Synchronized delivery of inspired nitric oxide

Effects on oxygenation and pulmonary tension during artificial ventilation

BY

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Nitric oxide (NO) is a mediator of vascular smooth muscle tension that metabolises rapidly in blood. NO delivered by inhalation can therefore be used as a selective pulmonary vasodilator to relieve pulmonary hypertension or to improve oxygenation with no systemic effects. In artificial ventilation nitric oxide has been administered in inspiration gas as a continuous gas flow or to form constant inspired concentration. Homogeneous inspired gas mixture has been regarded essential for successful therapy and the therapy has been characterized by the mixture NO concentration. The response in oxygenation on NO therapy has, however, been variable. Administration of NO as a short pulse synchronously with inspiration has been suggested to improve the response. In this study the NO administration was examined theoretically and experimentally with the aim to relieve pulmonary hypertension and improve oxygenation during artificial ventilation. For the experimental study a system for the synchronized administration was developed.

The effect on oxygenation was studied during equine anaesthesia where hypoxemia develops regularly secondary to left-to-right shunt caused by atelectasis. By administering the NO as a short pulse in early inspiration to well ventilated lung areas the oxygenation could be effectively improved. Delayed administration to low ventilated lung areas was found possible for a negative contribution on oxygenation, which reduces the improvement gained in the well-ventilated lung areas. When NO is delivered into the whole inspiration, the net effect on oxygenation is the sum of these negative and positive contributions, whereas with pulsed delivery to the early inspiration the negative contribution can be avoided. This finding may be the main explanation for the varying response in oxygenation detected in patients as a response to NO inhalation.

When the NO therapy aimed for the relief of induced pulmonary hypertension in pigs, no difference was observed between NO delivery as a short pulse or given to the whole inspiration. Maximum vasodilatation was observed with 105 nmol/min delivery rate. A larger delivery rate only contributed to an abrupt increase in pulmonary pressure at cessation of the delivery.

The NO uptake from alveoli to tissue depends on the alveolar NO partial pressure. In a simulation this partial pressure was shown to be independent of the administration mode. Also the relationship between the NO uptake and delivery setting was not explicit. With pulsed delivery, expired NO can be reduced which was confirmed by the experimental results. This is important when the NO therapy is given in rebreathing circuit.

**Key words:** nitric oxide, pulsed administration, pulmonary hypertension, oxygenation, equine
To my wife Minna
and my children
Essi, Paavo, and Kaisla
This thesis is based on the following publications and manuscript:


Paper II: Heinonen E, Högman M, and Meriläinen P. Theoretical and experimental comparison of constant inspired concentration- and pulsed delivery in NO therapy. *Int Care Med* 2000; **26**: 1116-23


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Abbreviations

ALI  Acute lung injury
ARDS  Acute respiratory distress syndrome
cGMP  Cyclic guanosine mono phosphate
CO₂  Carbon dioxide
COPD  Chronic obstructive pulmonary disease
CAO₂  Blood O₂ content, x = c for end-capillary, v for venous, a for arterial
ERDF  Endothelium derived relaxation factor
FₐNO  Alveolar gas NO fraction
FₑNO  Expired gas NO fraction
FₐNO̱peak  Peak NO fraction in expiration (y = E) or inspiration (y = I) gas
FₑTXX  End tidal fraction of gas XX in breathing gas
F₁NOAVG  Average NO fraction in inspired gas during the pulse administration
F₁O₂  Inspired gas O₂ fraction
FRC  Functional residual capacity
Hb  Blood haemoglobin content
I:E ratio  Ratio of inspiration and expiration times
IPV  Intermittent positive pressure ventilation
log SDQ  Pulmonary perfusion distribution
log SDV  Ventilation distribution
metHb  Methaemoglobin
MAP  Mean systemic arterial pressure
NO  Nitric oxide
NO₂  Nitrogen dioxide
O₂  Oxygen
PAP  Mean pulmonary arterial pressure
PCWP  Pulmonary capillary wedge pressure
PEEP  Positive end expiratory pressure
ppb  10⁻⁹, parts per billion
PPHN  Persistent pulmonary hypertension of the neonates
ppm  10⁻⁶, parts per million
PvO₂  Venous blood O₂ partial pressure
PₓXX  Blood partial pressure of gas XX. y = c: end-capillary, v: venous, a: arterial
Qs/Qt  True shunt, area where VA/Q < 0.005
Qi  Cardiac output
Qo₂/Qi  Venous admixture
RR  Respiration rate
SaO₂  Blood Hb O₂ saturation, y = c: end-capillary, v: venous, a: arterial
VA  Local ventilation
VA/Q  Ratio of local ventilation to local blood perfusion
VD/VT  Dead space ventilation (% of VT)
VDⁿ̅ʷ  Alveolar dead space, area where VA/Q >100
VDᵃⁿᵃᵗ  Anatomic dead space
VT  Tidal volume
Introduction

History of NO administration

Vasodilatation of pulmonary perfusion is an important regulator of the gas exchange in the lung. Over the years there has been a great interest in the physiology of vascular smooth muscle relaxation regulating the vasodilatation. The role of cyclic guanosine monophosphate (cGMP) in the relaxation was detected and explained in 1979.\(^1\) Shortly thereafter endothelium-derived relaxation factor (ERDF) regulating the cGMP production within the smooth muscle was discovered.\(^2\) This ERDF was found to be identical to nitric oxide (NO).\(^3\)\(^-\)\(^6\) The advantage of NO in contrast to intravenously administered vasodilatation drugs is the possibility to exert a local effect because of rapid metabolism in blood. Therefore NO added to the inspiration gas was proposed to relieve pulmonary hypertension and to improve gas exchange without systemic effects.\(^7\)\(^-\)\(^8\) The use of NO inhalation to relieve pulmonary hypertension has been studied since 1991 and to improve oxygenation since 1993.\(^9\)\(^-\)\(^14\)

The new therapy method led to the development of many different administration systems. At the beginning NO was delivered as a continuous flow into the inspiratory limb of the breathing circuit where it mixes with the breathing gas.\(^15\)\(^-\)\(^16\) The NO gas flow was regulated with a rotameter and the delivery safety was ensured with NO and nitrogen dioxide (NO\(_2\)) monitors connected to measure inspired gas concentrations at the Y-piece. These monitors were electrochemical fuel cells with a slow response time, thus measuring time averaged concentration. A variation in NO delivery resulting from the accumulation of NO into the inspiratory limb during expiration was observed 1993.\(^15\) The monitors could not detect this NO concentration bolus, which could be 150 ppm instead of the setting of 20 ppm.\(^17\) Nevertheless, still in 1997 almost all British intensive therapy units used the continuous NO flow delivery.\(^18\) To improve the continuous flow delivery, the NO flow to the breathing circuit was interrupted for the expiration period.\(^12\) With this intermittent delivery the accumulation of NO in the inspiratory limb could be avoided when constant flow, i.e. volume controlled, ventilation was used.\(^19\) However, with decelerating flow, i.e. pressure controlled, ventilation the NO concentration increases towards the end of inspiration. To provide a stable NO concentration to the breathing gas, NO was mixed with the other respiratory gases before entering the ventilator.\(^15\)\(^-\)\(^17\)\(^,\)\(^20\)\(^-\)\(^21\) High NO\(_2\) production is an inherent
property of this method due to long reaction time with oxygen before inhalation.\textsuperscript{17,22-24} To reduce the risk of NO\textsubscript{2} formation absorbers were added in the inspiration limb.\textsuperscript{21,25}

Nitric oxide has also been administered between the Y-piece and endotracheal tube as continuous or inspiratory phase flow.\textsuperscript{17,26,27} With this method the accumulation of NO and the NO\textsubscript{2} formation were reduced. However, when the NO flow was continuous, the NO delivered during expiration became directly expired, which made dose control difficult.\textsuperscript{24} When the NO flow was intermittent an expensive chemiluminescent NO analyser was required for monitoring.\textsuperscript{17}

To provide homogeneous inspired gas mixture and to reduce the NO\textsubscript{2} in inspiration gas, control of the NO flow injected to the inspiratory limb in relation to the inspiration was presented as an ideal in 1994.\textsuperscript{28} This delivery technique ensured constant and stable inspired gas NO concentration adapting to different ventilation modes and variations in ventilator settings. This constant inspired concentration delivery has been accepted as state of the art technique for NO administration and the homogeneous inspired gas mixture has become a basic requirement for the therapy, and the delivered concentration has been used to characterise the therapy.\textsuperscript{17,29-31}

Administration of NO as a pulse through a nasal adapter or a mask has been proven effective in relieving pulmonary hypertension in spontaneously breathing patients.\textsuperscript{32-34} In this delivery scheme the delivery was synchronised with the beginning of inspiration and the pulse width was 100 ms. The delivery has been characterised in moles/breath or by length of the pulse of known NO flow. With the pulsed delivery the NO consumption was reduced and NO expiration as well as NO\textsubscript{2} formation were eliminated, which reduce environmental contamination.\textsuperscript{32,35} With the pulsed delivery, inspired gas NO concentration became undefined. Monitoring of the NO concentration was not possible either from the nasal adapter. This stands in contrast to the requirements set for the effective NO delivery during artificial ventilation.

From a physiological point of view the NO delivery method has only been discussed briefly. The necessity of the constant inspired concentration has been questioned because the mixture is thought to become homogenous in the airways and lung.\textsuperscript{24} Whether the volume of NO delivered, the volume of NO consumed by the patient, or the inspired gas or
alveolar concentration are important for the therapeutic effect has been asked, but no answers have been presented.\textsuperscript{17,36}

**Indications for the NO inhalation therapy**

Nitric oxide has been approved as a drug for persistent pulmonary hypertension for neonates (PPHN).\textsuperscript{37,38} In adults the research has concentrated on acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) patients suffering from pulmonary hypertension and gas exchange problems caused by regional mismatch of ventilation and perfusion of the lung. Multicenter studies of ARDS patients have evaluated the contribution of NO inhalation to patient mortality and days of ventilation.\textsuperscript{21,39,40} These studies have been criticised, because only about 5% of the ARDS patients die of hypoxemic respiratory failure and therefore even a complete cure of the respiratory failure with the NO therapy would be unlikely to be discernable in these studies despite stringent patient entry criteria.\textsuperscript{41} While the focus was on ARDS, other patient groups suffering primarily hypoxaemia or pulmonary hypertension have been forgotten.

**Gas exchange and $V_A/Q$ distribution**

Oxygen (O\textsubscript{2}) from the inspired gas and carbon dioxide (CO\textsubscript{2}) from the blood are exchanged in the alveoli of the lung. The blood perfusion and lung ventilation are distributed in this region as presented in the schematic illustration of the Fig 1. These distributions are not equal and five regions can be separated based on the ratio of ventilation and perfusion ($V_A/Q$). In the areas of normal ventilation and perfusion, the $V_A/Q$ is near to unity. The perfusion exceeds ventilation in regions where $V_A/Q < 1$ and the ventilation exceeds perfusion where the $V_A/Q > 1$. True shunt ($Q_s/Q_l$) is perfusion of the lung region that has no ventilation ($V_A/Q = 0$), and alveolar dead space ($V_{D_{alv}}$) is ventilated area with no perfusion ($V_A/Q = \infty$). Overlapping of the ventilation and perfusion distributions tend to reduce in different disease states of the lung due to atelectasis, airway closure, oedema and fibrosis. Secondary to this reduction the gas exchange is impaired.

For various reasons the lung areas may have different compliance. Also the alveolar-pleural pressure gradient, which is vertically distributed, is different. Inspiration gas flow follows the path of the least impediment to the lung areas, which have high compliance a and high
alveolar-pleural pressure gradient. At the beginning of inspiration the compliance is high in open, elastic, and surfactant coated normal lung areas. The compliance of these areas will reduce when the alveoli become distended due to the gas flow. This will then redirect the flow to the lung areas having lower compliance, e.g. fibrotic areas. During expiration the alveolar-pleural pressure difference may disappear in the dependent lung regions causing airway closure and lung collapse. The pressure difference increases towards the end of inspiration and the collapsed areas and closed airways may open shortly before recollapse and close again at the beginning of next expiration. These intermittently opening lung areas form together with the rigid fibrotic areas a region of low ventilation.

Gravitation, hypoxia and NO guide the pulmonary perfusion to meet the ventilation. Perfusion of the dependent lung regions is limited by hypoxic pulmonary vasoconstriction caused by poor ventilation often present in these areas. Endogenously produced NO counteracts the hypoxic pulmonary vasoconstriction but its role in regulation of the perfusion is not totally understood. In addition to vascular endothelium, substantial amounts of NO is generated in the sinus cavities in humans, from where it is carried during natural breathing with the inspiration gas to the ventilated lung regions. The possible role in regulation of pulmonary perfusion of this exovascular NO is blocked when the patient is intubated.

Figure 1: Schematic illustration of perfusion and ventilation distributions in the lungs.
The alveolar NO uptake is fast compared to O₂ due to the high diffusion rate.\textsuperscript{46,47} Also the amount of NO required to induce vasodilatation is low compared to the amount of O₂ needed to oxygenate the increased blood flow. Thus the ventilation needed to deliver enough NO to induce vasodilatation is low compared to the ventilation needed to oxygenate the increased blood flow, and the lung areas determined as Qs/Qt for O₂ and CO₂ exchange may still have enough ventilation for effective NO delivery. In such areas NO inhalation counteracts the hypoxic vasodilatation. This increases the perfusion in these low Vₐ/Q areas, which results in impaired oxygenation.\textsuperscript{13,35,48}
Aims of the study

The overall aim was to investigate the administration of NO as a pulse synchronously with inspiration during artificial ventilation.

The specific aims were

- To develop an administration system for pulsed NO delivery.
- To theoretically simulate the NO delivery and the alveolar and end-tidal NO fractions and NO uptake.
- To evaluate the pulsed administration in relief of pulmonary artery hypertension.
- To evaluate the pulsed administration to enhance alveolar gas exchange.
- To study the effects of NO on ventilation-perfusion -relationship with various pulse timings.
Material and Methods

Description of the pulsed NO delivery system (paper I)

Delivery device

The delivery device was designed in Datex-Ohmeda Research Department (Helsinki, Finland) to administer the NO only into the alveoli, where the gas exchange with blood occurs. During inspiration, the gas is inspired in successive volumes starting from the gas located in the anatomic dead space. The volume in this dead space distal to the delivery point can not be reached by the administration and it forms delivery dead space (light grey zone, Fig 2). To minimise the delivery dead space, the administration point is designed to locate as near the airways of the patient as possible. The subsequent alveolar inhalation from the breathing circuit flows to the alveoli (hatched zone, Fig 2). The last part of this alveolar inhalation fills the possible low $V_A/Q$ lung areas. The gas following the alveolar inhalation remains in the dead space at the end of inspiration (dark grey zone, Fig 2), from where it is expired at the beginning of expiration. In order to deliver the NO into the inhalation, the administration must occur into the alveolar inhalation. To synchronise the administration with the inspiration, the breathing flow is measured with a patient flow sensor D-lite™, Datex-Ohmeda (Helsinki, Finland).

The delivery device regulates the NO gas flow rate to match the pulse volume with the target volume in the predetermined pulse duration. This is done with a valve, which is opened in proportion to the valve control (pulse flow control valve, Fig 2). When the inspiration is detected the pulse control valve is opened to start the NO gas flow. After the predefined pulse duration has passed, the valve is closed to stop the NO gas flow. With separate flow rate and pulse controls, fast delivery could be combined with the required large flow control range. The gas volumes between the valves determine the smallest pulse volume that is possible to deliver. This volume is minimised with a specific valve design.49

The NO delivery system uses carrier gas for rapid dose transport through the delivery line to the NO administration point to eliminate all control equipment in the most crowded working environment near the patient mouthpiece.50 With the carrier gas transport system NO gas is rapidly transported against the breathing circuit pressure, which increases during
inspiration. The carrier gas is suctioned through a suction line from the inspiratory limb by a carrier pump into a 100 mL container. By the use of breathing gas for the transport, the inspired gas mixture is not affected. The container overpressure at the beginning of the pulse is typically 50 kPa. The carrier transport is activated at the beginning of the pulse by opening the flush valve and the following gas flow of the order of 50 ml·s⁻¹ flushes the NO to the administration point in less than 100 ms.

The control system consists of a personal laptop computer equipped with an analogue and digital input/output board DACcard 1200 (National Instruments, Austin, TX, USA). The control software is written in the LabView 4.0 (National Instruments) program.

![Diagram](image)

Figure 2: Pulsed nitric oxide delivery system in connection of artificial ventilation. The gas volumes of the patient airways and the inspiratory limb describe the breathing gas volumes at the beginning of inspiration. The light grey colour designates for delivery dead space. The gas in the hatched zone forms the alveolar inhalation that fills the alveoli, and the gas in the dark grey zone remains in the dead space at the end of inspiration.
Safety of the NO delivery

The design takes into account the safety risks present in inhaled NO delivery, such as an error caused by setting the control system for a different concentration than the supply gas is, delivery inaccuracy due to device malfunction, and NO₂ formation. The delivery device was designed to cover the whole dosage range in NO therapy from neonatal to adult patients with a single 1000 μL/L NO/N₂ supply mixture, which eliminates the safety risk involved when multiple supply concentrations are needed. Because the NO is metered volumetrically in nanomoles (nmol)/ breath, the delivery has to be monitored also with a volumetric measurement. For this purpose, a dose monitoring flow sensor is connected in series with the dose control flow sensor. Difference in the sensor outputs is an indication of a defected delivery device, which may trigger actions required to ensure patient safety.

Nitrogen dioxide can be formed within the delivery device during prolonged storage of NO, or when NO is mixed with the breathing gas. Because the internal volume of the delivery device is only about 3 mL, the volume can be purged easily by synchronising the delivery with patient expiration before the administration is started. When NO is mixed with O₂, NO₂ formation is proportional both to the O₂ concentration and the reaction time, and to the square of the NO concentration of the mixture. Proportionality factor is 0.86·10⁻⁶ when both NO₂ and NO concentrations are expressed in ppm (ppm = parts per million, 10⁻⁶), FIO₂ in %, and reaction time in seconds.⁵¹ In the delivery device the NO and O₂ are kept unmixed until the time for inspiration. At the time of delivery the 1000 μL/L NO/N₂ supply gas flow is diluted when it is mixed with the carrier gas to form a gas mixture with an NO concentration 100 ppm or less. This mixture is then transported in less than 100 ms to the administration point where inspiration gas dilutes the mixture to form a mixture of 20 ppm or less, which is immediately inspired into the alveoli. Calculated from these numbers for FIO₂ = 100%, the NO₂ concentration of the inhaled gas when it reaches the alveolar region is less than 0.1 ppm, which is well below the permissible exposure limit of 5 ppm set for the average 8-hour working environment concentration and the recommended exposure limit of 1 ppm.⁵²

Miniature NO analyser

Nitric oxide concentration can be analysed with a chemiluminescence technique. By a reaction with ozone (O₃) NO produces NO₂ in an exited state. During return to the ground
state a photon is emitted. For the reaction, the O₃ is produced in the analyser. The reaction photons are counted with a photomultiplier. The analyser converts the photon frequency to NO fraction. To reduce thermal error pulses, the photomultiplier is cooled. To reduce photon deactivation by collision with other molecules (quenching) the reaction chamber pressure is reduced with a pump. A reduction in chamber pressure, however, also reduces the number of NO and O₃ molecules in the reaction chamber, which is compensated by increasing the reaction chamber volume.

Large reaction chambers, ozone generation, the photomultiplier tube and its cooling, and a separate vacuum pump have made the commercial NO analysers too big, too noisy, too expensive, and are too cumbersome, and have too high power consumption for clinical use.

In the new miniature chemiluminescence analyser these problems have been solved by optimal design of the reaction chamber geometry and optimal ozone generation. The reaction chamber volume is reduced and photon collection efficacy improved, which reduces the quenching. This allows higher chamber pressure, which can be maintained with a miniature diaphragm pump. The cooling of the photomultiplier is limited to the measurement cathode, which is kept at +5 °C. These reduces the size, noise and power consumption of the equipment. The size of the prototype device is 12×14×41 cm and its weight is 3.8 kg including the pump. Signal response time is 200 ms and resolution 1 parts per billion (10⁻⁹, ppb).

Below 0.5 ppm the NO reading of the new analyser was 6% lower compared with the reading of Sievers NOA 280 (Sievers Instruments, Boulder, CO, USA) chemiluminescence NO analyser during forced prolonged exhalations (r=0.99). However, during measurement of fast breathing, the peak values of the new miniature NO analyser were 46% higher (r=0.99), which indicates a faster response to rapid changes.

**Study protocols**

*Evaluation of the pulsed delivery system (paper I)*

The delivery system pulse accuracy was evaluated in the laboratory. This was done by collecting 2-20 successive nitrogen pulses into a 1, 10, or 50 ml measuring chamber. The
accuracy was evaluated with selected combinations of pulse volume (3, 10, 33, 100, 330, 1000 nanomole (nmol)/pulse) and pulse duration (0.2, 0.3, 0.6, 1.2 s).

In a pig study the use of a fast responding NO analyser in monitoring pulsed delivery was evaluated. In this study NO was administered in 5, 10, 40 and 80 nmol pulse volumes for 5 min for each dose during the first 30% of the inspiration period.

Mathematical model of NO delivery (paper II)

The theoretical model consists of two elastic compartments that represent perfused and non-perfused lung sections. The lung volume ($V_L$) is set initially to functional residual capacity (FRC). The elasticity is determined by compliance. In the simulation, the compliance was selected for the tidal volume ($V_T$) to give 10-15 cmH$_2$O peak inspiratory pressure. The lung is ventilated with inspiratory flow through a channel representing the anatomic dead space volume ($V_{D\text{anat}}$). NO is administered into the inspired flow at a rate defined by the NO delivery mode and delivery rate. NO is transported through the $V_{D\text{anat}}$ into the lung. The gas entering the elastic compartment is divided to flows into the parallel perfused and non-perfused lung compartments in a ratio determined by the proportion of the alveolar dead space ($V_{D^{\text{alv}}}$) of the total lung volume ($V_{D^{\text{alv}}}.V_L^{-1}$). The gas is mixed with the gas already present in the compartments in the manner that the compartment mixture is always homogenous. From the perfused compartment the NO is taken up in a rate determined by the NO diffusion constant ($D_{LNO}$) and the alveolar NO partial pressure. The estimation of normal value for the $D_{LNO} = 6 \text{ ml/s.\,kPa}^{-1}$ is based on measurements in human lungs.$^{46}$ The NO partial pressure in blood is regarded zero. The expiration gas is derived from the two lung compartments in the proportion of the $V_{D^{\text{alv}}}$ and $V_L$.

The mathematical model is presented in detail in paper II and the reader is referred to the methods section of that paper. The model was used to determine the alveolar NO fraction ($F_{A\text{NO}}$), expired NO fraction ($F_{E\text{NO}}$) and NO uptake in any ventilation setting of $V_T$, I:E ratio, respiration rate (RR), FRC, lung mechanics, $D_{LNO}$, $V_{D^{\text{alv}}}.V_L^{-1}$, $V_{D\text{anat}}$, and NO administration mode. The derived set of differential equations was simulated by integration of infinitesimal changes in a computer program.
**Pulsed NO to relieve pulmonary hypertension (paper II)**

In this dose-response study hypoxic pulmonary vasoconstriction, which was induced by hypoxic inspired gas, was relieved by NO delivery in different administration modes.

The study comprised three phases: a) baseline recording, b) induction of the hypoxic vasoconstriction, and c) administration of NO while continuing with the hypoxic breathing gas of phase b. During 30 minute baseline (a) the mean pulmonary artery pressure (PAP) was 20-30 mmHg at an inspired gas oxygen fraction (FIO2) of 0.21. During vasoconstriction the inspiration gas O2 fraction (FIO2) was decreased to 0.13-0.18 to a target PAP of 30-40 mmHg. After 15 minutes of hypoxia (b), the NO was given in steps of different delivery modes and amounts for five minutes each (c). As one step the NO delivery was stopped to study the consequences of an abrupt cessation of inhaled NO delivery.

**Pulsed NO to improve oxygenation (papers III and IV)**

The effect of pulsed NO on improving oxygenation was studied in equine anaesthesia accompanied by impairment of pulmonary gas exchange and arterial blood oxygenation.\textsuperscript{53-55} Considerable changes in the ventilation and blood flow distribution in the equine lung during general anaesthesia have been detected.\textsuperscript{56} The most important change regarding arterial oxygenation is the development of a large right-to-left vascular shunt, with blood perfusion in unventilated lung areas. In dorsal recumbency, approximately 1/3 of the blood passes through the lung without being oxygenated.\textsuperscript{54}

In the protocols the therapeutic efficacy of NO delivery was evaluated with a set of haemodynamic and blood gas measurements repeated at each delivery step. Except for part of protocol 5 (see below), NO was administered during the first half of inspiration.

**The effective dose of NO (protocol 1)**

After the baseline measurements, the horses (n=5) were given a NO dose of 2.5 μmol with each inspiration during a period of 5 minutes. The dose was then increased to 4.9 μmol and this was given for another 5 minutes.
**Oxygenation response time on the NO delivery (protocol 2)**

After the baseline measurements the horses (n=5) were given 4.9 μmol NO per breath for 5 minutes. This dose was continued for another 5 minutes before delivery was stopped. The fading out of the therapeutic effect was monitored 5 and 15 minutes after cessation of NO delivery.

**Spontaneous breathing versus IPPV (protocol 3)**

In five horses the effect of NO was studied during spontaneous breathing and intermittent positive pressure ventilation (IPPV). With each of these modes of ventilation, a dose of 4.9 μmol NO was given with each inspiration for 5 minutes. Another set of measurements was performed 15 minutes after the cessation of NO delivery.

**Distribution of ventilation and perfusion in the lung (protocol 4)**

The multiple inert gas elimination technique was used to evaluate the distribution of ventilation and pulmonary perfusion in relation to VA/Q in three horses during spontaneous breathing. The distribution was measured at baseline, after 5 minutes of NO inhalation at a dose of 4.9 μmol with each inspiration, and 15 minutes after the NO inhalation.

**Effect of pulse timing relative to inspiration (protocol 5)**

To measure the effect of pulse timing, NO was administered to different parts of inspiration in six horses. After the first baseline measurements, NO was administered during the first 30% of inspiration (NOp1). Fifteen minutes after cessation of NOp1, another baseline was recorded and NO was administered during the first 60% of inspiration (NOp2). After completion of NOp2, there was another 15-min resting period, followed by a new baseline recording. NO was then administered during 50% to 80% of inspiration (NOp3) to reach the low VA/Q areas of the lung. The response was recorded after five minutes of NO delivery in all delivery modes. In three of the horses the ventilation and pulmonary perfusion distributions in relation to VA/Q were measured. Since NOp3 involved a risk of a severe increase in shunt and hypoxaemia, no randomisation was performed.
Animals, anaesthesia and preparation

The local Ethics Committee for Animal Experiments in Uppsala, Sweden, approved all protocols included in the study.

**Pig studies (papers I and II)**

In the study on monitoring of the pulsed delivery a pig of 30 kg was used (*paper I*). In the study of NO therapy in relieving pulmonary hypertension, nine healthy pigs of mixed breed (Hampshire, Yorkshire and Swedish landrace) with a body weight of 25-35 kg were used (*paper II*). Premedication was given by an intramuscular (i.m.) injection with the neuroleptic Stresnil (Janssen Pharmaceutical, Beerse, Belgium) before transport. Anaesthesia was induced with 0.5 mg atropine and a mixture of 100 mg Zoletil forte vet. (Virbac Laboratories, Carros, France) diluted in 5 mL Domitor 1 mg.mL$^{-1}$ (Orion Corporation, Farmos, Finland); 1 mL per 20 kg body weight i.m. The pigs were intubated with a cuffed endotracheal tube and ventilated mechanically in constant flow volume controlled mode with zero positive end-expiratory pressure (PEEP) (Dräger Evita, Drägerwerk AG, Lübeck, Germany in *paper I*; Siemens Servo 900C, Siemens-Elema AB, Solna, Sweden in *paper II*). The I:E ratio was 1:2. The $V_t$ was 10 mL per kg plus compensation for dead space volume and the RR adjusted to an end tidal CO$_2$ fraction ($F_{ET}CO_2$) of 5%. A bolus injection i.v. of 0.2 mg Fentanyl (Antigen Pharmaceuticals Ltd, Roscrea, Ireland) was given before the anaesthesia maintenance. Anaesthesia was maintained by infusion of 5 mL-kg$^{-1}$-h of 4 g Ketamin (Veterinaria AG, Zürich, Switzerland), 1 mg Fentanyl and Pavulon 48 mg in 1000 mL Rehydrex with glucose (Pharmacia & Upjohn, Stockholm, Sweden).

A thermodilution catheter (7Fr, Swan-Ganz) inserted to pulmonary artery was used to measure the mean pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP) and thermodilution cardiac output ($Q_t$).

**Horse studies (papers III and IV)**

In *paper III* five standardbred trotters, three geldings and two mares, with an age between 3 and 12 years and with a body weight of 442 to 520 kg, were studied in dorsal recumbency. A second study on the three geldings was performed 2 months later. In *paper IV* six
standardbred trotters, five mares and one gelding, with an age of 2-8 years and a weight of 417 to 581 kg were examined in lateral recumbency.

Food was withheld for 12 hours before anaesthesia. Access was given to straw bedding and water. In the protocols 1-3 of paper III the horses did not receive any premedication. In protocols 4 and 5 acepromazine (Plegicil vet., Pharmacia & Upjohn Animal Health, Helsingborg, Sweden) (0.05 mg·kg⁻¹) was given intramuscularly approximately 30 minutes before induction of anaesthesia. An intravenous infusion of 7.5% guaifenesin (Myolaxin® vet., Bayer, Göteborg, Sweden) was given until the horse became ataxic. Then anaesthesia was induced by the intravenous injection of thiopental sodium (Pentothal® Natrium, Abbott, Kista, Sweden) 5 mg·kg⁻¹. The horses were intubated and anaesthesia was maintained with 1.3%-1.7% Isoflurane (Forene®, Abbott) in oxygen with a fresh gas flow of 5-6 L·min⁻¹ except protocols 4 and 5 where a flow rate corresponding to minute ventilation was used. A large-animal circle system was used for breathing, except during the Vₐ/Q measurement in protocols 4 and 5 where an open large-animal breathing circuit modified for the collection of expired inert gases was used.

The horses breathed spontaneously except in protocol 3 where mechanical ventilation with a large-animal pressure controlled bag-in-box ventilator (Stephen-Ventilator, Strömberg-Mika, München, Germany) was applied. With IPPV the peak inspiratory pressure was 25 cmH₂O. The ratio between inspiration and expiration times was 1:2. The ventilation was adjusted to obtain an arterial carbon dioxide partial pressure (PₐCO₂) of 5-6 kPa.

A thermodilution catheter (7Fr, Swan-Ganz) was inserted with an introducer kit (Arrow Int. Inc., Reading, PA, USA) through a right jugular vein to the pulmonary artery. This catheter was used for PAP and Qₐ measurement, and mixed venous blood sampling. A pig-tail, multi-hole catheter (Cook Europe A/S, Söborg, Denmark) was inserted with a similar technique into the same jugular vein, advanced to the right ventricle and retracted into the right atrium. This catheter was used as an injection port for Qₜ measurement. A catheter to measure mean arterial pressure (MAP) and to sample arterial blood was introduced percutaneously into the facial artery (Insyte-W, 18GA, Becton-Dickson, Ohmeda, Helsingborg, Sweden). The catheters were positioned under pressure-tracing guidance with simultaneous ECG monitoring and locked in position with a Luer-lock adaptor.
Measurements

Arterial and central venous blood was obtained and analysed with a standard electrode technique (ABL 5, Radiometer, Copenhagen, Denmark). The arterial (P_aO_2) and mixed venous (P_vO_2) oxygen partial pressures were measured at standard electrode temperature (37 °C). In paper II the haemoglobin oxygen saturation (S_aO_2 and S_vO_2, respectively) was measured with a hemoximeter used also to determine methaemoglobin (metHb) (OSM 3, Radiometer). In paper III and IV the S_aO_2 and S_vO_2 were calculated from the blood samples using the human P50 value 3.57 kPa, which is close to the equine value of 3.41 kPa.

In papers III and IV Q_t was measured with the thermodilution technique (Cardiac Output Computer Model 9520 A, Edwards laboratory, Santa Ana, California, USA); 20 mL 0°C 0.9 % saline was injected into the right atrium through the pig-tail catheter. In paper II the injectate volume was 10 ml and the Q_t was measured with AS/3-AM™ anaesthesia monitor (Datex-Ohmeda, Helsinki, Finland).

Mean systemic arterial blood pressure and PAP were measured by pressure transducers connected to an AS/3-AM™ anaesthesia monitor (Datex-Ohmeda) positioned at the level of the scapulo-humeral joint, which was considered to correspond to the level of the right atrium. The pressures, breathing gas composition and ventilation were recorded either on the AS/3- AM™ anaesthesia monitor or CS/3 TM (Datex-Ohmeda) patient monitor equipped with applicable monitoring modules. Data was collected with Collect software (Datex-Ohmeda).

NO and NO_2 monitoring

In paper II the exhaled NO was sampled for each NO delivery rate with a chemiluminescence monitor (Sievers NOA 280, Sievers Instruments). An electrochemical NO_2 analyser (Nomius, Dan-Sjö Medical, Stockholm, Sweden) was connected to the expiratory limb of the breathing circuit to measure the NO_2 fraction.

In paper I and IV the NO delivery was monitored with the miniature NO analyser (Datex-Ohmeda) connected to measure breath-by-breath both inspired and expired NO. The analyser was connected to a personal computer for data collection and display. Peak inspired NO fraction (F_{INo_{PEAK}}) and end-tidal NO fraction (F_{ETNO}) were determined from
the recording. Nitric oxide uptake was defined as the proportion of the delivered NO remaining in the body. This was determined by integrating the product of NO fraction and flow during expiration.

Average NO delivery during the pulse (F\textsubscript{1}NO\textsubscript{AVG}) was calculated as ratio of the NO-flow (pulse volume divided by pulse duration) to the inspiration gas flow (V\textsubscript{T} divided by inspiration time (t\textsubscript{i})). Linear regression analysis was used to test for correlation between F\textsubscript{1}NO\textsubscript{AVG} and F\textsubscript{1}NO\textsubscript{PEAK}.

Calculations

\textit{VA/Q distribution}

Distribution of ventilation and perfusion in relation to VA/Q was measured with a multiple inert gas elimination technique.\textsuperscript{57} Five gases, chemically inactive in blood (sulphur hexafluoride, ethane, cyclopropane, diethyl ether and acetone), were dissolved in isotonic sodium chloride solution and infused into the jugular vein at 30 ml/min. Arterial and mixed venous blood samples were drawn and mixed expired gas was collected after 60 minutes of infusion. Gas concentrations in the blood samples and the expired gas were measured using a gas chromatograph (Hewlett Packard 5880A, Palo Alto, CA, USA). The arterial/mixed venous and mixed expired/mixed venous concentration ratios were calculated for each gas and their solubility coefficients in blood were measured in each horse by a two-step procedure. These data were then used for deriving the distribution of ventilation (log SDV) and blood flow (log SDQ) in a 50-compartment lung model with each compartment having a specific VA/Q ratio. The Q\textsubscript{a}/Q\textsubscript{i} was defined as perfusion of the region where VA/Q < 0.005 and the dead space ventilation (V\textsubscript{D}/V\textsubscript{T} = (V\textsubscript{D}\textsuperscript{alv}+V\textsubscript{D}\textsuperscript{anat})/V\textsubscript{T}) was defined as the ventilation of the region where VA/Q > 100.

\textit{Venous admixture}

Venous admixture (Q\textsubscript{va}/Q\textsubscript{i}) is the calculated proportion of mixed venous blood (i.e. Q\textsubscript{a}/Q\textsubscript{i}) that would be needed to a particular content of O\textsubscript{2} in arterial blood. The method thus also measures the partial gas exchange in the low VA/Q regions in terms of mixed venous blood. Q\textsubscript{va}/Q\textsubscript{i} determination was based on the mixed venous and arterial blood O\textsubscript{2} contents and the alveolar O\textsubscript{2} partial pressure.\textsuperscript{58} The principle is presented in equations 1 and 2
\[ \frac{Q_{\text{soa}}}{Q_{l}} = \frac{C_c O_2 - C_a O_2}{C_c O_2 - C_v O_2} \]  

(1)

where the blood \( O_2 \) content is

\[ C_x O_2 = 0.0139 \cdot Hb \cdot S_x O_2 + 0.23 \cdot P_x O_2 \]  

(2)

where haemoglobin (Hb) is expressed in g L\(^{-1}\), \( S_x O_2 \) in % and \( P_x O_2 \) is in kPa.\(^{59}\) The subscript "c" stands for end-capillary, "a" for arterial and "v" for venous. To determine the \( C_c O_2 \), the \( S_x O_2 \) was assumed to 100% and the end-capillary blood \( O_2 \) partial pressure \( (P_c O_2) \) was estimated equal to alveolar \( O_2 \) partial pressure \( (P_A O_2) \) calculated from the measured end-tidal \( O_2 \) concentration \( (F_{Et}\text{O}_2) \).

**Statistics**

For all statistical calculations of this thesis the Statistica/w 5.0 software package (StatSoft Inc., Tulsa, OK, USA) was used. Repeated measurement ANOVA was used to compare data within the group on different study occasions. The Tukey honest significant difference test was used for post hoc comparisons and probability value calculation. Correlations was calculated with multiple regression analysis. Data is expressed as mean ± standard error of the mean if not otherwise stated.
Results

Evaluation of the pulsed delivery system (paper I)

Pulse volume accuracy

The evaluation of the NO pulse accuracy comprised measurements with 19 different dose volume-pulse duration combinations. Both the dose volume and the standard deviation of the doses were within 5% or 3 nmol of the dose setting (Table 1).

Table 1: Accuracy of the delivered NO dose measured with pulse lengths between 0.2-1.2 s. Average delivered dose is the mean of the doses measured with different pulse lengths. The standard deviation is the highest value measured in single pulse width.

<table>
<thead>
<tr>
<th>Dose setting nmol</th>
<th>Delivery ± SD nmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.36 ± 0.87</td>
</tr>
<tr>
<td>10</td>
<td>10.1 ± 1.6</td>
</tr>
<tr>
<td>33</td>
<td>30.4 ± 2.9</td>
</tr>
<tr>
<td>100</td>
<td>95.8 ± 3.6</td>
</tr>
<tr>
<td>330</td>
<td>317 ± 10</td>
</tr>
<tr>
<td>1000</td>
<td>990 ± 30</td>
</tr>
</tbody>
</table>

Monitoring the pulsed delivery

The monitoring of the pulsed administration was examined in a case study to learn the NO fraction pattern for a successful delivery. The NO fraction curve when the NO analyser was connected between the endotracheal tube and the NO administration point showed the characteristic low NO concentration from alveolar exhalation (A) (Fig 3). The inspiratory dose delivery spike (B) of next inspiration followed this. Notch (C) of zero NO concentration is formed by NO-free gas at the end of inspiration. The presence of notch C was an indication of a stop of the administration well before the end of inspiration. Delayed administration or administration at a distance from the monitoring site would shorten or remove the notch (C) and other notches (D) of NO free gas before the delivery pulse (B). This may show up as illustrated by the dotted line and indicate less successful delivery (Fig
3). Notch (C) may also disappear if the inspiration flow stops before the administration pulse. A spike (E) at the beginning of expiration indicates administration into the anatomic dead space whereas its absence indicates delivery to perfused alveolar regions.

In the same study the use of gas monitor to monitor the NO dose and NO uptake was also evaluated. \( \text{F}_{\text{NOPEAK}} \), \( \text{F}_{\text{ETNO}} \), and NO uptake derived from the NO curves when the NO was delivered in 10, 20, 40, and 80 nmol/breath to the first 30% of inspiration are presented in Table 2. The \( \text{F}_{\text{NOAVG}} \) was calculated from the NO delivery and ventilation settings. The \( \text{F}_{\text{NOPEAK}} \) was 1.6 fold compared to the \( \text{F}_{\text{NOAVG}} \) (r=0.99, p<0.001) and the NO uptake varied between 87% and 92%.

Figure 3: NO fraction and corresponding breath flow. The NO curve comprises the alveolar exhalation plateau (A), the inspiratory dose delivery spike (B), and the end-inspiration notch of zero NO concentration (C). The dotted line presents a less successful delivery when the administration is delayed relative to the inspiration flow. A new notch (D) at the beginning of inspiration and a pulse (E) at the beginning of expiration derived from the dead space are formed. \( RR=18 \text{ min}^{-1}, I:E=1:2 \)
Mathematical model

The effect of physiological properties and the ventilator settings on $F_{ANO}$, $F_{ETNO}$ and NO uptake rate both in constant inspired concentration delivery and in pulsed administration were studied with the simulation model. The values calculated for representative combinations of ventilator settings and physiological properties are presented in Table 3. Examples of the $F_{ANO}$ and $F_{ETNO}$ curve patterns simulated for 5 ppm constant inspired concentration and 5 nmol/breath pulsed in the first third of inspiration are presented in Fig 4a and 4b respectively. With constant inspired delivery mode an exhaust spike from the $V_{D^{\text{anat}}}$ can be seen. Correspondingly, in pulsed delivery the $F_{ETNO}$ is zero at the beginning of expiration. The differences between the $F_{ANO}$ and $F_{ETNO}$ resulting from continuous alveolar NO uptake during the transport through $V_{D^{\text{anat}}}$ are also clearly demonstrated. In the constant inspired concentration delivery the difference between the delivery concentration and the $F_{ANO}$ is also clearly seen.
Figure 4: Simulated alveolar (thick line) and expiratory (thin line) NO fraction for a) constant 5 ppm inspired concentration representing a typical clinical value and b) pulsed delivery 100 nmol·min⁻¹ used in the experiments of this study delivered into the first third of the inspiration period. In simulation RR=20 min⁻¹, $V_T=400$ ml, I:E=1:2, FRC=700 ml, $V_{D,\text{anat}}=150$ ml, $V_{D,\text{air}}$, $V_L^{-1} =0$, $C=22$ ml·cmH₂O⁻¹, R(airway)=12 kPa·L⁻¹·s⁻¹ and $D_{LNO}=6$ ml·s⁻¹·kPa⁻¹.
Table 3: Simulated NO delivery, uptake ratio, and mean $F_{A}NO$ and $F_{E}NO$ for different NO delivery modes. The selected NO delivery rates represent typical clinical settings (5 ppm NO at constant inspired concentration delivery) and the value of 5 nmol NO (pulse) providing the total vasodilatation in this study. Due to the linearity of the simulation model, similar results would be obtained with other dose rates also.

<table>
<thead>
<tr>
<th>Parameter Setting</th>
<th>Constant 5 ppm inspired concentration delivery</th>
<th>5 nmol pulse delivered to the first third of inspiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{LNO}$ ml s$^{-1}$. kPa$^{-1}$</td>
<td>$V_{p}^{V}{V}_{L}^{V}$ %</td>
<td>$RR$ min$^{-1}$</td>
</tr>
<tr>
<td>6 0 20 400 0.33 150 700</td>
<td>1786</td>
<td>558</td>
</tr>
<tr>
<td>6 0 30 267 0.33 150 700</td>
<td>1788</td>
<td>427</td>
</tr>
<tr>
<td>6 0 20 400 0.67 150 700</td>
<td>1786</td>
<td>572</td>
</tr>
<tr>
<td>6 0 20 400 0.33 100 700</td>
<td>1786</td>
<td>654</td>
</tr>
<tr>
<td>8 0 20 400 0.33 150 700</td>
<td>1786</td>
<td>432</td>
</tr>
<tr>
<td>10 0 20 400 0.33 100 700</td>
<td>1786</td>
<td>354</td>
</tr>
<tr>
<td>3 0 40 100 0.67 50 300</td>
<td>893</td>
<td>370</td>
</tr>
<tr>
<td>1 0 20 400 0.33 150 700</td>
<td>1786</td>
<td>2160</td>
</tr>
<tr>
<td>6 50 20 400 0.33 150 700</td>
<td>1786</td>
<td>677$^{a}$</td>
</tr>
<tr>
<td>18 0 10 700 0.33 250 4000</td>
<td>1563</td>
<td>193</td>
</tr>
<tr>
<td>18 0 10 700 0.33 250 4000</td>
<td>1563</td>
<td>196</td>
</tr>
</tbody>
</table>

$^{a}$ in the perfused compartment
$^{b}$ airway resistance fitted for 10 cmH$_{2}$O intrinsic PEEP.
Experimental verification of the mathematical model

To verify the mathematical model experimentally, the measured FENO was compared with the values obtained from the model. Examples of the experimental plots for pulsed delivery to the first third of inspiration and for whole inspiration administration are presented in Fig 5. Derived from these graphs for different pigs, the relationships between delivery rate and

![Figure 5: Expired NO fractions measured from pig expiration when the NO is delivered at 10 nmol per breath to whole inspiration (A) and the first third of inspiration (B). Respiration rate 19/min, constant flow ventilation with tidal volume 400 ml.](image)

![Figure 6: The experimental regression relationships derived from the pig experiments (paper I)(mean with 95% confidence interval) between the delivery rate (nmol/min) and the resulting expired peak (left bar) and end tidal (right bar) NO fraction for the different delivery modes. The values are determined for a RR= 20/min. For particular delivery rate, multiply the delivery rate with the corresponding bar value to get the expired value.](image)
the resulting peak and end-tidal NO fractions in the expiration gas were calculated (Fig 6). Using these experimentally derived values for the 5 ppm constant inspired concentration delivery (1786 nmol/min) the values for peak expired NO fraction ($F_{E\text{NO}_{\text{PEAK}}}$) and $F_{E\text{TNO}}$ are 4.7 ppm (range for the 95% confidence interval 3.8-6.3 ppm) and 890 ppb (800 ppb-1.1 ppm) respectively. For the 100 nmol/min pulse delivery to the first third of inspiration the corresponding values are 87 ppb (80-105 ppb) and 65 ppb (58-83 ppb). In the simulation (Fig 4), the $F_{E\text{NO}_{\text{PEAK}}}$ are 5 ppm and 820 ppb for the 5 ppm constant inspired concentration. For the 100 nmol/min pulsed delivery these are 90 ppb and 67 ppb, respectively.

**Efficacy in relieving pulmonary hypertension (paper II)**

At the baseline, vasoconstriction, and NO delivery the $Q_t$ was 4.6 ± 0.3, 4.7 ± 0.3, and 4.8 ± 0.4 L/min respectively. PCWP was 9.2 ± 0.6, 8.9 ± 0.5, and 8.8 ± 0.5 mmHg. NO delivery produces a significant effect on the PAP ($p<0.001$) (Fig 7). All the induced hypoxic vasoconstriction was relieved with 105 nmol·min$^{-1}$ NO delivery ($p<0.05$). According to Fig 7,

![Figure 7: The NO delivery response on relative PAP (×) and rebound effect dose dependency ( ) on the normalised scale. For the relative PAP scale description, see Table 4.](image)

half of the maximum effect was achieved with 50 nmol·min$^{-1}$ delivery, and even the
The smallest tested dose of 10 nmol·min⁻¹ produced a significant reduction in the PAP (p<0.001). Increasing the delivery from the 105 nmol·min⁻¹ did not give any further advantage. The PAP response on the NO delivery in the different delivery modes is presented in Table 4. No significant mode-dependent difference on the response was found.

When the NO delivery was stopped, there was a consistent increase in PAP. The maximum PAP was reached within two to four minutes after the interruption of the NO delivery. The magnitude of this rebound effect depended on the NO delivery rate (p<0.001) (Fig 7).

**MetHb and NO₂ formation (paper II)**

The metHb varied during the NO delivery phases between 0.6%-1.4% compared to the baseline variation of 0.8-1.2%. No NO₂ above the baseline noise level of 0.1 ppm could be detected.

<table>
<thead>
<tr>
<th>NO delivery rate nmol/min</th>
<th>NO delivery mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/3</td>
</tr>
<tr>
<td>21</td>
<td>0.54 ±0.04</td>
</tr>
<tr>
<td>52.5</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>105</td>
<td>0.25 ± 0.12</td>
</tr>
<tr>
<td>210</td>
<td>0.25 ± 0.21</td>
</tr>
</tbody>
</table>
When 8.5 ± 1.8 μmol min⁻¹ NO was delivered in the first 30% of inspiration, the increase in PaO₂ was significant (p = 0.033) from 26.7 kPa ± 6.7 kPa to 36.8 kPa ± 5 kPa. The Qva/Qt decreased (p = 0.045) at the same time from 27% ± 4% to 20% ± 2%. The increase in PaO₂ was dependent on the baseline value (r=0.94, p=0.005) (Fig 8). A similar relationship (r=0.94, p=0.006) was observed also for Qva/Qt (Fig 9). Regression analysis shows that with this NO therapy, Qva/Qt could not be reduced when the baseline Qva/Qt was below 14%. However, a baseline Qva/Qt above 14% could be reduced by 55%.

**Figure 8: Correlation between the improvement in and the baseline PaO₂ before the NO delivery. The regression line and its 95% confidence limits are based on data measured on horses on lateral recumbency (closed circles) when NO is pulsed on the first 30% of inspiration (paper IV, NOp1). The open circles designate data measured on dorsal recumbency when NO is delivered to the first half of inspiration (paper III, protocol for delivery duration).**

**Pulsed NO in improving oxygenation during horse anaesthesia (paper III and IV)**

When 8.5 ± 1.8 μmol min⁻¹ NO was delivered in the first 30% of inspiration, the increase in PaO₂ was significant (p = 0.033) from 26.7 kPa ± 6.7 kPa to 36.8 kPa ± 5 kPa. The Qva/Qt decreased (p = 0.045) at the same time from 27% ± 4% to 20% ± 2%. The increase in PaO₂ was dependent on the baseline value (r=0.94, p=0.005) (Fig 8). A similar relationship (r=0.94, p=0.006) was observed also for Qva/Qt (Fig 9). Regression analysis shows that with this NO therapy, Qva/Qt could not be reduced when the baseline Qva/Qt was below 14%. However, a baseline Qva/Qt above 14% could be reduced by 55%.
The effective dose of NO

During 5 minutes of NO inhalation with a dose of 2.5 μmol/breath, there was a significant increase in $P_aO_2$ ($p<0.01$) and a reduction in $Q_{va}/Q_t$ ($p<0.01$). No further improvement was achieved with the dose of 4.9 μmol/breath (Table 5).

Table 5. Effects of increasing the dose of NO inhaled at each breath during spontaneous breathing in five horses.

<table>
<thead>
<tr>
<th>NO delivery, μmol·min$^{-1}$</th>
<th>baseline</th>
<th>2.5 μmol NO</th>
<th>4.9 μmol NO</th>
<th>5 min after NO inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR, min$^{-1}$</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>$P_aO_2$, kPa</td>
<td>15.3 ± 4.7</td>
<td>31.1 ± 3.4*</td>
<td>33.2 ± 4.2*</td>
<td>14.3 ± 1.7</td>
</tr>
<tr>
<td>$P_aCO_2$, kPa</td>
<td>8.6 ± 0.1</td>
<td>8.8 ± 0.2</td>
<td>9.2 ± 0.4</td>
<td>9.0 ± 0.3</td>
</tr>
<tr>
<td>$Q_{va}/Q_t$, %</td>
<td>37 ± 8</td>
<td>23 ± 3*</td>
<td>20 ± 2*</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>29 ± 2</td>
<td>26 ± 1</td>
<td>26 ± 2</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>72 ± 9</td>
<td>88 ± 8</td>
<td>91 ± 10</td>
<td>88 ± 8</td>
</tr>
<tr>
<td>Heart rate min$^{-1}$</td>
<td>38 ± 2</td>
<td>39 ± 2</td>
<td>42 ± 1</td>
<td>37 ± 1</td>
</tr>
</tbody>
</table>

* $p<0.01$, RR = respiration rate. $P_aO_2$ = arterial oxygen partial pressure. $P_aCO_2$ = arterial carbon dioxide partial pressure. $Q_{va}/Q_t$ = venous admixture. PAP = pulmonary arterial mean blood pressure. MAP = mean arterial blood pressure.
Oxygenation response time on NO delivery

$P_aO_2$ increased significantly ($p<0.001$) during 5 minutes of inhalation of NO (4.9 $\mu$mol/breath). No further rise with prolonged NO inhalation was noted. After cessation of NO delivery the $P_aO_2$ decreased and after 5 minutes it did not significantly differ from the baseline value (Fig 10).

Figure 10: The arterial oxygen partial pressure ($P_aO_2$) response time on inhalation of 4.9 $\mu$mol/breath nitric oxide (NO).
**Spontaneous breathing and IPPV**

The improvement in $P_aO_2$ after treatment with NO was similar during spontaneous breathing and mechanical ventilation (Fig 11). The amount of NO given per minute was significantly greater during IPPV ($p<0.01$) than during spontaneous breathing, since the respiratory rate was higher during IPPV. The difference in $P_aCO_2$ between IPPV and spontaneous breathing ($p<0.001$, Table 6) was also due to the higher respiratory rate during IPPV. There was no difference in MAP, PAP or heart rate between the two modes of ventilation. The $Q_t$ was significantly lower during IPPV than during spontaneous breathing ($p<0.05$).

![Graph](image)

*Figure 11: The improvement in arterial oxygen partial pressure ($P_aO_2$) caused by 5 minutes of inhalation of nitric oxide (NO) at 4.9 $\mu$mol was similar during spontaneous breathing and intermittent positive pressure ventilation (IPPV). The amount of NO given per minute (closed triangle) was significantly greater during IPPV ($p<0.01$) than during spontaneous breathing because of a higher respiratory rate during IPPV.*

**Distribution of ventilation and perfusion in the lung**

Arterial oxygen tension increased during NO inhalation but returned to baseline values within 15 minutes after the administration was discontinued. No other changes in the physiological measurements were observed (Table 7).

At the baseline $Q_s/Q_t$ was $39\% \pm 4\%$. The log SDQ and log SDV were 0.46-1.15 and 0.44-0.92, respectively, and dead space ventilation ($V_D/V_T$) was 60-65%. During NO inhalation $Q_s/Q_t$ was reduced to $27\% \pm 6\%$. The log SDQ decreased in two horses and remained low in one horse (0.37-0.79), while log SDV was unchanged in two horses and increased in one
horse from 0.36 to 1.79. A high V\textsubscript{A}/Q mode was observed in the horse that showed an increased logSDV. V\textsubscript{D}/V\textsubscript{T} increased slightly to 63-68% (Fig 12).

Fifteen minutes after cessation of NO delivery the pulmonary gas exchange, including the distribution of ventilation and perfusion, returned to values almost identical to the baseline levels before NO inhalation. The mean RSS was between 0.3 and 5.6 in all study occasions.

Calculated for the six horses where the Q\textsubscript{s}/Q\textsubscript{t} was determined (Fig 12 and Fig 13), the Q\textsubscript{s}/Q\textsubscript{t} and Q\textsubscript{va}/Q\textsubscript{t} did not show statistically significant differences either at the baseline (Q\textsubscript{s}/Q\textsubscript{t} was 39\% ± 3\% and Q\textsubscript{va}/Q\textsubscript{t} was 35\% ± 4\%) or during NO inhalation (Q\textsubscript{s}/Q\textsubscript{t} was 28\% ± 3\% and Q\textsubscript{va}/Q\textsubscript{t} was 24\% ± 2\%).

---

Table 6. Effects of NO inhalation during spontaneous breathing and mechanical ventilation (IPPV). NO at a dose of 4.9 $\mu$mol was pulsed into the inspired gas at each inspiration.

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous breathing</th>
<th>IPPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>5 min NO inhalation</td>
</tr>
<tr>
<td>RR, min\textsuperscript{-1}</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>$P_aO_2$, kPa</td>
<td>13.9 ± 1.7</td>
<td>28.5 ± 3.4*</td>
</tr>
<tr>
<td>$P_aCO_2$, kPa</td>
<td>9.0 ± 0.3</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>$Q_{va}/Q_t$, %</td>
<td>32 ± 2</td>
<td>22 ± 2*</td>
</tr>
<tr>
<td>$Q_t$, L \cdot min\textsuperscript{-1}</td>
<td>33 ± 1</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>28 ± 1</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>88 ± 9</td>
<td>96 ± 11</td>
</tr>
<tr>
<td>Heart rate, min\textsuperscript{-1}</td>
<td>37 ± 1</td>
<td>36 ± 1</td>
</tr>
</tbody>
</table>

* p<0.05, † different from spontaneous breathing p<0.05. IPPV = intermittent positive pressure ventilation. $Q_t$ = cardiac output. For other abbreviations see Table 5.
Table 7. The effects of NO inhalation (4.9 µmol NO/breath) during spontaneous breathing on ventilation/perfusion parameters as measured by the multiple inert gas technique in three horses. No statistical computations were performed. Data are presented as mean values, with range in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>baseline</th>
<th>15 min NO inhalation</th>
<th>15 min after</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_T$, L</td>
<td>8.3 (6.0 – 9.6)</td>
<td>8.3 (6.0 – 10.0)</td>
<td>8.7 (6.2 – 10.0)</td>
</tr>
<tr>
<td>RR, min$^{-1}$</td>
<td>3 (2-4)</td>
<td>3 (2-4)</td>
<td>3 (2-4)</td>
</tr>
<tr>
<td>$P_aO_2$, kPa</td>
<td>14.3 (9.8 – 23.4)</td>
<td>34.2 (21.0 – 44.2)</td>
<td>14.3 (11.3 – 17.3)</td>
</tr>
<tr>
<td>$P_aCO_2$, kPa</td>
<td>9.2 (7.9 – 10.3)</td>
<td>9.6 (8.3 – 10.7)</td>
<td>9.4 (8.1 – 10.7)</td>
</tr>
<tr>
<td>$Q_t$, L·min$^{-1}$</td>
<td>36 (30 – 46)</td>
<td>35 (26 – 47)</td>
<td>33 (24 – 42)</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>26 (25- 27)</td>
<td>25 (24 – 27)</td>
<td>28 (24 – 34)</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>80 (72 – 86)</td>
<td>91 (84 – 99)</td>
<td>89 (82 – 94)</td>
</tr>
<tr>
<td>Heart rate, min$^{-1}$</td>
<td>41 (39 - 42)</td>
<td>39 (35 - 47)</td>
<td>41 (36 – 48)</td>
</tr>
</tbody>
</table>

For abbreviations see Table 5 and Table 6.
Figure 12: Ventilation and perfusion distributions in relation to ventilation-perfusion ratio during horse anaesthesia on dorsal recumbency at baseline (top panel), during NO administration to the first half of inspiration (middle panel), and 15 min after cessation of the NO administration (bottom panel).
Figure 13: Ventilation and perfusion distributions in relation to ventilation-perfusion ratio during horse anaesthesia on lateral recumbency at baseline (top panel), during NO administration to the first 30% of inspiration (upper mid panel), to the first 60% of inspiration (lower mid panel), and between 50% and 80% of inspiration (bottom panel).
Table 8: Tidal volume ($V_t$), respiration rate (RR), cardiac output (Qt), mean pulmonary artery pressure (PAP), NO delivery rate and fraction in the inhaled gas when administering NO during the first 30% (NOp1), during the first 60% (NOp2), and during the period between 50% and 80% of inspiration (NOp3). The NO fraction is calculated from the NO delivery, pulse duration, and ventilation.

<table>
<thead>
<tr>
<th></th>
<th>NOp1</th>
<th>NOp2</th>
<th>NOp3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_t$, L</td>
<td>5.4 ± 0.8</td>
<td>5.3 ± 0.8</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>RR min$^{-1}$</td>
<td>4.6 ± 1.2</td>
<td>4.6 ± 1.1</td>
<td>4.9 ± 1.4</td>
</tr>
<tr>
<td>Qt/min</td>
<td>28 ± 4</td>
<td>28 ± 4</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>PAP cmH$_2$O</td>
<td>28 ± 3</td>
<td>27 ± 1</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>NO µmol/min</td>
<td>8.5 ± 1.8</td>
<td>17.0 ± 4.4</td>
<td>8.1 ± 2.0</td>
</tr>
<tr>
<td>NO pulse ppm</td>
<td>26 ± 1</td>
<td>25 ± 2</td>
<td>25 ± 2</td>
</tr>
</tbody>
</table>

Effects of pulse timing relative to inspiration

The baseline values of $Q_{va}/Q_t$ and $P_aO_2$ in NOp1, NOp2 and NOp3 were all equal. At baseline the $Q_{va}/Q_t$ was 27 ± 4%, 29 ± 4% and 30 ± 3% and the $P_aO_2$ was 27 ± 7 kPa, 22 ± 5 kPa and 20 ± 4 kPa respectively. With NOp1 the $Q_{va}/Q_t$ decreased ($p=0.045$) and $P_aO_2$ increased ($p=0.033$). With NOp2 the $Q_{va}/Q_t$ decreased ($p=0.010$) and $P_aO_2$ increased ($p=0.021$). The delivered amount of NO in NOp2 was large compared to NOp1 ($p=0.011$).

Figure 14: The change in venous admixture ($Q_s/Q_t$) and $P_aO_2$ when NO is delivered as a pulse in different parts of inspiration. Administration of NO during the first 30% (NOp1), during the first 60% (NOp2), and during the period between 50% and 80% of inspiration (NOp3), respectively. (A) NOp1, (B) NOp2, (C) NOp3. * $p<0.05$. 
(Table 8). This reflects the longer pulse in NOp2 when the NO delivery on the affected lung zones was kept constant (Table 8). However, effects of NO on $Q_{va}/Q_t$ and $P_aO_2$ were equal. NOp3 did not improve either $Q_{va}/Q_t$ or $P_aO_2$. The changes in $Q_{va}/Q_t$ and $P_aO_2$ induced by the different delivery modes are presented in Fig 14.

The effect of the NO delivered in the different delivery modes on perfusion redistribution is presented in Fig 13. In two of the three measured horses the differences in $Q_s/Q_t$ and logSDQ between the different pulses were only minimal. In the third horse, the NO reduced the $Q_s/Q_t$ only when delivered at the early inspiration (NOp1). The log SDQ was reduced with NOp1 and NOp2. In this horse, delivery in both NOp2 and NOp3 increased the $Q_s/Q_t$. With NOp3 also the log SDQ was increased.

Expired nitric oxide

The expired peak NO fraction was higher during NOp2 ($p=0.047$) and NOp3 ($p<0.001$) than during NOp1. The end-tidal NO fraction was equally low in all NO delivery modes ($p=0.13$) (Fig 15).

Figure 15: The exhaled NO peak (closed bar) and end-tidal NO (open bar) fractions in the different delivery modes. (For explanation of NOp1, NOp2, and NOp3, see Fig 14.)
Discussion

Inhalation of NO is a new therapy in the treatment of pulmonary hypertension and hypoxaemia in humans. In the established delivery methods the NO delivery does not target the open lung areas, which may explain the fact that there are non-responders to this therapy. The object of this thesis has been to study NO administration as a pulse synchronously with inspiration to the open lung areas. NO administration as a pulse at early inspiration near the airways effectively improved oxygenation by reducing the right-to-left shunt perfusion during equine anaesthesia. This improvement was not obtained when NO was administered to the later phase of inspiration, and in a horse with dispersed perfusion and ventilation even a worse oxygenation was observed. In experiment with pigs where the therapy was aimed to relieve pulmonary hypertension induced by hypoxic breathing gas, no difference was observed whether the NO was administered in early inspiration or during whole inspiration. NO expiration was, however, strongly reduced with pulsed administration in early inspiration, which is an advantage when the therapy is given in rebreathing circuits. The relationship between the NO delivery setting and NO uptake was not explicit although the relation was closer with the pulsed delivery compared to the whole inspiration delivery.

Physiological efficacy of the pulsed administration

When NO is delivered in whole inspiration, all airways and all ventilated lung regions receive the gas. The local dose depends on the inspired gas NO concentration and the local ventilation ($V_A$). By increasing the inspired concentration the effective dose is extended towards the regions of lower $V_A$. Increased perfusion in the low $V_A/Q$ areas counteracts the oxygenation improvement obtained with the NO delivery to normal $V_A/Q$ areas as presented in paper IV. The net effect on oxygenation is then a balance of these negative and positive contributions. With the pulsed administration of NO in the normal $V_A/Q$ areas, the detrimental effect on oxygenation of the low $V_A/Q$ regions is avoided as shown in papers III and IV where a clear relationship between the initial $Q_{va}/Q_t$ and its reduction was observed.

The detrimental effect on oxygenation of the NO delivered to low $V_A/Q$ areas has also been observed in earlier animal studies. In a swine sepsis model (lipopolysaccharide), where a true shunt develops, the shunt was reduced with NO delivered to whole inspiration.60
Contrary to this, in a group B β-hemolytic streptococcus infusion model where the pulmonary blood flow becomes dispersed without increase in true shunt the dispersion could not be reduced.\textsuperscript{61} In a sheep smoke inhalation model, where decreased oxygenation was secondary to low $V_A/Q$ and only a modest increase in true shunt occurred, the dispersion was not reduced and the improvement in oxygenation was slight and clinically insignificant.\textsuperscript{62} These results indicate that NO inhalation does not normalise the blood flow dispersion when the pulmonary injury results in increased blood flow to low $V_A/Q$ areas instead of in a true shunt.\textsuperscript{63}

In paper IV the $Q_s/Q_t$ and the $Q_{va}/Q_t$ were observed to be equal. This indicates that the reduced oxygenation is due to true shunt instead of pulmonary blood flow dispersion. In anaesthesia airway closure is however the major contributor both to low $V_A/Q$ and $Q_s/Q_t$.\textsuperscript{64} The airway closure is opened shortly at the end of inspiration when the inspiratory pressure increases. When NO is delivered in whole inspiration, even the $Q_s/Q_t$ may receive NO resulting in increased perfusion. This may explain the differences between the results on oxygenation of the pulsed administration presented in papers III and IV and the administration in constant inspired concentration.\textsuperscript{65}

The presence of low $V_A/Q$ areas provide also a possible explanation for the reduced or unchanged gas exchange resulting from the NO therapy in ARDS, PPHN, and chronic obstructive pulmonary disease (COPD) patients.\textsuperscript{13;48;66-69} This is supported by the observation that the response of ARDS patients to inhaled NO can be improved with alveolar recruitment by the addition of PEEP.\textsuperscript{70}

NO administration may also increase the $V_D^{alv}$. The high $V_A/Q$ areas are filling first at the beginning of inspiration with the gas from the anatomic dead space. This gas contains NO only in alveolar end-expiratory concentration. This may be insufficient to increase the perfusion in the high $V_A/Q$ regions, and the dilatation in the normal $V_A/Q$ regions redirects the perfusion also from the high $V_A/Q$ regions making those into $V_D^{alv}$. The increase of the $V_D$ was demonstrated in Figs 11 and 13 when NO was administered to the first half of inspiration. This increase was, however, not statistically significant. This may be due to the inert gas measurement, which does not make a difference between the $V_D^{alv}$ and $V_D^{anat}$, and the latter often dominates in the $V_D$. The increase in $V_D^{alv}$ has been observed also on whole inspiration delivery.\textsuperscript{60;62}
The therapeutic efficacy in relieving pulmonary hypertension was observed independent of the pulse width in paper II, where the NO was administered to the first third, two thirds, or whole inspiration to relieve hypoxic vasoconstriction induced by low FIO2. No relationship between pulse width and the response in PAP has been detected in spontaneous breathing either.32,35 This indicates that the area of normal VA/Q is homogeneous and fills in parallel at the beginning of inspiration. Thus the NO delivered during this filling period is distributed to the whole area and the increase of the pulse width does not give any further advantage. However, the enhanced reduction in pulmonary hypertension as a response on increased dose observed in paper II is characteristic for NO therapy.13,20 In dispersed lungs, an increased dose decreases the Vl required to deliver the effective dose of NO. This increases the lung area where the capillaries are dilated, but may result to impairment in oxygenation as explained above and increase the rebound effect as shown in paper II. Because the dilatation occurs only in the non-shunting lung areas receiving NO, the true shunt decreases the therapeutic efficacy in reducing PAP.71 The smaller the non-shunting perfusion is in relation to the total perfusion, the smaller will the total effect on the PAP be. The reduction is enhanced by the increase in perfusion resistance in the shunt areas as a result of dilatation in the ventilated lung areas.72 This is due to the elasticity of the vessels, which allows constriction when the perfusion decreases.73

The risk of deteriorated oxygenation present with whole inspiration delivery can be avoided with pulsed delivery. Therefore the pulsed administration may enable simultaneous improvement in oxygenation and reduction in hypertension even in patients having a large low VA/Q region, although the ability to counteract pulmonary hypertension may be reduced.

**NO delivery in characterising NO therapy**

Nitric oxide uptake in the lung is divided between smooth muscle and blood. Thus, the effect of inhaled NO depends not only on its uptake but also on the division rate. In smooth muscle NO converts guanosine triphosphate (GTP) to cGMP, which mediates relaxation and contributes to gas exchange. Distribution of inhaled NO is presented in Fig 16.
In blood, NO is inactivated by haemoglobin and oxidation. If the NO division between smooth muscles and blood as well as the conversion of GTP to cGMP are constant, the therapeutic efficacy of NO inhalation correlates with its uptake. According to the simulation results of paper II, with unchanged delivery NO uptake may vary due to variations in dead space administration and in NO diffusion constant. When the NO is delivered with constant inspired concentration, ventilation settings affect the dead space administration. In continuous flow delivery also the site of administration affects the uptake, since the volume between administration point and lungs determines whether the bolus accumulated at the administration point is inhaled to alveoli or remains in the dead space. Therefore the delivery setting is insufficient to characterise the NO therapy. With pulsed delivery, administration to the anatomic dead space is avoided. Also, since the NO is delivered to well ventilated, compliant, healthy lung regions, variations in NO uptake caused by administration to alveolar dead space and variations in the diffusion constant are effectively

![Diagram of NO distribution in the lungs]

*Figure 16: Distribution of NO in the lungs.*
avoided. Therefore with pulsed administration, correlation between delivery and NO uptake is improved compared to modes where NO is delivered to whole inspiration.

Pulsed administration of NO in clinical use

Monitoring

NO

Pulse timing is essential for successful administration. For this purpose a fast responding analyser connected between the NO administration point and the airways of the patient is useful as proposed in paper I. A characteristic NO fraction chart includes an expiratory plateau representing the alveolar NO fraction and an inspired dose spike rising from the expiratory plateau and ending in a zero NO fraction. This notch of zero NO fractions represents the end of inspiration and the beginning of expiration, and is derived from the low $V_A/Q$ regions and anatomic dead space. The plateau of the alveolar expiration rises from this notch. A delay in pulse triggering would cause a notch between the expiration plateau and the delivery dose spike. If the pulse lasts too long, the zero notches after the dose delivery spike disappear and/or another spike at early expiration appears indicating dead space administration. To improve such delivery the delivery line can be connected closer to the airways of the patient, the delivery pulse can be shortened, the lung filling during inspiration can be delayed by using constant flow ventilation, or the inspiration time can be prolonged.

Monitoring of NO in inspired gas is mandatory when NO is delivered in constant inspired gas concentration. The inspired gas should be monitored at a distance from the delivery site to have a homogeneous inspired gas mixture. Usually the monitor is connected to measure the gas concentration at the inspiratory limb connection of the Y-piece. The monitor is generally an electrochemical fuel cell with a response time of more than 10 seconds. The continuous flow delivery of NO is often accompanied by an NO bolus accumulating at the administration point during expiration when the inspiratory flow is zero. The bolus passes the measurement site in less than one second and can not be detected with a slow responding NO monitor. When the NO is delivered in constant inspired concentration, even a slow monitor may give an accurate reading. However, the monitors
are used to safeguard the delivery. To detect spiked output caused by device malfunctions, a monitor of less than 1 s response time would be needed. The only fast responding NO analysing technique is chemiluminescence. This is seldom used because the commercially available devices are expensive and not designed for clinical use.

For administration at early inspiration, the administration site should be as near the patient as possible. This allows no mixing distance between the administration and monitoring sites, and the delivered dose cannot be predicted from the inspired concentration monitoring as shown in paper I. In volumetric administration of NO, also the monitoring has to be volumetric. Such a monitor can be conveniently integrated in the delivery device.

$NO_2$
The use of an $NO_2$ monitor is also compulsory in connection with NO therapy. This requirement can be motivated when the NO is mixed with the breathing gas upstream of the ventilator. Our data showing that no $NO_2$ could be detected with pulsed administration of NO confirms the theoretical calculations of $NO_2$ formation in pulsed delivery. Therefore, the requirement for mandatory $NO_2$ monitor in connection with pulsed administration can not be motivated.

$O_2$
Monitoring of inspired $O_2$ is also required in connection of NO therapies. When the supply gas of 1000 µL/L NO in N$_2$ is used, the $O_2$ dilution is less than 2%-units in the inspiration gas. This does not endanger oxygenation of the patient, and therefore monitoring of the $O_2$ is not necessary during NO therapy when high concentrate NO supply is used.

**NO therapy in rebreathing circuit**
In contrast to open breathing circuits, when rebreathing circuits are used the expired gas will be circulated through a CO$_2$ absorber back to inspiration. The ordinary soda-lime absorbers absorb NO only little and the expired NO will circulate back to inspiration resulting in an uncontrollable dose. In the breathing circuit the NO also reacts with $O_2$ forming $NO_2$. Therefore the elimination of the NO from the expired gas is advantageous when the NO therapy is given in a rebreathing circuit. As indicated in papers I and II, NO
uptake rate is of the order of 90% in the perfused alveoli. This is well in line with the previous observation of the 95%-100% alveolar NO uptake. Contrary to this, the uptake rate in dead space is zero. When NO is delivered to whole inspiration, NO expiration derived predominantly from the dead spaces has been recognised as a problem in connection with NO therapy in rebreathing circuit. In pulsed administration to early inspiration the dead space administration is avoided, and as a consequence of this the expired NO is reduced to an almost negligible level as shown in paper I, II, and IV. However, because of physiological reasons, such as reduced diffusion of NO, also the alveolar NO uptake may decrease resulting in increased NO expiration. This would result in elevation of the breathing circuit NO fraction. Therefore, the circuit NO fraction has to be monitored. The NO monitor connected between the administration point and the patient can be used also for this purpose. The breathing circuit fraction can be read as the notch fraction after the delivery pulse spike shown in paper I.

Applications of pulsed administration

Pulsed administration provides two advantages over whole inspiration delivery: 1) Avoiding the administration to low V/A/Q regions of the lung gives better efficacy in improving oxygenation, and 2) avoiding administration to the dead space eliminates NO expiration.

Pulsed administration of NO would provide a new non-invasive method to improve oxygenation during equine anaesthesia where hypoxaemia caused by atelectasis is frequently found. Previously, this problem has been tried to solve with IPPV or NO administration to whole inspiration without success. Selective mechanical ventilation of dependent lung regions has been more successful, but requires selective intubation of the lower lung lobes.

In human NO therapy the pulsed administration would benefit patients whose oxygenation deficit is secondary to low V/A/Q as in ARDS, COPD and pneumonia. A typical ARDS lung is inhomogeneous and consists of a normally aerated upper region and consolidated fluid-filled atelectatic dependent areas. In between these regions there is a region that may open and close in the course of tidal breathing. Alveolar damage and inflammation may exist also in the non-dependent areas, and this may progress in some patients to fibrosis. This will decrease lung compliance locally. By avoiding the NO delivery to these low
VA/Q regions, the response in oxygenation is improved. With the minimal NO expiration, pulsed delivery of NO would be optimal for maintenance of arterial oxygenation during anaesthesia in rebreathing circuit. In human anaesthesia closing volume is the major contributor both to low VA/Q and Qs/Qt in dependent lung areas, and it may increase by over 70% increasingly in elderly and overweight patients. Closely related to the development of atelectasis, FRC is observed to be reduced by 50% compared to the pre-anaesthesia value in morbidly obese patients. Common measures to compensate the impairment in oxygenation are use of higher FIO2 or increasing PEEP. However, the development of atelectasis correlates with high FIO2, and PEEP may increase the shunt perfusion or reduce Qt, leading to deteriorated oxygenation. With the pulsed administration even atelectasis formation could be reduced by allowing a reduction in FIO2. Furthermore, also pulmonary hypertension can be relieved with reduced risk of deterioration in oxygenation.

Further clinical and animal model studies are, however, still required before these potential applications may enter into the tool box of the clinician to save human or animal life.
Conclusions

The overall conclusion of this thesis is that NO administration in pulses synchronously with inspiration is feasible in connection with artificial ventilation. The constancy of inspired concentration is not required for effective therapy, and the delivery setting is not representative of the therapy either. With the pulsed NO delivery mode selective vasodilatation in well-ventilated lung areas can be effectively induced, and this can be applied to relieve pulmonary hypertension and to improve arterial oxygenation. With optimal delivery rates the maximum therapeutic effect can be achieved without rebound vasoconstriction when the therapy is ceased.

In successful therapy the NO should be administered near the airways of the patient. The pulse, which should be short compared to the inspiration, is synchronised with the beginning of inspiration. A fast NO analyser, which can be made feasible for clinical use, is advantageous in order to monitor the delivery timing.

The advantage of pulsed delivery compared to whole inspiration delivery is that administration to the low $V_A/Q$ region and dead spaces is avoided. This makes improvement of oxygenation possible even when the lung perfusion is dispersed. Also NO expiration is reduced, which is essential when the therapy is given in a rebreathing circuit during anaesthesia.
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