

Schwab SA, Kuefner MA, Anders K, Adamietz B, Heinrich MC, Baigger JF, Janka R, Uder M, Kramer M (2009) Peripheral intravenous power injection of iodinated contrast media: the impact of temperature on maximum injection pressures at difference cannula sizes. *Acta Radiol*. 16, 1502–1508.

Seccombe JF, Pearson PJ, Schaff HV (1994) Oxygen radical-mediated vascular injury selectively inhibits receptor-dependent release of nitric oxide from canine coronary arteries. *J Thorac Cardiovasc Surg.* 107, 505–509.

Shekerdemian L & Bohn D (1999) Cardiovascular effects of mechanical ventilation. *Arch Dis Child*. 80, 475–480.

Smith G, Cheney FW, Winter PM (1974) The effect of change in cardiac output on intrapulmonary shunting. *Br J Anaesth*, 46, 337-342.

Sorenson PR & Robinson NE (1980) Postural effects on lung volumes and asynchronous ventilation in anaesthetized horses. *J Appl Physiol*. 48, 97–103.

Stack A, Derksen FJ. Williams KJ, Robinson NE, Jackson WF (2014) Lung region and racing affect mechanical properties of equine pulmonary microvasculature. *J Appl Physiol*. 117, 370–376.

Staddon GE & Weaver BMQ (1981) Regional pulmonary perfusion in horses: a comparison between anaesthetised and conscious standing animals. *Res Vet Sci.* 30, 44-48.

Staffieri F, Bauquier SH, Moate PJ, Driessen B (2009) Pulmonary gas exchange in anaesthetised horses mechanically ventilated with oxygen or a helium/oxygen mixture. *Eq Vet J*. 41 (8), 747-752

Steffey EP, Kelly AB, Hodgson DS, Grandy JL, Woliner MJ, Willits N (1990) Effects of body posture on cardiopulmonary function in horses during five hours of constant-dose halothane anesthesia. *Am J Vet Res*. 51 (1), 11–16.

Steffey EP, Wheat JD, Meagher DM, Norrie RD, McKee J, Brown M, Arnold J (1977) Body position and mode of ventilation influences arterial pH, oxygen, and carbon dioxide tensions in halothane-anesthetized horses. *Am J Vet Res*. 38 (3), 379–382.

Steffey EP, Willits N, Woliner M (1992) Hemodynamic and respiratory responses to variable arterial partial pressure of oxygen in halothane-anesthetized horses during spontaneous and controlled ventilation. *Am J Vet Res*. 53 (10), 1850–1858.

Stegmann GF & Littlejohn A (1987) The effect of lateral and dorsal recumbency on cardiopulmonary function in the anaesthetised horse. *J S Afr Vet Assoc*. 58 (1), 21–27.

Stickland MK & Lovering AT (2006) Exercise-induced intrapulmonary arteriovenous shunting and pulmonary gas exchange. *Exercise Sport Sci Rev*. 34, 99-106.

Stickland MK, Lovering AT, Eldridge MW (2007) Exercise-induced arteriovenous intrapulmonary shunting in dogs. *Am J Respir Crit Care Med*. 176, 300-305.

Swanson CR & Muir WW (1986) Dobutamine-induced augmentation of cardiac output does not enhance respiratory gas exchange in anesthetized recumbent healthy horses. *Am J Vet Res*. 47 (7), 1573–1576.

Swanson CR & Muir WW (1988) Hemodynamic and respiratory responses in halothane-anesthetized horses exposed to positive end-expiratory pressure alone and with dobutamine. *Am J Vet Res*. 49 (4), 539–542.

Swanson CR, Muir WW, Bednarski RM, Skarda RT, Hubbell JA (1985) Hemodynamic responses in halothane-anesthetized horses given infusions of dopamine or dobutamine. *Am J Vet Res.* 46 (2), 365–370.

Tokics L, Hedenstierna G, Strandberg A, Brismar B, Lundquist H (1987) Lung collapse and gas exchange during general anesthesia: effects of spontaneous breathing, muscle paralysis and positive end-expiratory pressure. *Anesthesiol.* 66, 157-167.

Trim CM & Wan PY (1990) Hypoxaemia during anaesthesia in seven horses with colic. *J Ass Vet Anaesth*. 17, 45–49.

Turner CE, Barker-Collo SL, Connell CJW, Gant N (2015) Acute hypoxic gas breathing severely impairs cognition and task learning in humans. *Physiol & Behaviour*. 142, 104–110.

Tyberg JV, Grant DA, Kingma I, Moore TD, Sun YH, Smith ER, Belenkie I (2000) Effects of positive intrathoracic pressure on pulmonary and systemic hemodynamics. *Resp Physiol*, 119, 163-171.

Tyler DC (1983) Positive end-expiratory pressure: A review. *Crit Care Med*. 11 (4), 300–308.

Vaporidi K, Tsatsanis C, Georgopoulos D, Tsichlis PN (2005) Effects of hypoxia and hypercapnia on surfactant protein expression proliferation and apoptosis in A549 alveolar epithelial cells. *Life Sciences*. 78, 284–293.

Vidal Melo MF, Layfield D, Harris S, O'Neill K, Musch G, Richter T, Winkler T, Fischmann A, Venegas JG (2003) Quantification of regional ventilation-perfusion ratios with PET. *J Nucl Med*. 44, 1982-1991.

Vieira SRR, Puybasset L, Richecoeur J, Lu Q, Cluzel P, Gusman PB, Coriat P, Rouby JJ (1998) A lung computed tomographic assessment of positive end-expiratory pressure–induced lung overdistension. *Am J Respir Crit Care Med*. 158, 1571–1577.

Vizza CD, Della Rocca G, Di Roma A, Iacoboni C, Pierconti F, Venute F, Rendina E, Schmid G, Pietropaoli P, Fedele F (2001) Acute hemodynamic effects of inhaled nitric oxide, dobutamine and a combination of the two in patients with mild to moderate secondary pulmonary hypertension. *Crit Care*. 5, 355–361.

Wagner PD (1974) Simultaneous measurement of eight foreign gases in blood by gas chromatography. *J Appl Physiol*, 36, 600–605.

Walther SM, Domino KB, Glenny RW, Hlastala MP (1997) Pulmonary blood flow distribution in sheep: effects of anesthesia, mechanical ventilation, and change in posture. *Anesthesiol.* 87, 335-342.

Weinberger B, Laskin DL, Heck DE, Laskin JD (2001) The toxicology of inhaled nitric oxide. *Toxocol Sci*. 59, 5-16.

Wennmalm A, Benthin G, Edlund A, Jungersten L, Kieler-Jensen N, Lundin S, Westfelt UN, Petersson AS, Waagstein F (1993) Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. *Circ Res*. 73, 121-1127.

West JB (2008) In: West JB, ed. *Respiratory physiology: the essentials.* 8th ed. Lippincott Williams and Wilkins, Philadelphia, USA.

West JB & Dollery CT (1960) Distribution of blood flow and ventilation-perfusion ratio in the lung, measured with radioactive CO_2. *J Appl Physiol.* 15, 405-410.
West JB, Dollery CT, Naimark A (1964) Distribution of blood flow in isolated lung: relation to vascular and alveolar pressures. *J Appl Physiol*. 19, 713–724.
Wettstein D, Moens Y, Jaeggin-Schmucker N, Böhm SH, Rothen HU, Mosing M, Kästner SBR, Schatzmann U (2006) Effects of an alveolar recruitment maneuver on cardiovascular and respiratory parameters during total intravenous anesthesia in ponies. *Am J Vet Res*. 67 (1), 152–159.
Whitehair KJ, Steffey EP, Woliner MJ, Willits NH (1996) Effects of inhalation anesthetic agents on response of horses to three hours of hypoxemia. *Am J Vet Res*. 57 (3), 351–360.
Whitehair KJ & Willits NH (1999) Predictors of arterial oxygen tension in anesthetized horses: 1,610 cases (1992 – 1994). *J Am Vet Med Assoc*. 215 (7), 978–981.
Wiklund M, Granswed I, Nyman G (2017) Pulsed inhaled nitric oxide improves arterial oxygenation in colic horses undergoing abdominal surgery. *Vet Anaesth Analg*. 44 (5), 1139–1148.
Wildsmith JAW, Drummond GB, MacRae WR (1975) Blood-gas changes during induced hypotension with sodium nitroprusside. *Br J Anaesth*, 47, 1205-1211.
Wolfkiel CJ & Rich S (1992) Analysis of regional pulmonary enhancement in dogs by ultrafast computed tomography. *Invest Radiol.* 27, 211-216.
Wrigge H, Zinserling J, Neumann P, Muders T, Magnusson A, Putensen C, Hedenstierna G (2005) Spontaneous breathing with airway pressure release ventilation favors ventilation in dependent lung regions and counters cyclic alveolar collapse in oleic-acid-induced lung injury: a randomized controlled computed tomography trial. *Crit Care*. 9 (6), 780-789.
Young LE, Blissitt KJ, Clutton RE, Molony V (1998) Temporal effects of an infusion of dobutamine hydrochloride in horses anesthetized with halothane. *Am J Vet Res*. 59 (8), 1027–1032.
Young LE, Marlin DJ, McMurphy RM, Walsh K, Dixon PM (1999) Effects of inhaled nitric oxide 10 ppm in spontaneously breathing horses anaesthetized with halothane. *Br J Anaesth*. 83 (2), 321–324.
Young SS & Taylor PM (1993) Factors influencing the outcome of equine anaesthesia: a review of 1,314 cases. *Equine Vet J*. 25, 147–152.
Zapol W (2019) Nitric oxide story. *Anesthesiol*. 30, 435-440
Zhao Y, Hubbard L, Malkasian S, Abbona P, Molloi S (2020) Dynamic pulmonary CT perfusion using first-pass analysis technique with only two volume scans: Validation in a swine model. *PLoS ONE*. 15:1-16. https://doi.org/10.1371/journal.pone.0228110

9. The lungs of the anaesthetised horse: a story of a match made in hell

Imagine if the risk of you dying during an anaesthetic was 1 in 100. Would you sign that consent form? In healthy humans, the risk of dying is approximately 1 in 300,000, but in healthy horses, it really is 1 in 100. If we could make anaesthesia safer for horses, it would be a huge leap forward, and by delivering a tiny puff of gas, we might actually be able to!

Horses are not humans and so, to perform procedures, they frequently have to be anaesthetised. This means giving drugs to make the horse unconscious. Normally, horses do not lie down for long periods of time. As they are animals that predators eat, they have evolved to run fast, and to be able to sleep standing up! Yes, that really is true! If they do lie down, its only for a short period of time (a few minutes), and horses never sleep on their backs. They have also developed to run away from predators, so they have to be faster than lions, and tigers, and bears, oh my! For this, the lungs are very important. The lungs deliver oxygen to, and collect carbon dioxide from, the blood. The cells of the body use oxygen to perform all their tasks, and they produce carbon dioxide as a waste product. The lungs inflate and deflate, filling with air then emptying again. In the normal lung, the amount of air breathed in (or ventilation) is matched to the amount of blood (or perfusion) associated with it. In the parts of the lung that don't receive much air, blood is diverted away to the parts that receive more air, because the blood vessels (tubes that carry blood), get smaller. This balance of air to blood is called ventilation-perfusion matching. This is really important so that the blood can soak up as much oxygen as possible from the air that we breathe. The oxygen is then delivered to all the tissues of the body. Without oxygen, cells will die and, in particular, brain, heart and kidney cells are very much at risk. For horses, which like to run, muscle oxygenation is also very important. A muscle that doesn't have enough oxygen can become injured and this is known as a myopathy.

When we anaesthetise horses for procedures, we make them lie down, sometimes for 2 or 3 hours. As the horse is pretty heavy and have HUGE intestines

so they can digest grass, the lungs get squashed. This is especially true of the lung that is underneath and, of course, blood will pool there too due to gravity. So now there is lung that doesn't get any air, and lots of blood that doesn't receive any oxygen, and this is known as ventilation perfusion MISMATCH. Adding to the problem is that anaesthetic drugs stop the blood vessels becoming smaller so they can longer help to match ventilation with perfusion. The long and short of this is that it will lead to low oxygen levels in the blood, and this is called HYPOXAEMIA.

Hypoxaemia is one of the biggest problems that a horse anaesthetist (yes, there are such people!), faces. Many horse deaths associated with anaesthesia can be related to myopathy. If we could treat hypoxaemia effectively, it would be revolutionary. Nitric oxide (NO) is a molecule that occurs in the body naturally, and it opens up blood vessels by making them relaxxxx…..ahhhh. But…. we can also administer it as a gas when an animal breathes in. Pulsed inhaled nitric oxide, fondly known as PiNO (don't mistake this for pinot noir!), is a treatment that can be given to a horse when it is unconscious; it is given as a tiny puff of gas down the breathing tube, only when the horse breathes in. When we administer PiNO, it targets lung that is ventilating properly. It simply cannot get to the lung that is squashed. The NO travels across the lung into the blood vessels that are close by. The blood vessels relax, and open up and this allows more blood from the squashed lung to move AGAINST gravity and supply the lung that is better ventilated. We know this is how it works because we have measured lung perfusion using a fancy technique called computed tomography angiography – you may have heard of computed tomography as a CT scan. When perfusion is better matched to ventilation, oxygen levels in the blood dramatically improve.

So now we need to see if this reduces the number of horses that die when we anaesthetise them. Maybe we have created a match made in heaven with a little bit of PiNO!

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*"Research is exactly that: you have to **re**-search and **re**-search, to get to the answer!"* G. Nyman - Uppsala

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By adapting computed tomography angiography and the maximum slope model, regional perfusion of the lung was quantified in anaesthetised horses. During PiNO administration, aerated lung perfusion increased, atelectatic lung perfusion decreased and oxygenation indices improved. This selective pulmonary vasodilation optimised ventilation perfusion matching. The effect was similar during spontaneous breathing and mechanical ventilation unless the horse was concurrently hypotensive.

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