PULMONARY BLOOD FLOW DISTRIBUTION AND HYPOXIC PULMONARY VASOCONSTRICTION IN PENTOBARBITAL-ANESTHETIZED HORSES

DISSERTATION

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By

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ABSTRACT

Anesthetized horses commonly develop undesirable hypoxemia when dorsally recumbent. The major reason for this is development of ventilation/perfusion (V/Q) mismatching associated with atelectasis of dependent lung tissue. Improving ventilation frequently does not improve oxygenation, suggesting that pulmonary blood flow distribution is abnormal during anesthesia. Perfusion is normally matched to ventilation by hypoxic pulmonary vasoconstriction (HPV). This mechanism causes pulmonary arterioles to constrict in areas where alveolar oxygen (O₂) tension is low, redirecting blood flow to better-ventilated alveoli, and is believed to be modulated by nitric oxide (NO). The purpose of this study was to evaluate blood flow distribution in the anesthetized horse and to investigate the role of NO as a regulator of HPV in the anesthetized dorsally recumbent adult horse.

Six adult horses anesthetized with pentobarbital were intubated via tracheostomy with a double-lumen tube, which separated gas flow to left and right lungs. Each lung was individually ventilated via a dual-lung ventilator, and 100% O₂ was delivered to both lungs. A hypoxic/hyperoxic state was then induced by ventilating the left lung with 100% nitrogen (N₂) while 100% O₂ was delivered to the right lung. Nitric oxide (NO) production was manipulated by administration of L-arginine (a NO precursor) and L-
NAME (a NO synthase inhibitor). Each horse was instrumented for collection of pulmonary and arterial blood O₂ tensions. Pulmonary blood flow distribution was determined by the pattern of distribution of fluorescent microspheres, which were injected intravenously at strategic time points. Lungs were harvested, dried and sectioned prior to neutron activation and spectrographic analysis of the microspheres.

Blood flow was influenced somewhat by gravity, however distribution was mainly heterogeneous in isogravitational planes, and highest in the central lung. Hypoxic pulmonary vasoconstriction was not active in this model, as evidenced by persistent systemic hypoxemia after ventilation of the left lung with 100% N₂. Manipulation of NO produced appropriate changes in pulmonary arterial pressure, but had minimal effect on blood flow distribution and systemic arterial oxygenation.

Our study suggest that manipulation of NO is unlikely to be helpful in correction of severe hypoxemia due to V/Q mismatching seen during anesthesia in clinical equine patients.
Dedicated to Peter and Irene
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CHAPTER 1

INTRODUCTION

1.1 Hypoxemia in anesthetized horses

Anesthesia of the horse, and the athletic horse in particular, presents veterinary anesthetists with considerable challenges compared with other domestic species. Complications can occur during the induction, maintenance and/or recovery phases of anesthesia (table 1). [Muir and Hubbell 1991] Many of the problems can be overcome with technical support, consideration of physical characteristics (adequate positioning and padding to avoid nerve and muscle injury), training of support personnel and anesthetists (appropriate handling and use of anesthetic and other drugs), and reliance on equipment such as ventilators and demand valves to support ventilation. Almost 40 years ago anesthetized horses were shown to develop hypoxemia during the maintenance phase of anesthesia. [Hall et al 1968, Gillespie et al 1969, Weaver 1970, Hall 1971]. Despite appropriate ventilatory strategies to maintain normocapnia, hypoxemia remains a common complication of equine anesthesia. [Nyman et al 1989, Nyman et al 1990, Day et al 1995] Additionally, many surgical approaches require that equine patients are
placed in dorsal recumbency during anesthesia which exacerbates hypoxemia. [Nyman et al 1990] Hypoxemia is one of the two most common complications during the maintenance phase of anesthesia in horses, the other being hypoventilation. [Hall 1971, Muir and Hubbell 1991]

1.2 Sequelae of hypoxemia

Hypoxemia results in tissue hypoxia and leads to anaerobic metabolism. Tissues vary in their vulnerability to hypoxemia. [Lumb 2000] Oxygen delivery to an organ is a product of blood flow and oxygen carried in the blood (dissolved in plasma and carried as oxyhemoglobin). Organs with a greater requirement for the high-energy compounds produced by oxidative phosphorylation, such as the central nervous system and heart, are not able to function optimally in the absence of such compounds when compared to tissues that have a lesser requirement such as liver or kidney. Lactate and hydrogen ions, the byproducts of anaerobic metabolism, are retained within the brain as the blood-brain barrier is relatively impervious to [H⁺] and [HCO₃⁻] ions, resulting in intracellular acidosis, edema and cell death. Retinal hemorrhage, convulsions and brain damage are more severe sequelae of acute brain hypoxemia.

The sympathetic nervous system is activated in the face of hypoxemia. Hypoxemia also stimulates ventilation via peripheral chemoreceptor reflexes when PaO₂ is less than 60 mmHg. Hypoxic pulmonary vasoconstriction occurs in the lung which allows pulmonary blood flow distribution to be altered so that there is increased blood flow towards areas of
lung that are better oxygenated, although this frequently causes pulmonary hypertension. [Marshall et al 1981]

In addition, hypoxemia can lead to impaired function of the diaphragm. Decreased O₂ delivery decreases the time to muscle fatigue, which can lead to development of diaphragm muscle failure and exacerbation of hypoventilation. [Nunn 1987]

Severe hypoxemia during the maintenance phase of anesthesia that continues into the recovery period may lead to increased respiratory effort and ultimately respiratory embarrassment, and result in pulmonary edema and death. [Borer 2005] It has also been speculated that development or exacerbation of acute laryngeal paralysis in the recovery period may be due to decreased oxygen delivery to the nerves that abduct the laryngeal cartilage. [Abrahamsen et al 1990] Muscle and liver enzymes are also increased following hypoxemia, and may contribute to the development of post-anesthetic myopathy. [Steffey et al 1992] The reduced ability to provide oxygen support to a horse recovering from anesthesia coupled with the extreme muscular exertion in moving from lateral recumbency to standing result in periods of hypoxemia (PaO₂ less than 60 mmHg) in the equine recovery stall. [Steffey et al 1977, Mason et al 1987] Most horses can cope with a brief period of hypoxemia during return to the standing position, however if this follows a prolonged period of hypoxemia during anesthetic maintenance the outcome will likely be less than optimal.
1.3 Causes of hypoxemia

Hypoxemia is defined as an abnormally low PO$_2$ in arterial blood (P$_{aO_2}$), <60mmHg. [Muir and Hubbell 1991, West 1999] The five classically described causes of hypoxemia are hypoventilation, decreased fraction inspired oxygen (FiO$_2$) tension, diffusion impairment, shunting of blood to the arterial system without passing through ventilated lung tissue, and ventilation-perfusion inequality (mismatching). [West 1999]

1.3.1 Hypoventilation

Alveolar ventilation (V$_A$) is always some percentage of the total ventilation (V$_T$) because gas that is inspired must pass through non-alveolar structures (conducting airways), or dead space (V$_D$), therefore V$_T$ = V$_A$ + V$_D$.

V$_A$ can also be related to CO$_2$ concentration since the vast majority of CO$_2$ in the expired tidal volume (V$_E$) comes from alveolar gas exchange. [West 1999] This relationship is known as the alveolar ventilation equation:

\[ V_A = \frac{V_{CO2}}{P_{CO2}} \times K \]

\( V_{CO2} \) = volume of CO$_2$ exhaled per unit time
\( P_{CO2} \) = partial pressure of CO$_2$ in arterial blood (which normally approximates P$_{aCO2}$)
K = constant
From this equation it can be seen that by definition a decrease in alveolar ventilation, or hypoventilation, without a change in CO₂ production, will cause an increase in PaCO₂.

The relationship between ventilation, O₂ and CO₂ is described by the alveolar gas equation:

\[ PAO₂ = PΙO₂ - (PACO₂/R) + F \]

A=alveolar
I=inspired
R=respiratory quotient
F=correction factor (typically small enough to ignore)

Therefore, as PA_CO₂ rises, PAO₂ falls. When the inspired gas is room air, hypoventilation may easily lead to hypoxemia. However, the majority of horses anesthetized for procedures lasting over 60 minutes receive 100% oxygen as a carrier gas for a volatile anesthetic, so hypoventilation generally will not play a significant role in causing hypoxemia, and in any event can be corrected using mechanical ventilation if necessary. Equipment such as mainstream capnometers increase dead space (as equipment dead space), although excessive dead space is typically an issue in small patients (<10kg) or those with significant respiratory disease compared to horses.
1.3.2 Decreased FiO\textsubscript{2}

From the alveolar gas equation it is apparent that a decrease in FiO\textsubscript{2} will decrease the PA\textsubscript{O\textsubscript{2}}. This can occur naturally at high elevations above sea level. [Nunn1989] In anesthesia this can occur with inappropriate delivery of an incorrect anesthetic gas to the patient. [Dorsch and Dorsch 1999]

1.3.3 Diffusion impairment

In order to move from the alveoli into the blood, oxygen molecules must diffuse cross the alveolar-capillary or blood-gas barrier. Diffusion across a tissue barrier is governed by Fick’s law, which states that the rate of diffusion is proportional to the surface area of the tissue and the concentration gradient across it, and inversely proportional to the thickness of the tissue. Normally the barrier is very thin (0.5\textmu m in the human lung), but in states of disease such as pneumonia, chronic bronchitis, or pulmonary edema, this distance is much greater. [West 1997] Horses that present for general anesthesia always undergo thoracic auscultation to evaluate the pulmonary system, so usually the presence of diseases that would cause hypoxemia via this mechanism is known or expected prior to the anesthetic episode.

1.3.4 Shunt

A shunt occurs when venous blood bypasses the alveoli. The effect of shunted blood is a decrease in PaO\textsubscript{2} because poorly oxygenated blood is added to oxygenated blood prior to the blood returning to the systemic circulation. A small amount of clinically irrelevant shunting occurs normally because the bronchial veins empty into the pulmonary veins,
thus bypassing the lung, and the coronary circulation empties into the left ventricle via the Thebesian veins. Pathologic shunts leading to hypoxemia are commonly congenital in nature, and include pulmonary arterio-venous fistula and intra-cardiac defects. West [1999] describes the derivation of the shunt equation:

\[
\frac{Q_S}{Q_T} = \frac{(C_c' O_2 - C_a O_2)}{(C_c' O_2 - C_v O_2)}
\]

\(Q_S\) = shunt blood flow  
\(Q_T\) = total blood flow  
\(C_c' O_2\) = oxygen content of end-capillary blood (equivalent to \(P_{a O_2}\))  
\(C_a O_2\) = oxygen content of arterial blood  
\(C_v O_2\) = oxygen content of mixed venous blood

The presence of a severe shunt is usually known or suspected by the equine anesthetist based on physical examination and history.

1.3.5 Ventilation-perfusion mismatch

Ventilation-perfusion (V/Q) mismatch refers to the situation that arises when blood flow and ventilation are not uniform throughout the lung. The ideal ratio for V/Q is 1, in other words ventilation and perfusion are evenly matched. Unventilated alveoli that are perfused will have a ratio of zero, whereas ventilated alveoli with no perfusion have a ratio of infinity, and there is V/Q scatter between these two extremes. V/Q mismatching results in hypoxemia because (a) the underventilated alveoli tend to outnumber the
underperfused alveoli, and (b) the underperfused alveoli cannot increase the oxygen saturation of the blood passing through them beyond 100%, and thus are not able to compensate for the low saturation of blood coming from underventilated alveoli. [Nunn 1989] In the absence of a shunt, the presence of V/Q mismatching can be determined from the alveolar gas equation by substituting \( P_{\text{a}} CO_2 \) for \( P_{\text{a}} CO_2 \) and comparing the expected \( P_{\text{a}} O_2 \) with the actual \( P_{\text{a}} O_2 \). [West 1999]

Adult horses commonly develop V/Q mismatching under anesthesia. Dependent lung areas in the anesthetized horse have been shown to develop regions of atelectasis in lung tissue that is compressed by the weight of the abdominal viscera. [Nyman et al 1990] Compression atelectasis occurs in both lateral and dorsal recumbency, the degree of compression being worse in dorsal recumbency. The development of atelectasis in the anesthetized horse is more dramatic than in other domestic species. The marked decrease in lung volume caused by recumbency and anesthesia decreases tension on alveoli allowing them to collapse. [McDonell et al 1979, Nyman et al 1990] Atelectasis is also caused by the anatomy of the horse’s thoracic and abdominal organs: in dorsal and lateral recumbency the abdominal viscera lie within the dome-shaped diaphragm and compress dependent lung tissue (figure 1.1). [Hall et al 2001] The large vertical height of the horse’s lung promotes congestion of the dependent lung, thus exacerbating V/Q mismatch. [Stegmann 1986, Nyman et al 1990]
1.4 Blood flow and ventilation distribution

1.4.1 Distribution of ventilation

The first study of distribution of ventilation in the human lung was conducted by West in 1962. West studied the distribution of ventilation to horizontal slices of lung using radiolabeled $O_2$, and reported that lower slices had better ventilation than upper slices. This distribution, however, can be diminished by increasing the flow rate, such that the distribution of ventilation is more uniform. [Hughes et al 1972] In man, during anesthesia in lateral recumbency, the non-dependent lung receives a greater proportion of total ventilation regardless of mode of breathing. [Rehder and Sessler 1973] A marked decrease in lung volume occurs in laterally and dorsally recumbent horses, which decreases tension on alveoli, thus allowing them to collapse. [McDonell et al 1979, Sorenson and Robinson 1980, Nyman et al 1990] The decrease in lung volumes seen with recumbency in horses is not related to general anesthesia per se. [Sorenson and Robinson 1980] Ventilation is distributed equally to left and right lungs in dorsally recumbent anesthetized horses. [Moens et al 1995] When anesthetized horses are placed in lateral recumbency, body conformation dictates ventilation distribution (see 1.7.1 below).

1.4.2 Distribution of blood flow

Vascular resistance within the lung, and thus blood flow distribution, is largely determined by the pulmonary capillaries. [Nunn 1989, West 1999] Pulmonary vascular
resistance, and thus blood flow distribution, is impacted by passive and active mechanisms.

1.4.2.1 Passive mechanics

The effect of gravity on blood flow distribution is described as a zonal model. [West 1999] At the top of the lung zone 1 may exist where alveolar pressure (P_A) is greater than pulmonary arterial pressure (P_a), and P_a is greater than pulmonary venous pressure (P_v). This zone would have no blood flow, since the P_A would force the capillaries to close, and therefore no gas exchange would occur. The resulting unperfused ventilated lung is referred to as alveolar dead space. Normally zone 1 is a theoretical zone since P_a is sufficient to perfuse the uppermost alveoli. Further down the lung, in zone 2, P_a increases to the point where it is greater than P_A, but P_v is still low and remains less than both of the aforementioned pressures. In this zone blood flow is determined by the P_a-P_A difference, and has the pressure-flow characteristic of a Starling resistor. Zone 3 flow is determined by the P_a-P_v difference, since P_v is increased in the lower lung to the point where it exceeds P_A.

Recruitment of closed capillaries occurs in response to increased blood flow through the lung, and is most likely to occur in the upper lung (zones 1 and 2) where capillary pressure is lowest. These thin-walled pulmonary capillaries also have the ability to distend passively in the face of increased blood flow.
Inflation of the lung to total lung capacity increases the pulmonary vascular resistance by compressing alveolar capillaries. Pulmonary vascular resistance is lowest at functional residual capacity. [Nunn 1989]

1.4.2.2 Active mechanisms

There are numerous receptors in the pulmonary vasculature, both on and in smooth muscle cells as well as endothelial cells, which can control blood vessel tone. These include the adrenergic receptors of the sympathetic nervous system, purine, eicosanoid, and peptide receptors. The major mechanism responsible for regulating regional blood flow such that perfusion matches ventilation is unique to the pulmonary circulation, and is termed hypoxic pulmonary vasoconstriction. [Moudgil et al 2005]

1.5 Hypoxic pulmonary vasoconstriction (figures 1.2, 1.3)

Hypoxic pulmonary vasoconstriction (HPV) is prevalent in small resistance pulmonary arterioles of less than 200µm diameter, which are anatomically adjacent to and in close contact with the alveoli. [Kato and Staub 1966] The stimulus required to trigger HPV is a function of mixed venous and alveolar oxygen tension and does not require neurohumoral input. [Marshall et al 1994] The O₂-sensing mechanism is thought to be located within the electron transport chain of pulmonary arteriole smooth muscle cells, although the precise O₂ sensor has not yet been identified. [Moudgil et al 2005] The primary HPV response occurs in pulmonary arteriole smooth muscle cells, and, although modulated by endothelial cells, will occur in the absence of endothelium. [Murray et al}
1990, Madden et al 1992] The HPV response results in shunting of blood from unventilated lung areas to ventilated areas, thus optimizing matching of ventilation and perfusion, and improving oxygenation. In people the HPV response occurs within minutes of hypoxia developing, and is maximal after fifteen minutes. [Bindslev et al 1985] Persistent hypoxia results in sustained HPV, and HPV resolves rapidly upon return to normoxia. [Archer and Michelakis 2002]

Vasoactive factors from the endothelium have been shown to have a modulating role on HPV, having been implicated in both vasoconstriction and vasodilation. [Aaronsen et al 2002] The degree of the HPV response has been shown to be significantly reduced in the absence of endothelium in isolated equine pulmonary arterial rings. [MacEachern et al 2004] Blockade of endothelin receptors greatly reduces the HPV response and, although other vasoconstrictors have been implicated in HPV, it seems likely that endothelin is a primary mediator of the vasoconstriction response to hypoxia. [Sato et al 2000, Aaronsen et al 2002, Moudgil et al 2005] The endothelial-derived relaxing factor, as it was known until positively identified, is nitric oxide (NO). [Furchgott and Zawadski1980, Moncada et al 1991] Endothelin is thought to be the major mediator in vasoconstriction. [Moudgil et al 2005]

1.6 Nitric oxide

NO, a gas molecule with poor water solubility, is produced constitutively in pulmonary endothelial cells through activation of constitutive nitric oxide synthase (cNOS) by
receptor stimulation. [Lumb 2000, Michalekis 2003] This enzyme converts L-arginine to N-hydroxyarginine, and then N-hydroxyarginine to L-citrulline. The first step requires the cofactors calmodulin and flavine adenine dinucleotide, and in the process also converts NADPH and O₂ to NADP⁺ and H₂O. In the second step O₂ is also converted to NO. [Anggard 1994] NO then diffuses from the endothelial cell to the smooth muscle cell where it activates guanylate cyclase to produce cyclic guanosine monophosphate which decreases intracellular calcium via protein kinase activity, resulting in relaxation of the cell and vasodilation. Citrulline enters the urea cycle where it is converted back into arginine.

NO is a reactive oxygen species and, in vivo, is unstable in the presence of O₂, other reactive oxygen species such as hydrogen peroxide, and oxidizing agents. [Wolin 2000] In the presence of hemoglobin (Hb), NO rapidly binds to heme. [Toothill 1967] When NO combines with deoxyhemoglobin HbNO is formed. The combination of NO with the heme of oxygenated Hb results in the displacement of O₂, oxidation of Hb to methemoglobin and the production of a nitrate ion. [Jia et al 1996] NO can also combine with sulphydryl groups to form S-nitrosohemoglobin, which remains active as a vasodilator. [Jia et al 1996, Stamler 2004] Peroxynitrite forms when NO reacts with H₂O₂. [Michelakis 2003]
1.7 Investigation into the cause and resolution of hypoxemia in the anesthetized horse

Following the early observations of veterinary anesthetists that hypoxemia in anesthetized horses was likely due to V/Q mismatching, investigators sought to account for the predilection for this occurrence in the equine species.

1.7.1 Body position

Horses anesthetized with halothane in 100% oxygen and placed in dorsal recumbency have been reported as having significantly lower PaO$_2$ values (155mmHg) compared to those placed in lateral recumbency (320mmHg). [Steffey et al 1977] Additionally, oxygenation was better in horses in lateral recumbency that were mechanically ventilated (PaO$_2$=446 mmHg) compared to those breathing spontaneously (320 mmHg). Anesthesia in lateral recumbency increases the pulmonary shunt fraction from 4-7% (in conscious recumbent ponies) to 32-34%. [Hall 1984, Weaver and Walley 1975] Dorso-ventral radiographs of the lungs of laterally recumbent anesthetized horses showed that the dependent lung was poorly ventilated, with atelectasis developing after 20 minutes of anesthesia, whereas there was increased ventilation in the non-dependent lung. [McDonell et al 1979] In lateral recumbency the diaphragm is displaced cranially by the weight of the abdominal organs on the dependent lung, whereas in dorsal recumbency it is the caudal lobes of both lungs that are compressed by the abdominal viscera. [McDonell et al 1979] The lungs of a dorsally recumbent horse that died under anesthesia showed severe congestion and alveolar collapse in the caudal lobes that was attributed to compression atelectasis. [Nyman et al 1987, Nyman et al 1990] Horses that
have a flat abdominal outline consistently have higher PaO$_2$ values than those which have a convex outline, regardless of whether they are placed in dorsal or lateral recumbency. [Moens et al 1995]

1.7.2 Ventilation strategies

Selective mechanical ventilation of dependent lung regions with positive end-expiratory pressure (PEEP) of 20cmH$_2$O significantly improved oxygenation. [Nyman et al 1987] This technique requires specialized equipment, and intubation was performed via a tracheostomy, and has not been useful in clinical anesthetic practice as it is not practical.

The effects of 20cmH$_2$O PEEP in the anesthetized horse have been reported as increasing the functional residual capacity and PaO$_2$, however O$_2$ delivery was decreased via the negative effects of PEEP on cardiac output. [Wilson and Soma 1990]

1.7.3 Drugs

Delivery of NO in a pulsatile manner timed to occur with inspiration (4.9µmol/breath) resulted in increased oxygenation. [Heinonen et al 2001] The improvement in gas exchange observed can be explained by the fact that blood vessels in the region of ventilated lung will be exposed to NO, resulting in vasodilation and increased perfusion of the ventilated alveoli, thus improving the V/Q ratio. This is in contrast to continuous delivery of NO at 2.9µmol/breath which failed to affect PaO$_2$, presumably because the dose was inadequate. [Young et al 1999] The delivery of NO in this way has no systemic
effect because NO is rapidly metabolized and/or scavenged by Hb as it enters the pulmonary blood.

Aerosolized albuterol has been shown to improve oxygenation in anesthetized horses, although the mechanism by which this occurred is uncertain. [Robertson and Bailey 2002] Albuterol causes bronchodilation via $\beta_2$ adrenergic agonist activity, but also has low $\beta_1$ activity. Neither cardiac output nor V/Q ratios were measured in this study, so it remains possible that the increased oxygenation could have been due to either improved ventilation or an improvement in both ventilation and perfusion. Systemic activity (cardiovascular effect) is certainly possible as most horses in this study were seen to sweat following albuterol administration. Despite the findings of this study, albuterol does not consistently result in improved PaO$_2$. [Gunkel et al 2004]

Despite the fact that increasing cardiac output using dobutamine has been shown to have no effect on oxygenation, individual patients sometimes do show improvement in PaO$_2$ which, in the absence of other interventions, must be as a result of improved perfusion of ventilated alveoli. [Swanson et al 1986, Muir and Hubbell 1991]

1.8 Methodologies to investigate blood flow distribution

The multiple inert gas technique (MIGET) is used to assess the V/Q relationship, and has been used in horses. [Wagner et al 1974, Nyman et al 1989, Heinonen et al 2001] The MIGET technique is complex, and has been reviewed elsewhere. [Roca and Wagner
Briefly, six inert tracer gases with different solubilities are inhaled. After equilibrium is reached, arterial and end-tidal samples are obtained and retention and elimination of the gases is calculated. A distribution curve is then created for the spectrum of V/Q ratios, consisting of 50 points: 0 (no ventilation), infinity (no perfusion) and 48 points inbetween. This technique gives useful information about V/Q, but does not provide information regarding topographic blood flow distribution. Two V/Q distribution curves are shown in figure 1.4. The upper curve shows a normal scatter of V/Q ratios. The lower curve shows V/Q inequality with increased perfusion to poorly ventilated areas. These two theoretical curves shown have been drawn for illustrative purposes, and are based on curves obtained in horses by other investigators. [Nyman and Hedenstierna 1989, Heinonen et al 2001]

Topographic information about pulmonary function in the horse has been obtained using radiography [McDonell et al 1979, Moens et al 1995], radioactive gases [Amis et al 1984, Hornof et al 1986, O’Callaghan et al 1987] and computerized tomography [Nyman et al 1990]

The use of radioactive and fluorescent microspheres (approximately 15µ diameter) has allowed investigators to characterize blood flow distribution in the equine lung under various conditions, with the major finding common to all studies being the fact that the pattern of perfusion is not solely influenced by gravity. [Hughes et al 1996] This technique has also been used to examine pulmonary blood flow distribution in anesthetized horses. Blood flow distribution has been shown to be greater in the non-
dependent lung, with a distribution gradient from hilus to periphery in anesthetized
horses using scintigraphy. [Staddon et al 1981] Ponies anesthetized with halothane in one
study, and pentobarbital sodium in another, placed in the prone position, and
mechanically ventilated, have been shown to have preferential blood flow to the
caudodorsal lung, as well as to the hilar area. [Dobson et al 1985, Jarvis et al 1992] The
hypoxic pulmonary vasoconstriction response to one-lung hypoxia has been shown to be
active in pentobarbital-anesthetized ponies placed in sternal recumbency. [Elliott et al
1991]

1.9 Study and hypothesis

The adult horse is unique in its development of severe hypoxemia when dorsally
recumbent and anesthetized. Hypoxemia can occur in any horse, including those horses
that are healthy and those that are breathing 100% O₂, and is most commonly caused by
V/Q inequality. Despite strategies to improve ventilation as well as perfusion, no strategy
is available to the equine anesthetist that will consistently improve oxygenation. Hypoxic
pulmonary vasoconstriction normally diverts blood flow away from underventilated
alveoli, and if this mechanism were functioning normally in anesthetized supine adult
horses breathing 100% O₂, PaO₂ would be maintained at values approaching 500mmHg.
The fact that moderate to severe hypoxemia commonly develops in anesthetized horses is
a significant clinical issue which remains incompletely resolved.
The HPV response is different in magnitude among species, and the horse has been shown to have an intermediate response between pigs (which have a strong response) and dogs (weak response). [Elliott et al 1991] To date, the only study of pulmonary blood flow in the supine adult horse was performed during halothane anesthesia. [Dobson et al 1985] Inhalant anesthetics are known to inhibit HPV. [Marshall et al 1984] It is possible that the increased perfusion of the diaphragmatic lung lobes seen in the halothane-anesthetized horses was a result of greater NO activity in the diaphragmatic lobes as suggested by Pelletier et al [1998] or failure of the HPV mechanism due to inhalant anesthetic-induced alteration of NO activity.

Our specific aim was to study the HPV response to hypoxia in anesthetized supine adult thoroughbred horses using a one-lung hypoxic model to initiate HPV, and to determine the role of NO in that response via manipulation of NO production and inhibition. Pulmonary blood flow distribution was evaluated by injection of microspheres into the pulmonary circulation at specific time points.

Our hypothesis was that pulmonary blood flow would be preferentially distributed away from the hypoxic lung in anesthetized supine adult thoroughbred horses. Further, increased synthesis of NO would shift pulmonary blood flow distribution more evenly between the two lungs, and inhibition of NO synthesis would re-establish the HPV seen initially.


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<td>11. Excitement</td>
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Table 1.1 Complications associated with equine anesthesia. [adapted from Muir and Hubbell 1991]
Figure 1.1: Topography of the horse showing position of abdominal viscera relative to the lungs. Top: normal topography in the standing horse. Bottom: anesthetized dorsally recumbent horse showing the abdominal viscera overlying the caudal lung fields.
Figure 1.2: Role of nitric oxide in vasodilation of pulmonary artery smooth muscle cell in the presence of oxygen.
Figure 1.3: Role of endothelin in hypoxic pulmonary vasoconstriction of pulmonary artery smooth muscle cell.
Figure 1.4: Ventilation/perfusion ratio curves produced by the multiple inert gas elimination technique. Top: normal scatter of V/Q ratios. Bottom: V/Q inequality with increased flow to poorly ventilated areas.
CHAPTER 2

MATERIALS AND METHODS

2.1 Horses

Six adult thoroughbred horses, three geldings and three mares, were the subjects of this study. Horses weighed 480±21 kg. Each horse was determined to have normal cardiovascular and respiratory function based on physical examination, including thoracic auscultation, electrocardiogram (ECG), and thoracic radiographs. Hematologic and biochemical blood profiles were normal for all horses in the study. The protocol for the study was approved by the animal care and use committee of The Ohio State University.

2.2 Equipment and calibration

2.2.1 Ventilator and breathing system

A synchronizable dual-lung ventilator was specifically designed and built for use in this study (figure 2.1). The ventilator consisted of 2 separate ventilator bellows and housings electronically synchronized to deliver simultaneous breaths of different or equal tidal volumes to 2 different outlets. Tidal volume delivered from the ventilator was confirmed.
using a respirometer. Each ventilator outlet was attached to the circle system inlet of a small animal anesthetic machine. One anesthesia machine was configured to deliver oxygen only, whereas the second machine was configured to deliver either oxygen or nitrogen. Both ventilators and their respective anesthesia machines were checked for leaks prior to the start of each experiment.

2.2.2 Endotracheal tubes

A double-lumen endotracheal tube was designed and built for use in this study (figure 2.2). The tube consisted of two 18mm internal diameter silicone endotracheal tubes bonded together with silicone. A large endotracheal tube cuff (tracheal cuff) encompassed the entire tube at approximately 2/3 of its length. The distal ends of the individual tubes were separated by a silicone wedge. One distal tube was longer than the other, and was fitted with a small endotracheal tube cuff (bronchial cuff). The distal end of the right tube was partially cut away laterally so as not to occlude the opening of the right apical bronchus. [Moens et al 1992] Integrity of both cuffs was checked prior to each experiment.

2.2.3 Airway gas analysis

The fractional concentration of oxygen (FiO\(_2\)), nitrogen (FiN\(_2\)) and carbon dioxide (FiCO\(_2\)) were continuously monitored using a side-stream respiratory gas analyzer. The

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1 Hallowell Engineering and Manufacturing Corp, Pitsfield, MA
2 Wright Respirometer, Ferraris Medical, Orchard Park, NY
3 Bivona Medical Technologies, Inc, Gary IN
4 Capnomac Ultima, Datex Medical Instrumentation Inc., Tewksbury MA
gas analyzer was calibrated with known concentrations of oxygen, nitrogen and carbon
dioxide, according to specified guidelines.\textsuperscript{5}

2.2.4 Blood gas analysis

A blood gas analyzer was used to determine pH, PO\textsubscript{2} and PCO\textsubscript{2} of arterial
blood.\textsuperscript{6} The analyzer was calibrated according to specified guidelines.\textsuperscript{7} Blood was
drawn anaerobically into heparinized syringes and either placed in an ice bath or
analyzed immediately. Blood from syringes placed in the ice bath was analyzed as soon
as possible and never more than 1hr after having been drawn.

2.2.5 Physiologic data collection

The pulmonary artery pressure, ECG, and systolic, diastolic and mean arterial pressure
were recorded using a data acquisition system.\textsuperscript{8} Pressure transducers were calibrated
prior to each experiment at 0 mmHg (open to room air) and 100 mmHg using a mercury
manometer.\textsuperscript{9} Cardiac output was measured by a cardiac output monitor using the
thermodilution technique.\textsuperscript{10}

\textsuperscript{5} Datex Medical Instrumentation Inc., Tewksbury MA
\textsuperscript{6} ABL-500, Radiometer, Copenhagen, Denmark
\textsuperscript{7} Radiometer, Copenhagen, Denmark
\textsuperscript{8} PO-NE-MAH Digital Acquisition, Analysis, and Archive System version 1.21f, Gould Instrument
Systems, Valley View OH
\textsuperscript{9} Labtron Sphygmomanometer, Kappa Medical, Prescott, AZ
2.3 Experimental design (figure 2.3)

2.3.1 Preparation

Horses were fasted for 12 hours prior to each experiment and allowed free access to water at all times. On the day of the experiment each horse was instrumented while quietly standing in stocks. The area over the right jugular furrow was clipped and aseptically prepared for placement of intravascular and intracardiac catheters. Each catheter site was blocked with 1ml of 2% lidocaine hydrochloride. Two 8-F catheter introducers were placed approximately 20cm apart into the right jugular vein.11 A 150cm 7-F thermodilution catheter was advanced through the distal introducer until positioned in the pulmonary artery.12 Polyethylene tubing was inserted via the proximal introducer into the right atrium.13 A 4cm 20-G Teflon catheter was placed in the facial or transverse facial artery for measurement of arterial blood pressure.14 All catheters were connected to fluid-filled transducers with the scapulohumeral joint used as the reference point in the standing horse.15 Correct positioning of all catheters was determined by observing characteristic pulse waveforms and pressures. Heart rate and rhythm were monitored using a base-apex ECG.

2.3.2. Anesthesia

10 9520A Cardiac output Computer, American Edwards Labs
11 Percutaneous Sheath Introducer Set, Arrow, Erding, Germany
12 thermodilution cath
13 PE240, Becton Dickinson & Co., Sparks, MD
14 Surflo IV Catheter, Terumo Medical Corporation, Elkton, MD
15 TruWave Disposable Pressure Transducer, Edwards Lifesciences, Irvine CA
Following collection of baseline data, each horse was induced to anesthesia with a bolus of sodium pentobarbital (30mg/kg IV). Anesthesia was then maintained by an infusion of 5-15mg/kg/hr of sodium pentobarbital. Once anesthetized, each horse was positioned on its back and a tracheotomy performed. Each horse was intubated via the tracheostomy incision with a double-lumen endotracheal tube. An endoscope was used to confirm correct positioning of the endobronchial portion of the tube. The distal and proximal cuffs of the tube were then inflated. The double lumens were then attached at their proximal ends to the synchronized dual-lung ventilator and intermittent positive pressure ventilation commenced using 100% oxygen to both lungs with the breathing rate set at 6 breaths per minute and tidal volume set at 15ml/kg. Inspiratory pressure was not allowed to exceed 40cmH₂O.

2.3.3 Establishment of one lung hypoxia.

Hypoxic ventilation was initiated in the left lung by changing the gas delivered to the left bellows of the dual-lung ventilator from 100% oxygen to 100% nitrogen. Composition of gas in the separate lung fields was verified using a gas analyzer.  

2.3.4 Manipulation of the nitric oxide pathway

L-arginine (200mg/kg dissolved in 1L of 0.9% sodium chloride), a nitric oxide precursor, was rapidly infused IV in order to antagonize the hypoxic pulmonary vasoconstriction response. N-nitro-L-arginine methyl ester (20mg/kg dissolved in 1L of 0.9% sodium

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16 Nembutal, Abbott Laboratories, North Chicago, IL
17 L-arginine, Sigma-Aldrich, St Louis, MO
chloride), a nitric oxide inhibitor, was infused rapidly IV to promote hypoxic pulmonary vasoconstriction.\textsuperscript{18} [Mills \textit{et al} 1997]

2.3.5 Measurement of pulmonary blood flow distribution

Pulmonary blood flow distribution was determined by injecting 45-60 million inert 15\textmu m fluorescent microspheres were injected into the right atrium over 15 seconds.\textsuperscript{19} [Sinclair \textit{et al} 2000] A reference blood sample was drawn from the pulmonary artery at a rate of 60ml/min to be used for the calculation of blood flow in ml/min during injection of microspheres. A different microsphere color was infused for each measurement, and the order of colors used was randomized. The colors of microspheres used were as follows: black (samarium), violet (antimony), orange (rhenium), yellow (iridium) and red (gold).

2.4 Data collection

Data was collected at five discreet time points. Baseline data were obtained in the standing horse after instrumentation and prior to general anesthesia, while the horses breathed room air. A period of time lasting between 30 and 60 minutes was allowed after induction of anesthesia for stabilization of blood pressures and depth of anesthesia with horses ventilated on 100% oxygen before the second set of data was recorded. The left lung was then ventilated with 100% nitrogen for the remainder of the experiment. Twenty to 30 minutes was allowed to elapse following creation of a hypoxic lung field, after which time data was collected. L-arginine was then administered, and data was recorded after visually observing a decrease in pulmonary artery pressure, which occurred within

\textsuperscript{18} L-NAME, Sigma-Aldrich, St Louis, MO
5-10 minutes, L-NAME was administered after a 20-minute interval, and the final set of data was recorded after visually observing an increase in pulmonary artery pressure, which also occurred within 5-10 minutes.

2.4.1 Cardiorespiratory parameters

Heart rate and heart rhythm as determined by ECG, respiratory rate, systolic, mean and diastolic arterial blood pressure, mean pulmonary artery pressure, arterial blood gas and cardiac output were measured at each time point. End tidal gases (oxygen, nitrogen, carbon dioxide) were measured from left and right lung fields during anesthesia.

2.4.2 Lung blood flow

Microspheres were injected into the right atrium and data were collected at each of the five time points.

2.5 Lung harvesting process

2.5.1 Euthanasia

After the last injection of microspheres, horses were heparinized with 200000 U/horse IV and exsanguinated via carotid artery cannulation until arterial pulse pressure waves were negligible in order to reduce the blood volume in the lungs. Horses were then euthanatized with an overdose of sodium pentobarbital (100ml of a 390mg/ml solution) IV. Biopsies of heart, muscle and kidney were obtained at this time. [Elliot et al 1991]

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19 BioPhysics Assay Laboratory, Inc., Worcester, MA
2.5.2 Lung removal, rinsing and drying

Once the horse was confirmed dead, 6-8 ribs were removed from the right thorax and the trachea, heart and lungs removed from the thoracic cavity. Plastic hoses were inserted in the pulmonary artery via the right ventricle as well as into the left atrium and the lungs were flushed with a sodium-free solution until the fluid from the left atrium was free of blood on gross appearance. The heart was then removed from the lungs. A 26mm internal diameter silicone endotracheal tube was then inserted into the trachea and fastened in place with the cuff inflated. The endotracheal tube and trachea were then suspended from a horizontal bar and the open end of the endotracheal tube connected to a small animal anesthesia machine. The lungs were then inflated to 30-40 cmH₂O with air delivered via the anesthesia machine (figure 2.4). This pressure was maintained for 7-10 days until the lungs were thoroughly dried. Fly deterrent spray was sprayed on the external surface of the lungs in order to prevent fly strike of the lung tissue.

2.5.3 Lung sectioning, sampling and analysis

Each dried set of lungs was encased in foam, which was allowed to harden for 7 days. Lungs were encased in the same anatomical position they would occupy in a standing horse. After hardening, each set of lung was cut in 10cm thick transverse sections using a band saw, resulting in 10 to 12 transverse sections per lung. A grid of 10x10cm squares was then superimposed on each transverse section. Lung tissue was then harvested from each grid square, being careful to extract only lung parenchyma and not cartilaginous or fibrous tissue. Harvested tissue was then placed in pre-weighed plastic sample tubes.

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4 Heparin Sodium, American Pharmaceutical Partners, Schaumburg, IL
Approximately 200 samples were harvested from each horse. Plastic sample tubes were weighed empty and full using a calibrated scientific scale.\textsuperscript{22} The scale was calibrated against a known weight prior to use. Samples were then sealed and analyzed by neutron exposure activation by an analytical laboratory.\textsuperscript{23} [Reinhardt et al 2000] Briefly, samples are exposed to a field of neutrons. The activated samples are then stored for 2 days to allow short-lived activation products to decay. Spectrographic analysis was then performed to measure the concentration of radioactive nuclei present in each sample.

Laboratory analysis reported blood flow results as absolute blood flow per gram of dried tissue according to the following equation:

\[
\text{Absolute blood flow (ml/min/g)} = \frac{[(\text{dpm} / \text{mass})_{\text{segment}} / (\text{dpm} \times \text{time} / \text{volume})_{\text{reference sample}}] \times \text{dpm}} \]

\[\text{dpm} = \text{decays per minute} \]

\[\text{reference sample} = \text{reference blood sample drawn during microsphere injection} \]

2.6 **Statistical Analysis**

Cardiovascular and respiratory data as well as lung blood flow values were analyzed using a two-way analysis of variance for repeated measures. Differences within and among groups were detected with Tukey’s multiple comparison post hoc test. Results were considered to be significant where P<0.05.

\[\text{Euthasol, Virbac Animal health, Fort Worth, TX} \]
\[\text{SansSaLine, BioPhysics Assay Laboratory, Inc., Worcester, MA} \]
\[\text{Mettler AM50, Mettler-Toledo Inc, Columbus, OH} \]
\[\text{Interactive Medical Technologies, San Diego, CA} \]
2.7 References


Figure 2.1: Schematic of dual-lung ventilator. Insp = inspiratory, Ex = driving gas exhaust port.
Figure 2.2: Schematic of double lumen endotracheal tube emphasizing distal portion (not to scale). L=left, R=right.
Figure 2.3: Timeline of events during the in vivo phase of the experiment

- 60 min: Instrumentation while standing breathing room air
  1st color of microspheres injected

- 60 min: Anesthesia, intubation
  Both lungs ventilated with 100% oxygen
  2nd color of microspheres injected

- 30 min: Left lung ventilated with 100% nitrogen
  Right lung ventilated with 100% oxygen
  3rd color of microspheres injected

- 10 min: L-arginine infusion administered
  4th color of microspheres injected

- 20 min: Washout period

- 10 min: L-NAME infusion administered
  5th injection of microspheres
  Euthanasia, biopsies, lungs harvested and rinsed
  Lungs dried
Figure 2.4: Position of suspended lung during air drying, showing the diaphragmatic surface of the lungs.
CHAPTER 3

RESULTS

3.1 Cardiovascular data (table 3.1)

Heart rate (HR) did not change significantly during the experiment. The HR did, however, tend to increase with anesthesia. Cardiac output (CO) was significantly different from baseline at all four subsequent time points (p<0.001). CO after the onset of hypoxic ventilation of one lung differed significantly from anesthesia with 100% oxygen and after L-arginine and L-NAME infusions (p<0.001). Systolic arterial blood pressure (ABP) did not differ significantly over time. ABP showed a decreasing trend with the onset of anesthesia followed by an increasing trend following establishment of hypoxic ventilation of one lung. Mean pulmonary artery pressure (PAP) was significantly decreased after anesthesia, establishment of one lung hypoxia and L-arginine infusion compared to baseline (p=0.002). PAP was significantly increased after establishment of one lung hypoxia, L-arginine infusion and L-NAME infusion compared to after anesthesia (p=0.002). PAP was significantly increased after L-NAME infusion compared
to after L-arginine infusion (p=0.002). Right atrial pressure was not different during the experiment.

3.2 Blood gas data (table 3.2)

No significant differences were detected for pH, PaCO$_2$, HCO$_3^-$, tCO$_2$ or BE. PaO$_2$ was significantly increased after anesthesia, and significantly decreased after establishment of one lung hypoxia, L-arginine infusion and L-NAME infusion compared to baseline (p<0.001). PaO$_2$ was significantly decreased at the three time points subsequent to anesthesia (p<0.001). PaO$_2$ was also decreased after L-arginine infusion compared to establishment of one lung hypoxia.

3.3 Pulmonary blood flow data

Pulmonary blood flow data is presented in a series of 22 figures representing transverse slices of lung (from cranial to caudal)(fig 3.1 to 3.11 and 3.18 to 3.28), a series of 12 figures representing longitudinal slices (from dorsal to ventral)(fig 3.12 to 3.17 and 3.29-34), and a series of 5 figures showing the six longitudinal slices of the whole lung at each time point (fig 3.35-3.39).

Figures were constructed from average blood flow for each unique cube of lung tissue. Blood flow was color coded according to the following key:
Grey – no blood flow
Purple – 0-25 ml/min/g
Blue – 25-50 ml/min/g
Cyan – 50-75 ml/min/g
Green – 75-100 ml/min/g
Yellow – 100-125 ml/min/g
Orange – 125-150 ml/min/g
Red – 150-175 ml/min/g
Fuschia – greater than 175 ml/min/g

Blood flow was analyzed in two ways. First, as an absolute value, i.e. that recorded from microsphere analysis (figures 3.1-3.17), and secondly normalized for cardiac output (figures 3.18-3.34). The second analysis was performed since cardiac output changed significantly during the experiment.

A standard letter code is observed throughout figures 3.1-3.34 indicating statistical significance as follows for the absolute blood flow data:

a – different from baseline (standing breathing room air)
b – different from anesthesia (anesthetized in dorsal recumbency breathing 100% oxygen)
c – different from establishment of one lung hypoxia (anesthetized in dorsal recumbency; one lung ventilated with 100% O₂ and one lung ventilated with 100% N₂)
d – different from L-arginine infusion (L-arginine infused with horse anesthetized in dorsal recumbency; one lung ventilated with 100% O₂ and one lung ventilated with 100% N₂)

An asterisk (*) indicates that blood flow is significantly different between left and right lung fields within the same section.

A standard letter code was used to denote statistical significance in figures 3.35-3.39 where differences due to vertical height were analyzed for each time point:

a – different from longitudinal section 1
b – different from longitudinal section 2
c – different from longitudinal section 3
d – different from longitudinal section 4

Longitudinal section 6 was excluded from statistical analysis for differences due to vertical height because the number of data points in this slice was too small for analysis. This section is shown in all figures for the sake of completeness.

3.3.1 Absolute pulmonary blood flow

Absolute blood flow was calculated from averages for all cubes of left or right lung in a given section.
3.3.1.1  Transverse section 1 (figure 3.1)

Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline (p<0.001).

Average blood flow to the right lung was also significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline (p<0.001).

Blood flow was not different between left and right at any time point.

3.3.1.2  Transverse section 2 (figure 3.2)

Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline (p<0.001).

Average blood flow to the right lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline (p<0.001).

Average blood flow was not different between left and right at any time point.

3.3.1.3  Transverse section 3 (figure 3.3)

Left lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline (p<0.001).
Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline (p<0.001).

Blood flow was greater to the right side compared to the left at baseline (p=0.005), after L-arginine infusion (p=0.03) and after L-NAME infusion (p=0.008).

3.3.1.4 Transverse section 4 (figure 3.4)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline.
Blood flow was increased after establishing one lung hypoxia to after anesthesia. Blood flow was decreased after L-arginine infusion compared to after establishment of hypoxia (p<0.001).

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishing one lung hypoxia to after anesthesia (p<0.001).

Average blood flow was not different between left and right at any time point.

3.3.1.5 Transverse section 5 (figure 3.5)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood
flow was increased after establishment of hypoxia, and decreased after L-arginine and L-NAME infusions, compared to after establishment of hypoxia. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was decreased after L-arginine and L-NAME infusions compared to after anesthesia and after establishment of one lung hypoxia. (p<0.001)

Average blood flow was not different between left and right at any time point.

3.3.1.6 Transverse section 6 (figure 3.6)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Average blood flow to the left lung was significantly increased after establishment of hypoxia, and decreased after L-arginine and L-NAME infusions compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. Blood flow was also decreased after L-NAME infusion compared to after L-arginine infusion. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of hypoxia, and decreased after L-arginine and L-NAME infusions
compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. (p<0.001)

Average blood flow was not different between left and right at any time point.

3.3.1.7 Transverse slice 7 (figure 3.7)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Average blood flow to the left lung was significantly decreased after L-arginine and L-NAME infusions compared to after anesthesia and after establishment of hypoxia. Blood flow was also decreased after L-NAME infusion compared to after L-arginine infusion. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of hypoxia, and decreased after L-NAME infusions compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. (p<0.001)

Average blood flow was not different between left and right at any time point.
3.3.1.8 Transverse section 8 (figure 3.8)

Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Average blood flow to the left lung was significantly increased after establishment of hypoxia, and decreased after L-arginine and L-NAME infusions compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of hypoxia, and decreased after L-NAME infusions compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. (p<0.001)

Average blood flow was greater to the right side than to the left after L-NAME infusion (p=0.048).

3.3.1.9 Transverse section 9 (figure 3.9)

Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. (p<0.001)
Right lung: Average blood flow was significantly decreased after anesthesia and after L-arginine and L-NAME infusions compared to baseline. Blood flow was increased after establishment of hypoxia compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. (p=0.001)

Average blood flow was greater to the right side than to the left after L-arginine (p=0.04) and L-NAME (p=0.047).

3.3.1.10 Transverse section 10 (figure 3.10)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of hypoxia compared to after anesthesia. Blood flow was decreased after L-arginine infusion compared to after establishment of hypoxia. (p<0.001)

Right lung: Average blood flow was significantly decreased after anesthesia and after L-arginine and L-NAME infusions compared to baseline. Blood flow was increased after establishment of hypoxia and after L-NAME infusion compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after establishment of hypoxia. (p<0.001)
Blood flow was greater to the right side than the left after anesthesia (p=0.032) and after L-NAME infusion (p=0.039). Blood flow difference between left and right approached significance after L-arginine (p=0.051), with the trend that flow to the right side was higher.

3.3.1.11 Transverse section 11 (figure 3.11)
Left lung: Average blood flow to the left lung was significantly decreased after anesthesia and L-arginine infusion when compared to average blood flow at baseline. Blood flow was increased after establishment of hypoxia compared to after anesthesia. Blood flow was decreased after L-arginine infusion compared to after establishment of hypoxia. (p=0.013)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. (p<0.001)

Blood flow to the right side was higher than flow to the left at all time points. p values ranged between p<0.001 and p=0.02

3.3.1.12 Longitudinal section 1 (figure 3.12)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of one lung hypoxemia compared to after
anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after hypoxia. (p=0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of one lung hypoxia and decreased after L-arginine and L-NAME infusions compared to after anesthesia. (p<0.001)

Average blood flow was not different between left and right lungs.

3.3.1.13 Longitudinal section 2 (figure 3.13)

Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of hypoxemia, and decreased after L-arginine and L-NAME infusion compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after hypoxia. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of one lung hypoxia and decreased after L-arginine and L-NAME infusions compared to after anesthesia. (p<0.001)
Average blood flow was greater to the right lung than to the left after L-arginine infusion (p=0.038) and after L-NAME infusion (p=0.021).

3.3.1.14 Longitudinal section 3 (figure 3.14)

Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased after establishment of hypoxemia, and decreased after L-arginine and L-NAME infusion compared to after anesthesia. Blood flow was decreased after L-arginine and L-NAME infusions compared to after hypoxia. Blood flow was decreased after L-NAME infusion compared to after L-arginine infusion. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood flow was increased decreased after L-arginine and L-NAME infusions compared to after hypoxia. (p<0.001)

Average blood flow trended higher on the left side of this section after establishment of hypoxia, but was not significant (p=0.054).

3.3.1.15 Longitudinal section 4 (figure 3.15)

Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. Blood
flow was decreased after L-arginine and L-NAME infusion compared to after anesthesia and after hypoxia. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. (p<0.001)

Average blood flow was not different between left and right lungs.

3.3.1.16 Longitudinal section 5 (figure 3.16)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. (p<0.001)

Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. (p<0.001)

Average blood flow was not different between left and right lungs.

3.3.1.17 Longitudinal section 6 (figure 3.17)
Left lung: Average blood flow to the left lung was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. (p<0.001)
Right lung: Average blood flow was significantly decreased at all time points subsequent to baseline when compared to average blood flow at baseline. (p<0.001)

Average blood flow was not different between left and right lungs.

3.3.2 Blood flow distribution normalized for cardiac output

Absolute blood flow for each lung section was normalized for cardiac output. The absolute blood flow of each lung cube was divided by the cardiac output at each time point and multiplied by the cardiac output at baseline.

3.3.2.1 Transverse section 1 (figure 3.18)

Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all time points subsequent to baseline when compared to average ratio at baseline (p<0.001).

3.3.2.2 Transverse section 2 (figure 3.19)

Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all time points subsequent to baseline when compared to average ratio at baseline (p<0.001).
3.3.2.3 Transverse section 3 (figure 3.20)

Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all time points subsequent to baseline when compared to average ratio at baseline (p<0.001). Average blood flow : cardiac output ratio of right lung was significantly decreased after hypoxia, L-arginine infusion and L-NAME infusion compared to after anesthesia baseline when compared to average blood flow at baseline (p<0.001).

3.3.2.4 Transverse section 4 (figure 3.21)

Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all time points subsequent to baseline when compared to average ratio at baseline (p<0.001). Average blood flow : cardiac output ratio of right lung was significantly decreased after hypoxia, L-arginine infusion and L-NAME infusion compared to after anesthesia baseline when compared to average blood flow at baseline (p<0.001).

3.3.2.5 Transverse section 5 (figure 3.22)

Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all time points subsequent to baseline when compared to average ratio at baseline (p<0.001). The ratio was increased in the left lung after hypoxia compared to after anesthesia, and decreased after L-arginine and L-NAME compared to after hypoxia. In the right lung the ratio was decreased after hypoxia, L-arginine and L-NAME compared to after anesthesia.
3.3.2.6 Transverse section 6 (figure 3.23)
Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all time points subsequent to baseline when compared to average ratio at baseline (p<0.001), except in the right lung after anesthesia when the ration was not different, and in the left lung after hypoxia when the ration was higher. In the right lung the ratio was decreased after hypoxia, L-arginine and L-NAME compared to after anesthesia. In the left lung the ration was decreased after L-arginine and L-NAME compared to after hypoxia, and decreased after L-NAME compared to after L-arginine.

3.3.2.7 Transverse section 7 (figure 3.24)
Average blood flow : cardiac output ratio of the left lung was significantly decreased after L-arginine and L-NAME compared to baseline, after anesthesia and after hypoxia (p<0.001). In the left lung the ratio was also decreased after L-NAME compared to after L-arginine. Average blood flow : cardiac output ratio of the right lung was significantly decreased after hypoxia, L-arginine and L-NAME compared to baseline (p<0.001). The ration was also decreased after L-arginine and L-NAME compared to after anesthesia and after hypoxia (p<0.001).

3.3.2.8 Transverse section 8 (figure 3.25)
Average blood flow : cardiac output ratio of the left lung was the same after anesthesia, significantly increased after hypoxia, and significantly decreased after L-arginine and L-NAME compared to baseline (p<0.001). The ration was increased after hypoxia and decreased after L-arginine and L-NAME compared to after anesthesia. The ration was
also decreased after L-arginine and L-NAME after hypoxia, and after L-NAME compared to after L-arginine.

Average blood flow : cardiac output ratio of the right lung was significantly increased after anesthesia and hypoxia, and significantly decreased after L-arginine and L-NAME compared to baseline (p<0.001). The ratio after L-NAME was also increased compared to after L-arginine.

3.3.2.9 Transverse section 9 (figure 3.26)
Average blood flow : cardiac output ratio of the left lung was significantly decreased after L-arginine and L-NAME compared to after baseline, anesthesia and hypoxia (p<0.001).

Average blood flow : cardiac output ratio of the right lung was significantly increased after hypoxia, L-arginine and L-NAME compared to after baseline (p<0.001).

3.3.2.10 Transverse section 10 (figure 3,27)
Average blood flow : cardiac output ratio of the left lung was significantly decreased after anesthesia compared to after baseline (p<0.001).

Average blood flow : cardiac output ratio of the right lung was significantly increased after hypoxia compared to after baseline and anesthesia (p<0.001). The ratio was decreased after L-arginine and L-NAME compared to after hypoxia.
3.3.2.11 Transverse section 11 (figure 3.28)

Average blood flow : cardiac output ratio of the right lung was significantly decreased after anesthesia, hypoxia and L-NAME compared to after baseline (p<0.001). The ratio was also decreased after L-NAME compared to after L-arginine.

3.3.2.12 Longitudinal section 1 (figure 3.29)

Average blood flow : cardiac output ratio of the left and right lung was significantly increased at all time points subsequent to baseline when compared to average ratio at baseline (p<0.001).

The ratio was increased in the left lung after hypoxia when compared to anesthesia. The ration was decreased on the left after L-arginine and L-NAME compared to after hypoxia.

3.3.2.13 Longitudinal section 2 (figure 3.30)

Average blood flow : cardiac output ratio of the left lung was significantly increased after hypoxia when compared to average ratio at baseline (p<0.001). The ratio was decreased after anesthesia, L-arginine and L-NAME when compared to average ratio at baseline and after hypoxia.

Average blood flow : cardiac output ratio of the right lung was significantly increased after anesthesia and hypoxia when compared to average ratio at baseline (p<0.001). The
ratio was decreased after L-arginine and L-NAME compared to after baseline, anesthesia and after hypoxia.

3.3.2.14 Longitudinal section 3 (figure 3.31)
Average blood flow : cardiac output ratio of the left lung was significantly decreased after anesthesia, L-arginine and L-NAME, and increased after hypoxia when compared to average ratio at baseline (p<0.001). The ratio increased after hypoxia compared to after anesthesia. The ratio decreased after L-arginine and L-NAME compared to after anesthesia and hypoxia, and also decreased after L-NAME compared to after L-arginine.

Average blood flow : cardiac output ratio of the right lung was significantly decreased at all subsequent time points when compared to average ratio at baseline (p<0.001). The ratio was decreased after L-arginine and L-NAME compared to after anesthesia.

3.3.2.15 Longitudinal section 4 (figure 3.32)
Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all subsequent time points when compared to average ratio at baseline (p<0.001). The ratio in the left lung was also decreased after L-arginine and L-NAME compared to after anesthesia and after hypoxia. The ration in the right lung was decreased after hypoxia, L-arginine and L-NAME compared to after anesthesia.
3.3.2.16 Longitudinal section 5 (figure 3.33)
Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all subsequent time points when compared to average ratio at baseline (p<0.001).

3.3.2.17 Longitudinal section 6 (figure 3.34)
Average blood flow : cardiac output ratio of the left and right lung was significantly decreased at all subsequent time points when compared to average ratio at baseline (p<0.001).

3.3.3 Differences due to vertical height

Blood flow was heterogeneous in the majority of longitudinal isogravitational slices regardless of time point.

3.3.3.1 Standing breathing room air (figure 3.35)
Average blood flow to the 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th} and 5\textsuperscript{th} longitudinal sections was increased compared with the 1\textsuperscript{st} (uppermost) longitudinal section.

3.3.3.2 Anesthetized with both lungs ventilated with 100% oxygen (figure 3.36)
Blood flow to the 2\textsuperscript{nd} and 3\textsuperscript{rd} longitudinal sections was increased compared to the 1\textsuperscript{st} (lowest), 4\textsuperscript{th} and 5\textsuperscript{th} sections. Blood flow was decreased in the 5\textsuperscript{th} section compared to sections 1 to 4.
3.3.3.3 Anesthetized with left lung ventilated with nitrogen (figure 3.37)

Blood flow was increased in sections 2 and 3 compared to section 1 (lowest section).

Blood flow in section 4 was decreased compared to sections 1-3, and decreased in section 5 compared to sections 1-4.

3.3.3.4 Anesthetized with left lung hypoxia and L-arginine infusion (figure 3.38)

Blood flow was decreased in section 4 compared to sections 1-3, and decreased in section 5 compared to sections 1-4.

3.3.3.5 Anesthetized with left lung hypoxia and L-NAME infusion (figure 3.39)

Blood flow was decreased in section 4 compared to sections 1-3, and decreased in section 5 compared to sections 1-4.

3.3.4 Differences horizontally within the lung.

Average blood flow for each transverse section was compared in the standing horse and in the anesthetized horse. The arrows indicate whether the average blood flow was significantly increased or decreased (p=0.05) compared with the preceding section. A “-“ symbol indicates that blood flow did not differ from the preceding section.

3.3.4.1 Standing breathing room air (figure 3.40)

Blood flow was highest in transverse sections 5,6 and 7 and lowest in transverse sections 1 and 11.
3.3.4.2 Anesthetized with both lungs ventilated with 100% oxygen (figure 3.41)

Blood flow was highest in transverse sections 7 and 8, and lowest in sections 1, 2, 10 and 11.

3.4 Kidney and heart biopsies

No blood flow was detected for any of the kidney or heart biopsies, indicating that an undetectable amount of microspheres left the pulmonary vasculature.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>HR bpm ± sd</th>
<th>CO L/min ± sd</th>
<th>SAP mmHg ± sd</th>
<th>MAP mmHg ± sd</th>
<th>DAP mmHg ± sd</th>
<th>PAP mmHg ± sd</th>
<th>RAP mmHg ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (air)</td>
<td>59.67 ± 30.22</td>
<td>26.78 ± 6.59</td>
<td>125.17 ± 18.41</td>
<td>103.67 ± 9.37</td>
<td>88.00 ± 7.07</td>
<td>28.84 ± 8.98</td>
<td>2.08 ± 4.81</td>
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<tr>
<td>Anesthesia (O2/O2)</td>
<td>86.00 ± 29.74</td>
<td>14.10a ± 7.19</td>
<td>104.83 ± 29.97</td>
<td>91.17 ± 31.04</td>
<td>82.67 ± 31.34</td>
<td>13.02a ± 4.07</td>
<td>0.84 ± 2.79</td>
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<tr>
<td>Hypoxia (O2/N2)</td>
<td>85.00 ± 46.59</td>
<td>18.37ab ± 6.03</td>
<td>146.17 ± 51.13</td>
<td>128.67 ± 48.67</td>
<td>121.83 ± 52.58</td>
<td>21.02ab ± 2.12</td>
<td>1.04 ± 2.93</td>
</tr>
<tr>
<td>L-arginine (O2/N2)</td>
<td>87.00 ± 39.22</td>
<td>15.18ac ± 3.28</td>
<td>131.83 ± 33.59</td>
<td>115.17 ± 31.98</td>
<td>110.17 ± 33.70</td>
<td>19.10ab ± 2.81</td>
<td>1.20 ± 3.49</td>
</tr>
<tr>
<td>L-NAME (O2/N2)</td>
<td>98.17 ± 62.46</td>
<td>14.64ac ± 4.32</td>
<td>128.17 ± 44.17</td>
<td>113.50 ± 43.83</td>
<td>103.67 ± 42.07</td>
<td>24.02bd ± 5.34</td>
<td>2.48 ± 4.63</td>
</tr>
</tbody>
</table>

Table 3.1: Results for HR (heart rate), CO (cardiac output), SAP, MAP, DAP (systolic, mean and diastolic arterial blood pressure), PAP (mean pulmonary artery pressure) and RAP (mean right atrial pressure). a – different compared to baseline, b – different compared to anesthesia, c – different compared to establishment of one lung hypoxia, d – different compared to L-arginine.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH ± sd</th>
<th>PaCO₂ mmHg ± sd</th>
<th>PaO₂ mmHg ± sd</th>
<th>HCO₃⁻ mmol/L ± sd</th>
<th>tCO₂ mmol/L ± sd</th>
<th>BE mmol/L ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (air)</td>
<td>7.41 ± 0.02</td>
<td>41.84 ± 4.55</td>
<td>92.78 ± 11.89</td>
<td>26.70 ± 2.46</td>
<td>27.96 ± 2.56</td>
<td>2.50 ± 2.03</td>
</tr>
<tr>
<td>Anesthesia (O₂/O₂)</td>
<td>7.41 ± 0.03</td>
<td>40.16 ± 5.97</td>
<td>277.41ᵃ ± 179.50</td>
<td>25.60 ± 2.71</td>
<td>26.82 ± 2.86</td>
<td>1.56 ± 2.05</td>
</tr>
<tr>
<td>Hypoxia (O₂/N₂)</td>
<td>7.36 ± 0.03</td>
<td>46.86 ± 8.62</td>
<td>48.62ᵇˢ ± 10.56</td>
<td>26.42 ± 3.32</td>
<td>27.86 ± 3.55</td>
<td>1.10 ± 2.24</td>
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<tr>
<td>L-arginine (O₂/N₂)</td>
<td>7.46 ± 0.04</td>
<td>41.28 ± 5.04</td>
<td>38.94ᵃᵇˢ ± 3.30</td>
<td>29.68 ± 2.62</td>
<td>30.96 ± 2.72</td>
<td>5.82 ± 2.28</td>
</tr>
<tr>
<td>L-NAME (O₂/N₂)</td>
<td>7.39 ± 0.06</td>
<td>45.30 ± 6.12</td>
<td>42.94ᵃ ± 4.69</td>
<td>27.12 ± 0.95</td>
<td>28.48 ± 0.83</td>
<td>2.12 ± 2.20</td>
</tr>
</tbody>
</table>

Table 3.2: Results for pH, PaCO₂ (arterial carbon dioxide tension), PaO₂ (arterial oxygen tension), HCO₃⁻ (bicarbonate), tCO₂ (total carbon dioxide) and BE (base excess). a – different compared to baseline, b – different compared to anesthesia, c – different compared to hypoxia, d – different compared to L-arginine.
Figure 3.1: Absolute blood flow in transverse lung section 1
Figure 3.2: Absolute blood flow in transverse lung section 2
Figure 3.3: Absolute blood flow in transverse lung section 3
Figure 3.4: Absolute blood flow in transverse lung section 4
Figure 3.5: Absolute blood flow in transverse lung section 5
Figure 3.6: Absolute blood flow in transverse lung section 6
Figure 3.7: Absolute blood flow in transverse lung section 7
Figure 3.8: Absolute blood flow in transverse lung section 8
Figure 3.9: Absolute blood flow in transverse lung section 9
Figure 3.10: Absolute blood flow in transverse lung section 10
Figure 3.11: Absolute blood flow in transverse lung section 11
Figure 3.12: Absolute blood flow in longitudinal lung section 1
Figure 3.13: Absolute blood flow in longitudinal lung section 2
Figure 3.14: Absolute blood flow in longitudinal lung section 3
Figure 3.15: Absolute blood flow in longitudinal lung section 4
Figure 3.16: Absolute blood flow in longitudinal lung section 5
Figure 3.17: Absolute blood flow in longitudinal lung section 6
Figure 3.18: Blood flow in transverse lung section 1 corrected for cardiac output
Figure 3.19: Blood flow in transverse lung section 2 corrected for cardiac output
Figure 3.20: Blood flow in transverse lung section 3 corrected for cardiac output
Figure 3.21: Blood flow in transverse lung section 4 corrected for cardiac output
Figure 3.22: Blood flow in transverse lung section 5 corrected for cardiac output
Figure 3.23: Blood flow in transverse lung section 6 corrected for cardiac output
Figure 3.24: Blood flow in transverse lung section 7 corrected for cardiac output
Figure 3.25: Blood flow in transverse lung section 8 corrected for cardiac output
Figure 3.26: Blood flow in transverse lung section 9 corrected for cardiac output
Figure 3.27: Blood flow in transverse lung section 10 corrected for cardiac output
Figure 3.28: Blood flow in transverse lung section 11 corrected for cardiac output
Figure 3.29: Blood flow in longitudinal lung section 1 corrected for cardiac output
Figure 3.30: Blood flow in longitudinal lung section 2 corrected for cardiac output
Figure 3.31: Blood flow in longitudinal lung section 3 corrected for cardiac output
Figure 3.32: Blood flow in longitudinal lung section 4 corrected for cardiac output
Figure 3.33: Blood flow in longitudinal lung section 5 corrected for cardiac output
Figure 3.34: Blood flow in longitudinal lung section 6 corrected for cardiac output
Figure 3.35: Differences in blood flow due to vertical height standing breathing room air.
Figure 3.36: Differences in blood flow due to vertical height anesthetized and both lungs ventilated with 100% oxygen.
Figure 3.37: Differences in blood flow due to vertical height anesthetized with left lung hypoxic.
Figure 3.38: Differences in blood flow due to vertical height anesthetized with left lung hypoxic after L-arginine infusion.
Figure 3.39: Differences in blood flow due to vertical height anesthetized with left lung hypoxic after L-NAME infusion.
Figure 3.40: Differences in blood flow in successive transverse slices in the standing horse breathing room air.
Figure 3.41: Differences in blood flow in successive transverse slices in the anesthetized horse ventilated with 100% oxygen.
4.1 Summary of findings

The major findings of the study are:

1. Blood flow distribution in the standing horse showed isogravitational heterogeneity rather than a gravitational gradient.

2. The isogravitational pattern of blood flow distribution pattern was similar in the anesthetized horses.

3. Hypoxic pulmonary vasoconstriction was not effective in shunting blood from the hypoxic to the hyperoxic lung, and did not prevent systemic hypoxemia from developing.

4. Administration of L-arginine did not alter blood flow distribution, and minimally affected PaO$_2$.

5. Administration of L-NAME caused blood flow redistribution from the hypoxic to the hyperoxic lung some areas of the caudal lung field, but did not affect PaO$_2$ significantly.
4.2 Blood flow distribution in the standing horse.

Blood flow was greatest in the 3rd and 4th longitudinal lung sections, and least in the 1st section. This overall pattern only somewhat follows the gravitational distribution suggested by West [1999]. This is consistent with previous findings in standing horses that demonstrate gravity is a minor determinant of blood flow distribution, and that there is not a consistent vertical gradient to pulmonary blood flow. [Hlastala et al 1996]

Hlastala et al [1996] also reported that lung blood volume was lowest in the most cranial part of the lung and, although we did not measure lung blood volume in our study, blood flow to the cranial lung was decreased compared to the middle of the lung, and similar to the most caudal part of the lung in some longitudinal slices (1 and 2). The 6th longitudinal slice consists solely of cranial lung, and, although the number of samples from this area is small, blood flow to this area was lower than in slices 2-5 despite the fact that this is the lowest part of the lung in the standing horse and thus under the highest gravitational force.

When each longitudinal slice is considered separately, it is apparent that blood flow is relatively homogeneous in the first slice, whereas in the 2nd to 5th slices blood flow shows markedly preferential distribution to the hilar region. Microsphere studies of blood flow in the standing and exercising horse have been reported which show that blood flow within isogravitational planes of the lung are heterogeneous. [Hlastala et al 1996, Sinclair et al 2000] These studies showed that the heterogeneity of pulmonary blood flow can be characterized by fractal dimension, which decreases with exercise. It is not surprising
that pulmonary blood flow should have fractal properties, since the anatomic nature of
the bronchial tree is also fractal. [McNamee 1991] It was not possible to perform
analysis of fractal dimension in our study since the distance between sample lung pieces
was too great.

Blood flow was greatest in horizontal sections 5, 6, and 7 compared to cranial and caudal
sections.

2.4 Blood flow distribution pattern in the anesthetized horse

Absolute blood flow was generally decreased to all areas of the lungs during anesthesia
with both lungs supplied with 100% O\textsubscript{2} compared to the standing horse. This can be
attributed to the decrease in cardiac output observed during anesthesia. A vertical
gradient existed in the anesthetized horses, with blood flow lowest in the uppermost
(ventral) longitudinal slice (slice 6) and increasing down the lung, although the lowest
longitudinal slice (slice 1) did not have higher blood flow than slice 2. Blood flow was
also heterogeneous in the isogravitational plane, with blood flow distributed away from
the cranial lung to the hilus and the caudal areas in slice 5, and 4. Blood flow was greatest
in horizontal sections 7 and 8 compared to cranial and caudal sections. The pattern is
similar to that seen in the standing horse, but more marked. This could be attributed to the
decreased cardiac output, which would tend to favor blood flowing to the areas of lung
closest to the hilus. Another factor that could impact blood flow is the fact that
throughout anesthesia intermittent positive pressure ventilation (IPPV) was used. This
mode of ventilation increases intrathoracic pressure and decreases venous return to the heart, thus decreasing cardiac output further. [Hodgson et al 1986] Atelectasis of alveoli has been shown to develop in the dependent portions of the lungs within 20 minutes of anesthesia in the dorsally recumbent horse, and lasts for the duration of anesthesia. [Nyman et al 1990] It is likely that this occurred in the anesthetized horses since average PaO₂ at this time point was 277mmHg, and not the expected value of approximately 500mmHg. This indicates that ventilation/perfusion inequality was present. Blood flow to the lowest portions of the lung, where atelectasis is most likely to occur due to compression by the overlying abdominal organs, was higher than to more dorsal areas in our horses. Nyman et al [1990] showed that the atelectatic lung tissue was also congested, indicating that blood was not shunted away from the physiologic deadspace, and that this was largely responsible for the mismatch of ventilation and perfusion.

2.5 Blood flow distribution in the hyperoxic/hypoxic lung model

In order to evaluate the effectiveness of HPV in the lung of the anesthetized horse we elected to use a model where one lung is hypoxic (ventilated with 100% N₂) and one lung is hyperoxic (ventilated with 100% O₂). The general pattern of blood flow after establishing hypoxia in the left lung was similar to that seen when both lungs were ventilated with 100% oxygen, which was unexpected as there was no redirecting of blood flow away from the hypoxic lung. The first point to note is that the PaO₂ after establishing hypoxia in the left lung was 48mmHg, in other words the horses were systemically hypoxemic at this time. Systemic hypoxemia (PaO₂ <50mmHg) causes the
pulmonary arteries that are supplied by the bronchial arteries to vasodilate. [Marshall et al 1991] This occurs in pulmonary arteries >500µ in diameter. [Marshall et al 1994] Pulmonary arterial vessels <500µ in diameter are the effectors of the HPV response, and vasoconstriction occurs as a function of alveolar oxygen tension and mixed venous oxygen tension. [Marshall et al 1983] Thus in our model it is apparent that failure of HPV lead to hypoxemia which resulted in generalized vasodilation of the pulmonary arterial vasculature. The HPV response decreases in proportion to the number of alveoli that are hypoxic. As the hypoxic segment increases, the effectiveness of blood flow diversion decreases, and if the entire lung is hypoxic there is no blood flow diversion. [Marshall et al 1994] The fact that the entire left lung was hypoxic in our study may have limited the effectiveness of the HPV response, thus resulting in systemic hypoxemia. This is in contrast to pentobarbital-anesthetized ponies where the left lung was ventilated with 4% O₂ and the right lung with 100% O₂ where a large percentage of blood was diverted to the hyperoxic lung and PaO₂ decreased from 520 to 396 mmHg. [Elliot et al 1991] Elliot’s study differed from ours in that ponies were used in comparison to thoroughbred horses, whose lungs have an increased vertical lung height and therefore the lungs are under increased hydrostatic forces. [Hlastala and Glenny 1999] The ponies were also suspended in sternal recumbency, compared to our dorsally recumbent horses. This means that the abdominal viscera of the ponies were in the normal position and not compressing their caudal lung fields. Additionally, the ponies’ lungs were exposed to hydrostatic forces in their normal position, rather than upside down. Areas of lung that had high blood flow and low blood flow in the supine position in dogs retained their blood flow characteristics when placed in dorsal recumbency. [Glenny and
Robertson1990] It is possible that by placing horses in dorsal recumbency areas of lung
retained some of their blood flow characteristics seen in the standing position regardless
of the distribution of ventilation.

Cardiac output increased compared to when both lungs were ventilated with 100% O₂,
which can be attributed to hypoxemia-induced stimulation of the sympathetic nervous
system which increases heart rate and cardiac contractility. [Lumb 2000]

2.6 Blood flow distribution in response to NO synthesis

The blood flow distribution pattern was largely the same as under 4.4. This is most likely
due to the fact that, despite administration of L-arginine to increase production of NO,
HPV had not shunted blood away from the hypoxic lung. Additionally, the presence of
systemic hypoxemia likely caused pulmonary vasodilation (discussed above) prior to this
stage of the experiment, rendering the vasodilatory effects of NO redundant. [Marshall et
al 1991]

Administration of L-arginine decreased pulmonary arterial pressure compared to when
horses were ventilated with 100% O₂, indicating that the infusion likely impacted
pulmonary vascular tone through increased NO production. This decrease in pulmonary
artery pressure could explain the decrease in the blood flow (corrected for cardiac output)
seen in many of the horizontal sections compared to when both lungs were ventilated
with 100% O₂.
A drop in PaO$_2$ did, however, occur following L-arginine administration, which was significantly lower (38 mmHg) compared to the hyperoxic/hypoxic situation without L-arginine (48 mmHg). This may be due to small alterations in blood flow distribution towards unventilated alveoli which our model was not sensitive enough to detect. Sensitivity of microsphere blood flow studies is related to the number of pieces of the organ under investigation sampled, and, had we increased our sample number, we may have been able to detect more subtle shifts in blood flow distribution.[Glenny and Robertson 1990, Sinclair et al 2000]

2.7 Blood flow distribution in response to NO synthase inhibition

Blood flow distribution after administration of L-NAME produced the same general pattern as seen after ventilating the left lung with nitrogen and after administration of L-arginine, i.e. a hilus-to-periphery gradient. In some of the transverse sections (sections 6, 7, and 8) blood flow was decreased in the left (hypoxic) lung compared to after L-arginine administration, but only increased in the right (hyperoxic) lung in transverse section 8. Despite the influence of systemic hypoxemia on pulmonary arterial vessel tone, an increase in pulmonary arterial pressure concomitant with L-NAME infusion was seen, indicating that the infusion was responsible for the change in vascular tone via L-NAME’s ability to block NO synthase. It is likely that inhibition of NO production with resultant reinstatement of HPV (albeit a weak HPV response) accounted for the alteration in blood flow in the left lung. Although PaO$_2$ showed an increasing trend from 38 mmHg after L-arginine to 43 mmHg after L-NAME this difference was not significant, nor did it
differ significantly from the PaO$_2$ after creation of one lung hypoxia (48mm Hg). This can be attributed to the fact that, although blood flow decreased in some parts of the left (hypoxic) lung this did not necessarily translate to increased flow in the hyperoxic lung. It is possible that the arteries in the oxygenated lung were maximally dilated as they were under the influence of oxygenated alveoli and systemic hypoxemia, and thus any vasoconstriction in the left pulmonary tree merely translated into increased pulmonary arterial pressure. [Marshall et al 1994] It is also possible that the dose of L-NAME was not high enough to return blood flow distribution to the pre-L-arginine state, and thus a higher, albeit still hypoxemic, PaO$_2$. Finally, since this manipulation was the last in the series to be conducted, and atelectasis of the lung in the dorsally positioned anesthetized horse progresses with time, this inadequate response may have been due to further atelectasis developing thus increasing V/Q inequality. [Nyman et al 1990]

2.8 Assessment of the model

Our findings were unexpected in that we had not anticipated that inducing total hypoxia in one lung would cause severe systemic hypoxia, given the PaO$_2$ when the horses were ventilated with 100% oxygen. The severity of the systemic hypoxemia undoubtedly created conditions (vasodilation) in the proximal pulmonary arterial tree that were not conducive to appropriately evaluating our ability to manipulate the NO pathway. [Marshall et al 1991] The fact that such severe hypoxemia developed after one lung hypoxia, and prior to any manipulation of NO production, attests to the fact that the HPV mechanism is unable to operate in an effective manner in horses under the conditions
created in this model. This was unexpected based on the findings of Elliott et al [1991] where 8% O₂ delivered to one lung created a marked shift in blood flow to the hyperoxic lung. The subjects in that study were ponies, rather than adult thoroughbred horses, and there are species differences in the HPV response. There are also known differences in the HPV response within a species, for example people who have lived at high altitudes for generations have a markedly reduced HPV response. [Archer and Michelakis 2002] Additionally, the ponies were in sternal recumbency as opposed to dorsal recumbency and thus their abdominal organs did not compress the caudodorsal lung field. It is likely that this physical compression played the largest role in creating V/Q mismatch that lead to the development of severe hypoxemia following reduction of total FiO₂ to 50%, as this has been implicated in severe hypoxemia and death in clinical patients. [Nyman et al 1987, Nyman et al 1990]

Another factor that may have played a role is the fact that we created complete hypoxia in the left lung. In people, the onset of the HPV response occurs when the gas within the alveoli is hypoxic rather than anoxic. [Archer and Michelakis 2002, Moudgil et al 2005] However, the greatest degree of redistribution of blood flow in ponies occurred when 4% O₂ was used in the hypoxic lung. [Elliott et al 1991] As far as we know the lowest percentage of alveolar O₂ that will trigger HPV in the horse has not been determined. It would be of interest to determine this value in the dorsally-anesthetized horse prior to conducting further studies investigating the mechanisms that lead to failure of the HPV mechanism to optimize PaO₂ in horses during anesthesia in the supine position.
We selected pentobarbital as our anesthetic agent based on its use in prior studies that investigated HPV in ponies and because it has been reported as having minimal effect on HPV compared with some inhalational agents. [Elliot et al 1991, Lennon and Murray 1996]

2.9 Conclusion

Similar to the findings of others, blood flow distribution in the standing horse is only partially determined by gravity, and shows isogravitational heterogeneity as well as a hilar-peripheral gradient in most transverse and longitudinal sections. [Hlastala et al 1996, Sinclair et al 2000] This pattern of blood flow distribution also occurs in the pentobarbital-anesthetized dorsally recumbent horse, with an overall decrease in blood flow to all areas of the lung which is most likely due to the decrease in cardiac output seen following anesthesia.

Ventilation of the left lung with 100% N₂ and the right lung with 100% O₂ caused severe systemic hypoxemia in the pentobarbital-anesthetized dorsally recumbent horse. The lack of any significant change in blood flow distribution under these conditions indicates failure of the HPV mechanism to optimize matching of perfusion to oxygenated alveoli.

Attempts to manipulate the production and inhibition of NO minimally altered blood flow distribution and did not result in improved oxygenation.
4.9 Clinical relevance

Our study suggest that manipulation of NO is unlikely to be helpful in correction of severe hypoxemia due to V/Q mismatching seen during anesthesia in clinical equine patients.
4.10 References

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