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# Gas Exchange in the Normal Lung

Experimental studies on the effects of  
positive end-expiratory pressure  
and body position

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**To my family**



# Abstract

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**BACKGROUND:** The principal function of the lung is gas exchange requiring adequate ventilation and perfusion at the level of the alveoli. The efficiency of gas exchange is depending on the distributions of regional ventilation (V) and pulmonary blood flow (Q) and their correlation.

**AIMS:** To validate a high-resolution method to quantify regional V and to investigate the combined effect of positive end-expiratory pressure (PEEP) and body position on distributions of regional V and Q in the normal lung with mechanical ventilation. To assess the matching of V and Q by calculating ventilation-perfusion ratio (V/Q) heterogeneity, determining the spatial distribution of V/Q and to investigate the role of nitric oxide (NO) in regional V/Q matching.

**METHODS:** Anesthetized mechanically ventilated sheep were studied in prone or supine position with different levels of PEEP (0, 10 and 20 cmH<sub>2</sub>O). Measurements of regional V were done by determining the deposition of a wet aerosol of fluorescent microspheres (FMS) with a median mass aerodynamic diameter of 1.1 μm, and validated against Technegas. Radioactive microspheres, 15 μm in diameter, were used for determining regional Q. Nitric oxide synthase (NOS) was inhibited with Nω-nitro-L-arginine methyl ester (L-NAME) to evaluate the role of NO on regional V/Q matching. The right lung was dried at total lung capacity and diced in approx. 1000 regions tracking the spatial location of each region.

**RESULTS:** The deposition of FMS mirrored regional deposition of Technegas and thus regional ventilation well. In prone, with PEEP, only a small dorsal redistribution of V but not

of Q was observed. The vertical Q gradient was abolished with PEEP in prone position in conflict with the classical zonal model. In supine position both V and Q were distributed with a unimodal gradient and PEEP displaced the mode further dorsally. V/Q heterogeneity was greater in supine than in prone position with and without PEEP. Furthermore, PEEP generated regions with high V/Q in supine but not in prone position. Inhibition of NOS did not change the V/Q distribution in prone position.

**CONCLUSION:** There were marked differences in redistribution of regional ventilation and regional pulmonary blood flow between prone and supine position when PEEP was applied. NO was not an active mechanism for V/Q matching in normal sheep lungs.

# List of original papers

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This thesis is based on the following four papers, which will be referred to by their Roman numerals:

**I. Positive end-expiratory pressure affects regional redistribution of ventilation differently in prone and supine sheep.**

Mats J. Johansson, Andreas Wiklund, Torun Flatebø, Anne Nicolaysen, Gunnar Nicolaysen, Sten M. Walther. *Crit Care Med* 2004; 32: 2039 – 2044.

**II. Marked differences between prone and supine sheep in effect of PEEP on perfusion distribution in zone II lung.**

Sten M. Walther, Mats J. Johansson, Torun Flatebø, Anne Nicolaysen, Gunnar Nicolaysen. *J Appl Physiol* 2005; 99: 909 – 914.

**III. Minimal redistribution of regional ventilation perfusion ratios by 10 and 20 cmH<sub>2</sub>O positive end-expiratory pressure in prone sheep.**

Mats J. Johansson, Torun Flatebø, Anne Nicolaysen, Gunnar Nicolaysen, Sten M. Walther. *Manuscript*.

**IV. Inhibition of constitutive nitric oxide synthases does not influence ventilation – perfusion matching in normal prone adult sheep with mechanical ventilation.**

Mats J. Johansson, John-Peder Escobar Kvitting, Torun Flatebø, Anne Nicolaysen, Gunnar Nicolaysen, Sten M. Walther. *Submitted*.

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## ABBREVIATIONS

(A-a)O <sub>2</sub>	Alveolar-arterial O <sub>2</sub> tension differences
cNOS	Constitutive nitric oxide synthase
CT	X-ray computed tomography
CV	Coefficient of variation
eNOS	Endothelial nitric oxide synthase
EtCO <sub>2</sub>	End-tidal Pco <sub>2</sub>
FMS	Fluorescent microspheres
FRC	Functional residual capacity
HPV	Hypoxic pulmonary vasoconstriction
iNOS	Inducible nitric oxide synthase
MAP	Mean systemic arterial pressure
MIGET	Multiple inert gas elimination technique
MPAP	Mean pulmonary arterial pressure
MRI	Magnetic resonance imaging
nNOS	Neural nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
P <sub>A</sub>	Alveolar pressure
P <sub>a</sub>	Pulmonary artery pressure
Pco <sub>2</sub>	Partial pressure of carbon dioxide
PEEP	Positive-end expiratory pressure
PET	Positron emission tomography
PO <sub>2</sub>	Partial pressure of oxygen
P <sub>v</sub>	Pulmonary venous pressure
Q	Regional pulmonary blood flow
SaO <sub>2</sub>	Arterial oxygen saturation
SDlog(V/Q)	Standard deviation of logV/Q
SvO <sub>2</sub>	Mixed venous saturation
SPECT	Single photon emission computed tomography
TLC	Total lung capacity
V	Regional pulmonary ventilation
V <sub>A</sub>	Alveolar ventilation

$V_{CO_2}$	Volume carbon dioxide produced
$V_D$	Dead space volume
$V_{O_2}$	Volume oxygen consumed
$V/Q$	Ventilation to perfusion ratio
$V_T$	Tidal volume



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# 1. Introduction

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The history of gas exchange goes back to the speculations and thoughts of philosophers and physicians in ancient Greece. It was not until the 19<sup>th</sup> century, however, that technical innovations made it possible to measure the course of gas exchange. The German chemist, Heinrich Gustav Magnus (1802 – 1870), developed methods for the extraction of oxygen and carbon dioxide in blood, and showed that oxygen was more abundant in arterial than in venous blood. In a seminal paper from 1837 “*Über die im Blute Enthaltenen Gase, Sauerstoffe, Stickstoff, und Kohlensäure*” he concluded: “...it is probable that the inhaled oxygen is absorbed in the lungs by the blood, where, given up in the capillary vessels, it determines the formation of carbonic acid”. The German physiologist Eduard Pflüger (1829 – 1910) convinced other scientists in his thesis “*Über die physiologische Verbrennung in den lebendigen Organisme* (1875)” that respiration took place in the tissues themselves and that the function of the blood was simply to transport oxygen to and carbon dioxide from these tissues.

In the 19<sup>th</sup> century it was believed that the lung itself secreted oxygen. This paradigm changed in 1910 when August and Marie Krogh (Krogh and Krogh, 1910a, 1910b) in Copenhagen published several ground-breaking papers concluding that oxygen transfer from alveolar gas to capillary blood could be adequately explained by passive diffusion. In 1917, August Krogh and Johannes Lindhard (Krogh and Lindhard, 1917) published a paper where they speculated that pulmonary perfusion through each lung lobe should be in proportion to its ventilation. The modern era of understanding ventilation-perfusion relationships began

with works of Wallace Fenn, Artur Otis and Hermann Rahn at the University of Rochester, NY (Fenn *et al.*, 1946) and another group led by Richard Riley at the Johns Hopkins University in Baltimore, MD (Riley and Cournard, 1949). Fenn, Otis and Rahn were aviation physiologists working on breathing at high pressures. The group led by Riley examined the relationships between oxygen, carbon dioxide and haemoglobin in human blood. Insight into ventilation-perfusion relationships and the study of clinical respiratory physiology were revolutionised by the advent of the platinum PO<sub>2</sub> electrode introduced by Clark in 1953 (Clark *et al.*, 1953) and the PCO<sub>2</sub> electrode introduced by Severinghaus and Bradley a few years later (Severinghaus and Bradley, 1958).

### **1.1 Artificial ventilation**

Mechanical ventilation first developed in the 19<sup>th</sup> century, with the Cuirass-ventilator, as an apparatus for intermittent negative pressure ventilation. In the 1910s H.K. Giertz (Giertz, 1959) in Stockholm showed that artificial ventilation by rhythmic insufflation was superior to constant differential pressure breathing of the Sauerbruch type during thoracic surgery. Paul Frenckner (Frenckner, 1934) developed the first positive pressure ventilator, the Spiropulsator, in the 1930s in Stockholm. This was further modified as the “Frenckner-Crafoord-Andersson” ventilator, which was used by Clarence Crafoord during major thoracic surgery in the late 1930s (Andersson *et al.*, 1940). Ventilators eventually evolved to become respirators for use in intensive care.

### **1.2 Positive end-expiratory pressure**

Frumin *et al.* (Frumin *et al.*, 1959a and 1959b) demonstrated that alveolar-arterial oxygen gradients varied with pressure in the airways during exhalation and that closure of pulmonary units, and thus loss of functional residual capacity (FRC), causes a progressive decrease in

compliance in the lung. General anaesthesia and many pathological conditions result in a decrease in FRC, having a harmful effect on gas exchange. FRC can be increased by the application of positive end-expiratory pressure (PEEP), first described by Hill *et al.* (Hill *et al.*, 1965) on patients that had undergone open-heart surgery. PEEP maintains alveolar expansion (Glazier *et al.*, 1967). The best PEEP, or optimum PEEP, is defined as the level of PEEP giving maximal oxygen transport, which is the product of cardiac output and oxygen content (Suter *et al.*, 1975). This optimum PEEP level correlates with the highest total respiratory compliance, the highest mixed venous oxygen tension, and lowest dead space ventilation.

In current clinical practice PEEP is often used in the mechanical ventilation of intensive care unit patients in order to improve ventilation, and during general anaesthesia in order to prevent per- and postoperative atelectasis.

### **1.3 Effect of body position**

In experiments on humans where gas samples were drawn from different lobes in different body positions, Martin *et al.* (Martin *et al.*, 1953) concluded that the partial pressures of O<sub>2</sub> and CO<sub>2</sub> differed between lobes and body position. Froese and Bryan (Froese and Bryan, 1974) concluded that the diaphragm's position varied between positions and between the awake as well as the paralysed anaesthetised state. Prone positioning as a part of therapy for severe acute respiratory failure was first used in the 1970s. Piehl and Brown (Piehl and Brown, 1976) and Douglas *et al.* (Douglas *et al.*, 1977) noticed an increase in PaO<sub>2</sub> when their patients were turned onto their front. During 1990s and 2000s prone positioning, often together with PEEP, was used to increase gas exchange in intensive care unit patients.

## 2. Aims of the thesis

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The combined effects of PEEP and body position on ventilation and pulmonary perfusion has not been fully explored, neither in diseased nor in healthy lungs. Hence, this thesis aims to address the interaction of PEEP and position in the normal lung. We were specifically interested in examining the influence of PEEP and position on the distributions of ventilation (V) and perfusion (Q) and ventilation-perfusion relationships (V/Q).

The primary aims of this thesis were:

- [I] To validate a method for high resolution measurements of regional V.
- [II] To study the interaction of position and mechanical ventilation with PEEP on the distribution of regional V.
- [III] To study the interaction of PEEP and position on the distribution of pulmonary blood flow.
- [IV] To study the interaction of PEEP and position on V/Q distributions.
- [V] To study the role of endogenous nitric oxide production in the matching of V and Q in normal lungs.

## 3. Ventilation

---

Ventilation of the pulmonary functional unit occurs by both convection and diffusion. During inspiration, oxygen-rich air flows into the lung as a result of the negative intra-thoracic pressure developed by the respiratory muscles. During expiration, passive recoil of the thoracic wall leads to exhalation of O<sub>2</sub>-depleted but CO<sub>2</sub>-rich air. Deep in the lung, as the peripheral airways are approached, convection becomes weak and O<sub>2</sub> now diffuses towards the periphery driven by the PO<sub>2</sub> gradient caused by O<sub>2</sub> absorption at the alveolar surface. The higher PCO<sub>2</sub> in the capillaries than in the alveoli causes diffusion of CO<sub>2</sub> into the alveoli, and from the alveoli towards the proximal airways where convection begins.

### 3.1 Airway anatomy and physiology

The estimated human alveolar surface area is about the size of a tennis court (130 m<sup>2</sup>), and the number of alveoli approaches 480 million in the adult. The functional unit is larger than a single alveolus since studies of diffusion and convection show that the branched complex of alveolated airways that are derived from the same first order transitional bronchiole, the pulmonary acinus, is at diffusion equilibrium. The mean volume of an acinus in the human has been found to be 187 mm<sup>3</sup> with large inter-individual variations (Weibel *et al.*, 2005). The diameter of the transitional bronchiole, the stem of the acinus, is significantly related to the air volume it supplies (Haefeli-Bleuer and Weibel, 1988).

The human airways comprise multiple symmetric dichotomous branches beginning at the trachea and ending in the most peripheral alveolar sacs (Weibel, 1963), **Figure 1**. There

## Chapter 3

are in total 23 airway generations, where the first 14 generations serve as conducting airways where air moves by convection. The cross-sectional area decreases down to the third generation of bronchial airways thereafter the cross-sectional area increases. This is important since this decreases airway resistance and thereby airflow velocity. Resistance ( $R$ ) is highly dependent on the radius of the cross-sectional area ( $R \sim r^4$ ).

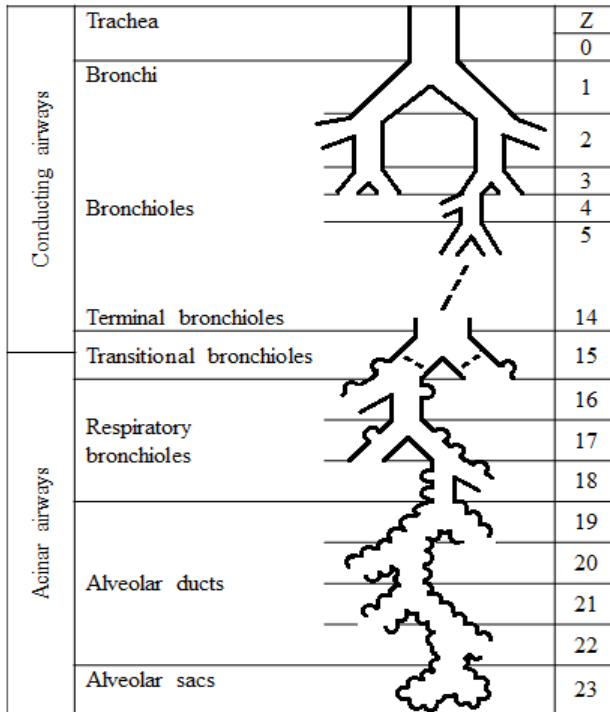


Figure 1. Model of the human airway system. Modified after Weibel (2005).

Since the conducting airways contain no alveoli, they do not participate in respiratory gas exchange. They do participate, however, in the warming and humidification of inspired air. At rest, movement of gas in the 14 – 16<sup>th</sup> generations occurs by both convection and diffusion. The driving force of diffusion in these most peripheral parts of the lung is the partial pressure difference for  $O_2$  and  $CO_2$  (i.e. between alveolar air and capillary blood). This difference in partial pressure can only be maintained if the capillaries are well-perfused, and

the alveolar gases are continuously replenished. The most distal generations of the human airway system consist of acinar airways, that consist of an axial channel, the alveolar duct, with alveoli arranged like cuplike chambers opening into the duct. This causes the alveolar surface area for gas exchange to be approximately five times greater than the surface of the duct itself. To ensure that the wall that separates alveolar gases and blood is tenable, the membrane consists of three layers; the alveolar epithelium and the capillary endothelium separated by a thin interstitial layer, together forming a very thin wall about 1µm thick.

The interstitial connective tissue fibre system supports the capillary network with which it is intertwined. It is a part of highly structured three-dimensional fibre continuum that extends from the pleura to the airway walls (Weibel *et al.*, 2005). In the periphery the septal fibres are suspended between interlobular connective tissue septa and with fibre rings around alveoli that form the actual walls of the acinar ducts. This intertwined fibre construction transmits the respiratory movements to the alveolar septa that thus remain well expanded in the parenchymal airspace. Since, large numbers of alveoli are interconnected with curved surface linings, there is a potential risk of collapse of small alveoli into a large alveolus, according to Laplace's law. The presence of surfactant in the alveolar liquid helps to prevent these alveoli from coalescing into large alveoli, because it considerably reduces surface tension. The function of surfactant is also to alter the surface tension in the alveoli as their size varies with inspiration and expiration. As the surface area of the alveolus reaches a minimum at the end of the expiration, the surfactant molecules are compressed into a smaller area thereby reducing surface tension.

### 3.2 Alveolar ventilation

Alveolar ventilation is dependent on respiratory frequency and the gas volume reaching the alveoli. Alveolar ventilation ( $V_A$ ) = respiratory frequency x (tidal volume – dead space)

$$V_A = f (V_T - V_D)$$

There is some uncertainty as to what constitutes  $V_D$  since there is a variation in alveolar dead space due to ventilation of relatively hypoperfused alveoli, and that anatomical dead space can vary in volume. The air that enters the alveoli during inspiration consists of stale air from conducting airways and fresh air. Due to diffusion this air is mixed when it reaches the alveoli.

The composition of alveolar gas depends on production and exchange of  $\text{CO}_2$ , barometric pressure, alveolar ventilation, oxygen exchange and nitrogen exchange. Since carbon dioxide is eliminated from the body by ventilation only and the  $\text{CO}_2$  inhaled is negligible, the volume of carbon dioxide produced ( $V_{\text{CO}_2}$ ) is the same as the volume expired ( $V_{\text{E}_{\text{CO}_2}}$ ) at steady state. Oxygen delivery depends on ventilation and the inspired  $\text{O}_2$  fraction. Oxygen removal from the alveoli is regulated by the oxygen gradient between alveolar gas and capillary blood. Higher tissue oxygen consumption ( $V_{\text{O}_2}$ ) increases this gradient. Tissue  $V_{\text{O}_2}$  varies with activity but under resting conditions it is approximately 250 ml/min for an average-sized person.

### 3.3 Compliance and regional ventilation

Ventilation is dependent on compliance, which is defined as volume/ $\Delta$ pressure. Compliance varies in the lung due to a number of factors. Compliance is different at different lung volumes, less volume per pressure unit is gained when approaching total lung capacity (TLC) (Hoffman, 1985). Lung volume is dependent on body position and thereby compliance is also dependent to some degree on position. In the prone position the lung volume is larger than in the supine (Henderson *et al.*, 2013). Lung compliance is different between spontaneous breathing and controlled intermittent positive pressure ventilation, here referred to as

mechanical ventilation. During mechanical ventilation with muscle relaxation the position of the diaphragm varies between positions since the abdominal content influences the diaphragmatic position differently (Froese and Bryan, 1974). Applying PEEP will increase FRC, which means that inspiration starts from a new lung volume. These variations in total and regional compliance will influence regional ventilation.

### 3.4 Ventilation heterogeneity

The existence of unevenly distributed ventilation in man was assumed by both Rahn (Rahn, 1949) and by Riley and Cournard (Riley and Cournard, 1949). Martin *et al.* (Martin *et al.*, 1953) studied lobar alveolar gas concentration differences between positions and found that ventilation varied between lobes and with position. A vertical gradient in pulmonary ventilation distribution was first described by West and Dollery (West and Dollery, 1960) and explained by the pleural pressure gradient due to the effect of gravity in the upright position. The pleural pressure at the apex is less than the atmospheric pressure, and increases, though still sub-atmospheric, towards the base of the lung. These ventilation heterogeneities are related to two features; the weight of the lung itself, and the differences in shape between the lung tissue and the surrounding pleural space. The less expanded basal lung tissue has a greater compliance and, consequently, greater relative ventilation, when inspiration starts from FRC with measurements made under static conditions. When, instead, the distribution of inspired gas was studied during inspiratory flow, especially high flows, regional differences were less than those seen with static or low flow inspiration (Bake *et al.*, 1974). Studies in humans by Rehder and colleagues (Rehder *et al.*, 1977 and 1978) showed that ventilation was more uniform in the prone than previously found in the supine position. When measurements of ventilation were made using techniques with higher resolution, larger iso-gravitational variations in regional ventilation were found. The vertical ventilation distribution gradient

differed between positions. In the prone position the gradient was much less or non-existent (Hubmayr *et al.*, 1987).

### 3.5 Apnoeic mass movement

Apnoeic mass movement oxygenation relies on the discrepancy between the rate at which oxygen is normally removed from the alveoli compared to that at which CO<sub>2</sub> is typically delivered (Enghoff *et al.*, 1951). In apnoeic man, V<sub>O<sub>2</sub></sub> averages 230 - 250 ml/min, whereas the output of CO<sub>2</sub> to the alveoli is limited to about 20 ml/min and the remaining CO<sub>2</sub> production is buffered within the body tissues. This means that the volume of gas in the lung decreases by 210 – 230 ml/min and a volume gradient is created between the upper airway and the alveoli. If the airway is patent with access to pure oxygen this will result in mass movement of oxygen down the airways to the alveoli. On the other hand CO<sub>2</sub> is not exhaled because of the mass movement of O<sub>2</sub>, and the alveolar CO<sub>2</sub> will therefore rise by about 0.4 – 0.8 kPa/min. Theoretically, humans can tolerate apnoea for about 100 minutes with maintained saturation provided that the airway remains patent and there is a constant supply of 100 % oxygen. Hypercapnia, however, is an inevitable feature in this situation and P<sub>a</sub>CO<sub>2</sub> values as high as 18.7 kPa have been reported in man (Payne, 1962).

## 4. PEEP, position and regional ventilation

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**PAPER I:** *Positive end-expiratory pressure affects regional redistribution of ventilation differently in prone and supine sheep.*

The ability to measure ventilation and to understand what happens when changes in ventilation are made is clinically important. Decisions concerning the use of PEEP and positioning are probably more valid if they are based on knowledge of how these interventions influence distribution of ventilation. All methods used to measure or even image regional ventilation aspire to twin goals that often are in direct conflict i.e. good spatial and temporal resolution. Since normal ventilation is inherently cyclic with periods of inspiration and expiration this conflict is obvious.

The pulmonary acinus is the functional unit of the lung. The acinus comprises the branched complex of alveolated airways that are connected to the same first order of transitional bronchioles and is also where ventilation begins to convert from convection to diffusion. For measuring or imaging clinically relevant changes in ventilation, techniques having this level of spatial resolution would be of value. The best current estimates of regional ventilation in humans are provided by techniques based on wash-in and wash-out of labelled gases.

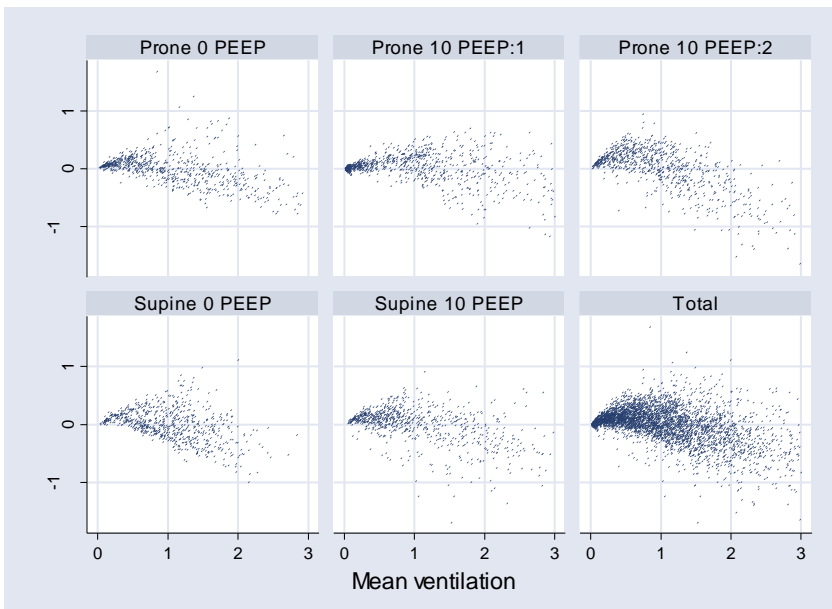
### 4.1 Validation of a microsphere method for ventilation measurements

There is no accepted gold standard for ventilation measurements, but measurements of regional ventilation with aerosolised fluorescent microspheres (FMS) reconcile the conflict

between spatial and temporal resolution fairly well provided that the ventilation is stable over a period of minutes. Spatial resolution depends on the volume of each lung region (i.e. the voxel size for non-invasive imaging methods and piece or weight of lung tissue for destructive slice-and-dice methodology) and the temporal resolution depends on the time the microspheres are given. The measurements reflect the mean ventilation during that time, including cyclic variations due to inspiration and expiration. By using different labels it is possible to measure regional ventilation repeatedly and, for instance, compare what happens in the same region when position and PEEP are repeatedly changed.

In the experiments on sheep that we report in **Paper I** we made the fluorescent aerosol from an aqueous solution of fluorescent polystyrene microspheres, 0.2  $\mu\text{m}$  in diameter. The solution was nebulised continuously over 8 minutes with a nebulizer that eliminated particles  $> 5 \mu\text{m}$ . Since the aerosol of fluorescent microspheres consisted of particles in the 1  $\mu\text{m}$  range, the aerosol was not equivalent to a gas. For that purpose a validation experiment was done comparing deposition of Technegas and FMS aerosol during mechanical ventilation. Technegas is an aerosol of minute particles of graphite covered with  $^{99\text{m}}\text{Tc}$  that form aggregates having a diameter of 30 – 160 nm, that primarily deposit by diffusion-mediated dispersion in gas-exchanging lung parenchyma (Senden *et al.*, 1997, Lloyd *et al.*, 1995, Lemb *et al.*, 1993). Even the smallest aerosol, however, will distribute in a different manner compared to gas molecules at the alveolar level (Henry *et al.*, 2002). Although, Hinz *et al.* (Hinz *et al.*, 2003) found that regional ventilation measured with Technegas and  $^{81\text{m}}\text{Kr}$  had an excellent correlation ( $R^2 = 0.98$ ) when measured with single-photon emission computed tomography (SPECT). We found similarly a high correlation ( $r = 0.95$ ; range 0.91-0.96) when deposition of Technegas was compared with aerosolised FMS in five animals. Agreement and precision assessed according to Bland and Altman (Bland and Altman, 1986) were fair but Bland-Altman plots revealed a gradual decrease in precision (increase in the standard

deviation of the mean difference) with higher ventilation. This pattern is typical for count data and was seen in repeated measurements of perfusion performed simultaneously in the same five animals. Ventilation measurements with FMS yielded a larger estimate of ventilation, however, than Technegas in regions with high mean ventilation. This deposition pattern was similar between animals irrespective of body position or PEEP level, **Figure 2**. In contrast, repeated perfusion measurements showed a uniform pattern within animals. The location of regions in which ventilation was overestimated by FMS aerosol compared to Technegas changed with posture showing that the phenomenon was not associated with a specific lung segment. The similarity in pleural and non-pleural regions indicates, moreover, that the two tracers were deposited in a similar pattern in gas exchanging and airway tissue, **Figure 3**. The very similar distributions of the two tracers for ventilation along the gravitational axis show that any influence of gravity as a result of particle size must be small. We thus found no straightforward explanation for the small difference in deposition of the two tracers.



*Figure 2. Plots of the differences between Technegas (TG) and fluorescent microspheres (FMS) in different positions and PEEP levels.*

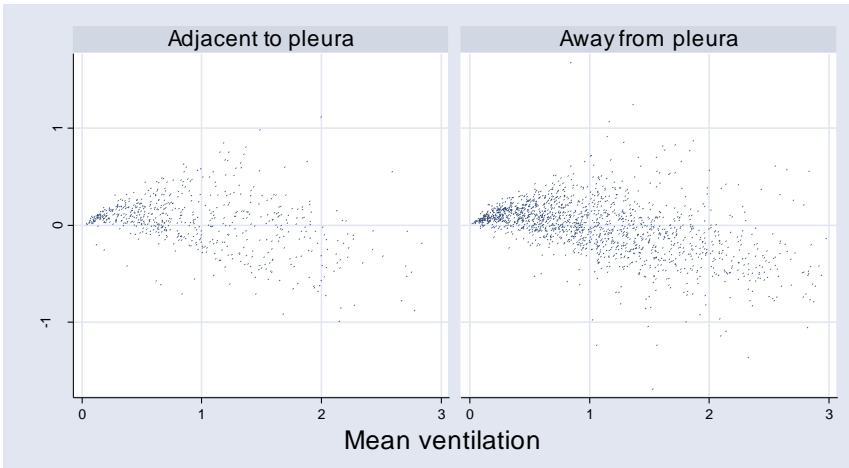


Figure 3. Plots of differences between Technegas (TG) and fluorescent microspheres (FMS) in relation to the pleura.

Studies validating measurements of regional ventilation in small lung regions are few. Melsom *et al.* assessed the correlation between the same tracers as in **Paper I** in standing spontaneously breathing, awake sheep (Melsom *et al.*, 1999). They found a high correlation (mean  $r = 0.82$ ,  $n=3$ ) and a similar deposition pattern from the center to the periphery of the lung. Altemeier *et al.* (Altemeier *et al.*, 1998) used a dry fluorescent aerosol together with intravenous radioactive microspheres to predict pulmonary gas exchange. They found a good prediction of gas exchange indicating the utility of the fluorescent microsphere technique for labeling regional ventilation. Coghe, Votion and Lekeux (Coghe *et al.*, 2000) showed that the images of Technegas and of  $^{81m}\text{Kr}$  obtained with  $\gamma$ -camera were highly equivalent in healthy calves. They, however, found a weaker agreement between a  $^{99m}\text{TcDTPA}$ -aerosol and the above mentioned two tracers. They observed that the  $^{99m}\text{TcDTPA}$ -aerosol had a significant deposition in larger airways. This is in conflict with the results reported by Melsom *et al.* (Melsom *et al.*, 1997) with either Venticoll-aerosol or the fluorescent microsphere aerosol as

used in **Paper I**. The  $^{99m}\text{TcDTPA}$ -aerosol used by Coghe *et al.* (Coghe *et al.*, 2000) had a relatively large fraction of particles larger than  $3\ \mu\text{m}$  and there is no information as to whether the aerosol generator had a system for eliminating larger particles. Melsom *et al.* (Melsom *et al.*, 1999) used a generator creating an aerosol where only 25 % of the particles were larger than  $1.4\ \mu\text{m}$ . Particles larger than  $5\ \mu\text{m}$  were eliminated. We conclude that both Technegas and the wet aerosol used in **Paper I** depict regional ventilation reliably.

To what extent does the variation in bias influence analysis of regional ventilation? When all animals were analyzed together the ventilation measured with FMS aerosol to regions with a normalized ventilation of less than 2.5 accounted for 85 % of total ventilation. Bias and precision in the determination of normalized regional ventilation in this large subset of regions was 0.04 and 0.26, respectively. Hence, we believe that the impact of the systematic variation of bias was small with respect to analysis of the vertical distribution of regional ventilation. This is also born out in the very close agreement between the two tracers for lung planes.

### 4.2 Redistribution of ventilation by PEEP and position

Ventilation was more homogeneously distributed in prone compared to supine position without PEEP, **Figure 4**. This was in accordance with earlier work by Mure *et al.* (Mure *et al.*, 2000) in pigs and Musch *et al.* (Musch *et al.*, 2002) in man. The uni-modal vertical ventilation gradient in supine position was reproduced by two recent SPECT studies in supine and prone anaesthetised and mechanically ventilated humans (Nyrén *et al.*, 2010 and Petersson *et al.*, 2010). However, the distribution in prone position differed.  $V$  decreased linearly from non-dependent dorsal to dependent ventral lung regions in sheep, while in humans the vertical distribution gradient was unimodal with lower mean ventilation in both

ventral dependent and dorsal non-dependent lung regions. These differences in redistribution of ventilation can be explained by anatomical and/or methodological differences.

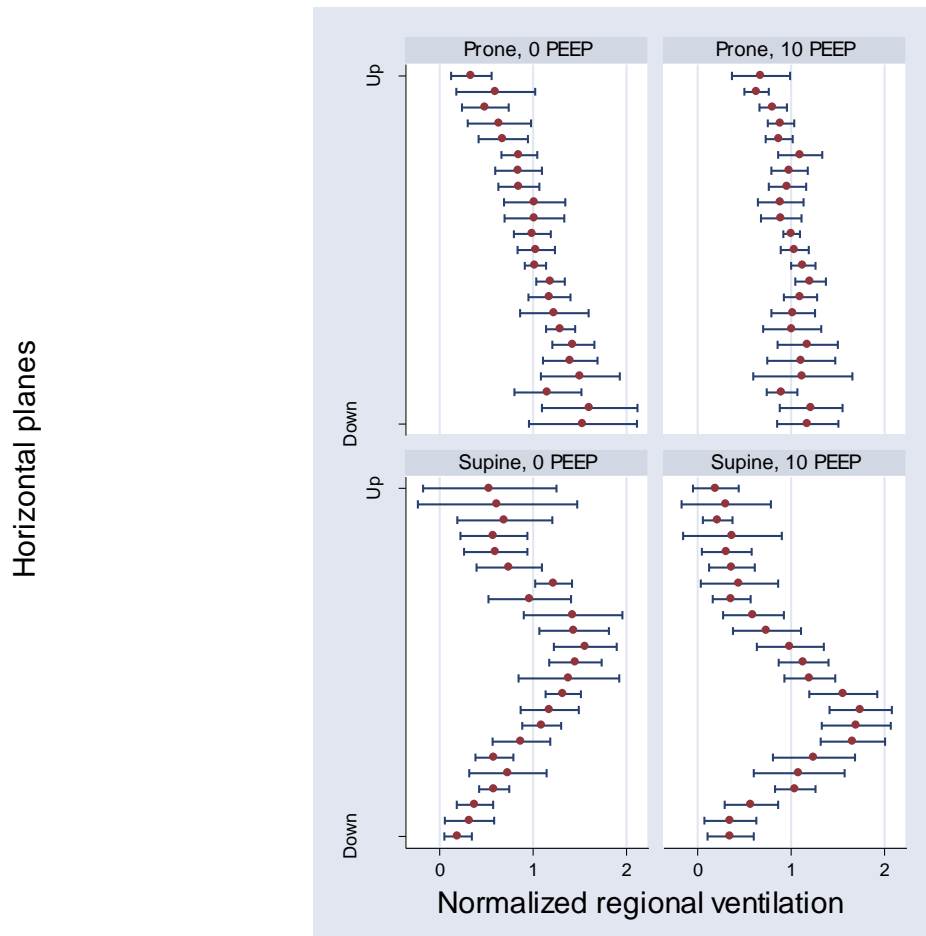


Figure 4. Normalized regional ventilation in horizontal planes (Johansson *et al.*, 2004).

The relationship between width and height of the rib cage is different between man and sheep; the human rib cage is wider and the sagittal distance is less than in sheep. In prone sheep the wide dorsal lung is non-dependent and may by its weight influence expansion of ventral dependent lung. This is probably not the case in prone man. The position of subjects in the human studies was not precisely given (Nyrén *et al.*, 2010), but they were placed in a

comfortable prone position. The sheep were placed in *sphinx* position, with their fore and hind legs bent and placed along the body and the head and neck fixed in an upright position. When Hoffman (Hoffman, 1985) examined lung expansion in anaesthetised dogs with increasing lung volumes and revealed more homogeneous expansion in prone position, his experiments were done with the animals suspended in six evenly spaced ties placed around the spinal ligaments. This position is more alike the position of the sheep than the humans prone position. The regional compliance and, as a result, ventilation in regions near the diaphragm could therefore differ. In addition, SPECT tends to underestimate ventilation in the periphery of the lung because of attenuation of photons near the chest wall (Pettersson *et al.*, 2007).

Applying 10 cmH<sub>2</sub>O PEEP in sheep had distinct position dependent effects. Ventilation became more uniformly distributed in prone position and the vertical distribution gradient disappeared, as a result of redistribution of V to non-dependent lung. When 10 cmH<sub>2</sub>O PEEP was applied in supine position, ventilation was redistributed to more dependent dorsal regions, and, as a result, the vertical gradient increased significantly.

The effect of 10 cmH<sub>2</sub>O PEEP on ventilation distribution in anaesthetised prone man was small, with some redistribution of V to dependent lung (Pettersson *et al.*, 2010). In supine, 10 cmH<sub>2</sub>O PEEP redistributed V further dorsally with an increasing vertical gradient, quite similar to the pattern in supine sheep.

Applying 10 cmH<sub>2</sub>O PEEP in sheep had distributed V different in prone and supine positions. In prone was V distributed to non-dependent dorsal regions and in supine was V further distributed toward dependent dorsal regions. These data underscore the power (and complexity) of a deceptively simple positional change on physiological variables of vital concern to the clinician (Marini, 2004).

## 5. Pulmonary blood flow

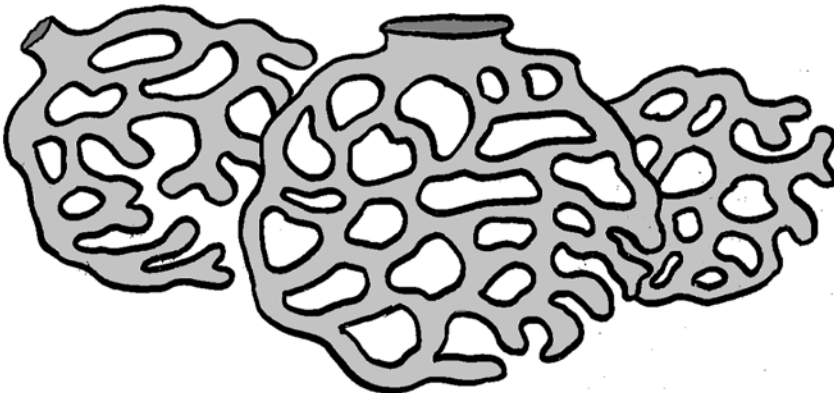
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**E**volution led to the development in mammals of a separate low-pressure blood flow system through the lungs coupled in series with a high-pressure system through the rest of the body. In man the mean pulmonary arterial pressure (MPAP) is in the range 17 – 22 mmHg compared to the mean systemic arterial pressure (MAP) of 80 – 100 mmHg. Other normal blood pressures in the pulmonary circulation relative to the atmosphere are:  $\approx$  15 mmHg in the arteriole;  $\approx$  10 mmHg in the capillaries;  $\approx$  8 mmHg in the veins; and  $\approx$  6 mmHg in the left atrium.

The gradual development of these two separate, but in series, circulatory systems were crucial to the evolution of warm-blooded mammals since they consumed higher quantities of oxygen than amphibians. One prerequisite for the high  $\text{VO}_2$  in mammals is that the exchange of  $\text{O}_2$  and  $\text{CO}_2$  can occur on a large scale. This depends on a thin blood-gas barrier between the capillaries and alveoli, and on very large alveolar and capillary surface areas where gas exchange can occur. The thickness of this barrier varies with the size of the species, the mean thickness in humans is  $0.62 \mu\text{m}$  (Maina and West, 2005), and consists of three layers; the capillary endothelium, a basement membrane with collagen, and the alveolar epithelium. This barrier is exposed to tensile stress from both capillaries and alveoli. The strongest layer and therefore the main obstacle to disruption is the basement membrane. During exercise the tensile stress on the blood-gas barrier can be  $5 \times 10^5 \text{ N/m}^2$  in humans. This is close to the maximum stress of  $1 \times 10^6 \text{ N/m}^2$  that the barrier can resist (West, 2011). There are about 480 million alveoli with capillaries between them in man (Ochs *et al.*, 2004).

The alveolar surface area of gas exchange is larger than the capillary surface area,  $130 \text{ m}^2$  and  $115 \text{ m}^2$  respectively. The capillary blood volume, 200 ml at rest, is distributed over this area (Gehr *et al.*, 1978 and Weibel *et al.*, 1993). A given blood cell passes 10 to 14 alveoli, providing ample opportunity for gas exchange (Staub and Schultz, 1968).

The airway and the pulmonary vascular trees follow each other and branch dichotomously down to the arterioles. At this level, which is at about the 16-17<sup>th</sup> generations of branching (Weibel *et al.*, 2005), the vascular geometry changes from a dichotomously branching tree to a meshwork of capillaries (Guntheroth *et al.*, 1992), **Figure 5**. In terms of flow distribution vessel dichotomous branching is not even since more blood flows in one branch than in the other. There are also extra blood vessels unaccompanied by an airway; the supernumerary vessels (Elliott and Reid, 1965). They branch at an angle of  $90^\circ$  to the axial branch. It is thought that these vessels are closed at rest and provide collateral blood flow during increased cardiac output or if vessels are occluded due to disease.



*Figure 5. Alveolar capillary meshwork. Illustration by Hanna Johansson.*

### **5.1 Perfusion heterogeneity**

Non-uniform pulmonary blood flow ( $Q$ ) was indirectly demonstrated in experiments analysing gas samples drawn from upper and lower lung lobes in dogs (Rahn *et al.*, 1956).

Differences in the vertical distribution of  $Q$  were proposed to explain the observation that pulmonary tuberculosis was most commonly located in the apical parts of the lung (Dock, 1946). The explanation for this was that less blood flow in the apical lung in the upright position results in higher airway oxygen concentrations in the apices, and this was thought to promote mycobacterial growth. West and Dollery (West and Dollery, 1960) demonstrated regional differences in  $Q$  in the lung by using external scintillation counters for measuring the distribution of inhaled radioactive  $C^{15}O_2$  in seated humans. The scintillation counters were placed at nine levels over each lung. Perfusion in the area of interest is proportional to the rate at which  $C^{15}O_2$  is cleared. A pattern emerged from the experiments where  $Q$  increased from the apex to the base of the lung. This distribution pattern disappeared when measurements were made with the subjects in the supine position and the scintillation counters placed on identical landmarks. During moderate exercise while in the upright position, the apical/basal difference in  $Q$  was reduced. Ball *et al.* (Ball *et al.*, 1962) also examined the vertical distribution of  $Q$  using  $^{133}Xe$  injected intravenously and using external scintillation counters to estimate regional blood flow in upright individuals. They demonstrated the same distribution pattern as reported by West and Dollery (West and Dollery, 1960), but their gradient was larger.

### 5.2 The zonal model

The mechanism of distribution of blood flow was likened to the flow created by different Starling resistors. In a Starling resistor, flow is dependent on the relationship between the difference in upstream and downstream pressures and the surrounding pressure, **Figure 6**. When applied to pulmonary physiology the pressure in the arterioles ( $P_a$ ), the pressure in the venules ( $P_v$ ) and the alveolar pressure ( $P_A$ ) are accounted for. Permutt *et al.* (Permutt *et al.*, 1962) proposed that if  $P_A$  remains constant, there would be three regions within the lung in

which  $P_a$  and  $P_v$  vary according to the vertical level in the lung. When  $P_A > P_a > P_v$  there is no flow because the capillary is collapsed. If instead  $P_a > P_A > P_v$ , the driving pressure for flow is now the difference between  $P_a$  and  $P_A$ . Finally, if  $P_a > P_v > P_A$ , the driving pressure for flow is now the difference between  $P_a$  and  $P_v$ . West and colleagues (West *et al.*, 1964) put these conditions together in a model that described the three conditions as three vertically arranged zones (Zone I =  $P_A > P_a > P_v$ , Zone II =  $P_a > P_A > P_v$ , Zone III =  $P_a > P_v > P_A$ ). Later a fourth zone was added by Hughes *et al.* (Hughes *et al.*, 1968) when they observed a decrease in  $Q$  in the most dependent parts of the lung, explained as the effect of gravity on the interstitial pressure.

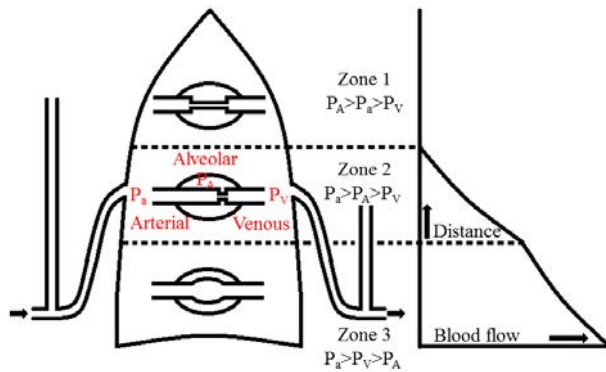


Figure 6. The zonal model with a Starling resistor. Modified after West *et al.* (1964).

### 5.3 Isogravitational heterogeneity

If gravity is the only factor that determines the distribution of  $Q$ , the blood flow to regions at the same vertical level (isogravitational) should be equal regardless of position, and under weightlessness these variations should be abolished. The gravitational model was questioned in the 1970s when Reed and Wood (Reed and Wood, 1970) determined regional blood flow in

different positions in animals using radioactive microspheres (that lodge in the capillaries in proportion to the blood flow). Analysis of small cylinders, 1 cm in diameter and 1 cm in height, cut out from excised dried lung, gave a three-dimensional picture of the distribution of blood flow. These results revealed a heterogeneous distribution of blood flow within isogravitational planes in addition to a vertical gradient. Hogg *et al.* (Hogg *et al.*, 1971) studied the effect of lung expansion and body position on pulmonary perfusion in dogs with albumin macroaggregates labelled with  $^{99m}\text{Tc}$ . They found that there was a wide range of lung expansion and blood flow within a given horizontal slice of the lung in upright position. Regional pulmonary perfusion was also examined by Amis *et al.* (Amis *et al.*, 1984) with a infused  $^{81m}\text{Kr}$  and a gamma camera and revealed horizontal Q gradients.

Based on the deposition of radioactive microspheres Nicolaysen *et al.* (Nicolaysen *et al.*, 1987) found that Q to regions at the same isogravitational level and at the same distance from the hilus could vary considerably. With similar techniques yielding greater spatial resolution Glenny *et al.* (Glenny *et al.*, 1991) were able to quantify the contribution of gravitational heterogeneity to overall perfusion heterogeneity and concluded that gravity was a minor factor in the distribution of Q. Hlastala *et al.* (Hlastala *et al.*, 1996) using the same technique found that differences in flow at the same gravitational level could be 10 times greater than the difference in flow at different levels. Pulmonary perfusion has been studied in both humans and animals under microgravity conditions induced by parabolic flight. Glenny *et al.* (Glenny *et al.*, 2000) studied mechanically ventilated pigs in different positions under different gravitational conditions. They found that there was a small Q gradient increasing from dorsal to ventral parts in the prone position under microgravity, but there was even greater isogravitational heterogeneity. This divergence of findings on the distribution of Q is probably due to different spatial resolutions between the methods that have been used. External scintillation counters measure Q in 6 – 10 regions compared to microsphere

techniques which provide Q data from  $\approx 1\,000$  regions. Measurements of Q in humans using other high-resolution techniques, such as SPECT, PET, CT and MRI, also confirm the presence of isogravitational heterogeneity (Hakim *et al.*, 1987, Musch *et al.*, 2002, Alford *et al.*, 2010, Prisk *et al.*, 2007).

There has gradually evolved a general consensus over recent decades that the spatial distribution of Q is determined by both gravity and the geometry of the vascular tree. The influence of gravity on flow distribution may be more important in humans than in quadruped animals. In a study on baboons, that spend most of their time in the upright position, 7 %, 5 % and 25 % of the variation in perfusion heterogeneity was attributed to gravity in the supine, prone, and erect positions, respectively (Glenny *et al.*, 1999).

## 6. PEEP, position and regional perfusion

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**PAPER II:** *Marked differences between prone and supine sheep in effect of PEEP on perfusion distribution in Zone II lung.*

Early studies using wash-in of radioactive gases indirectly indicated that distribution of  $\dot{Q}$  varied with body position: Rahn *et al.* (Rahn *et al.*, 1956) reported a redistribution of flow when changing from the supine to the upright position in dogs. West and Dollery (West and Dollery, 1960) revealed a vertical  $\dot{Q}$  gradient with perfusion increasing from the apex to the base of the lung in humans. Reed and Wood (Reed and Wood, 1970) explored differences in  $\dot{Q}$  distribution between the prone and supine positions that they had observed in dogs. In more recent work Walther *et al.* (Walther *et al.*, 1999) studied the effect of low PEEP in prone and supine lambs on  $\dot{Q}$  distribution and revealed position-dependent differences. There was, however, a lack of studies exploring the combined effect of high PEEP and prone positioning on regional  $\dot{Q}$ . A prerequisite for such studies was a method that measures differences in  $\dot{Q}$  distribution with accuracy between experimental situations. Ideally such a method must also allow determination of  $\dot{Q}$  in multiple experimental situations in the same subject, where each subject may be his own control. The microsphere technique fulfills these requirements.

## 6.1 The microsphere method for perfusion measurements

Microsphere methods provide information on total and regional perfusion between and within organs in more detail than do flow probes and external scintillation counters. Measurements of Q using radioactive microspheres were originally made in organs other than the lung (Rudolph and Heymann, 1967). The microspheres lodge in the capillary bed in proportion to the blood flow passing through the organ of interest. The organ is excised and radioactivity is measured. Relative blood flow per region is determined from radioactivity per milligram weight or per unit volume. Fluorescent microspheres can also be used; blood flow is then measured using a spectrophotometer, as fluorescence per milligram weight or unit volume (Prinzen and Glenny, 1994). The microsphere technique is deemed the gold standard for Q measurements in the lung (Richard *et al.*, 2002). Repeated injections of microspheres with different labels can be used to measure blood flow in different experimental situations.

When using a method based on intravascular injection of microspheres, it is important that the following six principles are fulfilled: 1) complete mixture in the central circulation; 2) complete extraction during first pass; 3) no leakage from the bloodstream; 4) no disturbance of native flow (reactive vasoconstriction or obstruction of vessels); 5) markers must remain fixed to the microspheres and should not penetrate the endothelium; and 6) enough deposition of microspheres to minimize signal-to-noise ratio allowing accurate measurements (Prinzen and Bassingthwaite, 2000). The method measuring Q in lung using 15  $\mu\text{m}$  radioactive microspheres injected into the right atrium of the heart was validated by Melsom *et al.* (Melsom *et al.*, 1995) with a molecular tracer N, N, N'-trimethyl-N-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine (HIPDM). The correlation coefficient was high 0.99 and when the difference between the methods was analysed there were narrow limits of agreement, 0.09. When the kidneys were examined for detection of incomplete extraction, no radioactivity was detected. Using video-microscopy, Lamm *et al.* (Lamm *et al.*, 2005)

examined 15  $\mu\text{m}$  fluorescent microsphere lodging sites and the effect of lodging on perfusion in the pulmonary microcirculation. They found that microspheres always entered the arterioles as singles. Blood flow continued unabated either around the microspheres or into the alveolar capillaries via adjacent capillary pathways, since the microspheres lodged at the inlets to capillaries from either alveolar corner vessels or small arterioles. They concluded that 15  $\mu\text{m}$  fluorescent microspheres have little impact on local pulmonary capillary blood flow. Young *et al.* (Young *et al.*, 1980) used multiple inert gas elimination technique (MIGET) to identify the level of resolution required for Q measurements when determining V/Q, for more details on MIGET consult section 7.3. Using graded embolisation of beads of different sizes (50  $\mu\text{m}$  – 500  $\mu\text{m}$ ) in dogs, they found that in terms of gas exchange the functional unit that is supplied by blood vessels is between 100 and 150  $\mu\text{m}$  in size. This corresponds to the portion of the lung supplied by a respiratory bronchiole, i.e. the airway located at or near the acinar entrance. An alternative for determining Q distribution is macroaggregates of albumin labeled with  $^{99\text{m}}\text{Tc}$ . The particle size is 10 – 150  $\mu\text{m}$ . Since the microsphere method is destructive it cannot be used in humans. Another disadvantage is that the particulate nature of the spheres probably causes slight overestimation of flow heterogeneity (Prinzen and Bassingthwaight, 2000).

In most experiments where microsphere methods are used to quantitate blood flow in the lungs, the spheres are injected *in situ* over a period of time and the lung is then excised and dried *ex situ* at total lung capacity (TLC). Expansion to TLC can distort measurements since the lung may expand into a shape that differs from that when the microspheres were given. In normal lungs in the prone position this is not a problem since the lung is homogeneously expanded *in situ* (Hoffman, 1985). In the supine position, however, dependent parts of the lung *in situ* will be compressed by the lungs own weight, the heart and the abdomen (Liu *et al.*, 1990), and thus dependent alveoli expand and their volume increases

*ex situ*. With increasing FRC this non-uniform lung expansion was decreased in the supine position (Hoffman, 1985). Thus, when PEEP is applied in the supine position, regional differences will be less pronounced. The microspheres are often given over several ventilation cycles and thereby the effect of lung expansion to TLC when measuring the deposition of microspheres will also disappear.

### 6.2 Redistribution of Q by PEEP and position

In **Paper II** in the prone position, Q was homogeneously distributed from dorsal non-dependent to ventral dependent regions with only a small vertical gradient, **Figure 7**. Applying 10 and 20 cmH<sub>2</sub>O PEEP in this position reduced this already small gradient even more. Even though the zonal model predicts a greater vertical gradient with increasing P<sub>A</sub>, here with PEEP, no such increase was seen in the prone position. In this position most of the lung was considered Zone II, and thus a pulmonary blood flow gradient from non-dependent to dependent regions was expected. The results presented in **Paper II** challenge the classical model, i.e. the vertical gradient was the same at all three levels of PEEP in the prone position. Q heterogeneity was significantly lower in the prone than in the supine position at all levels of PEEP. When Q heterogeneity was partitioned into gravitational and iso-gravitational components, both were smaller in the prone position at all PEEP levels. With this in mind, factors other than the effect of a hydrostatic pressure gradient and increased P<sub>A</sub> must be sought to explain Q distribution in the prone position.

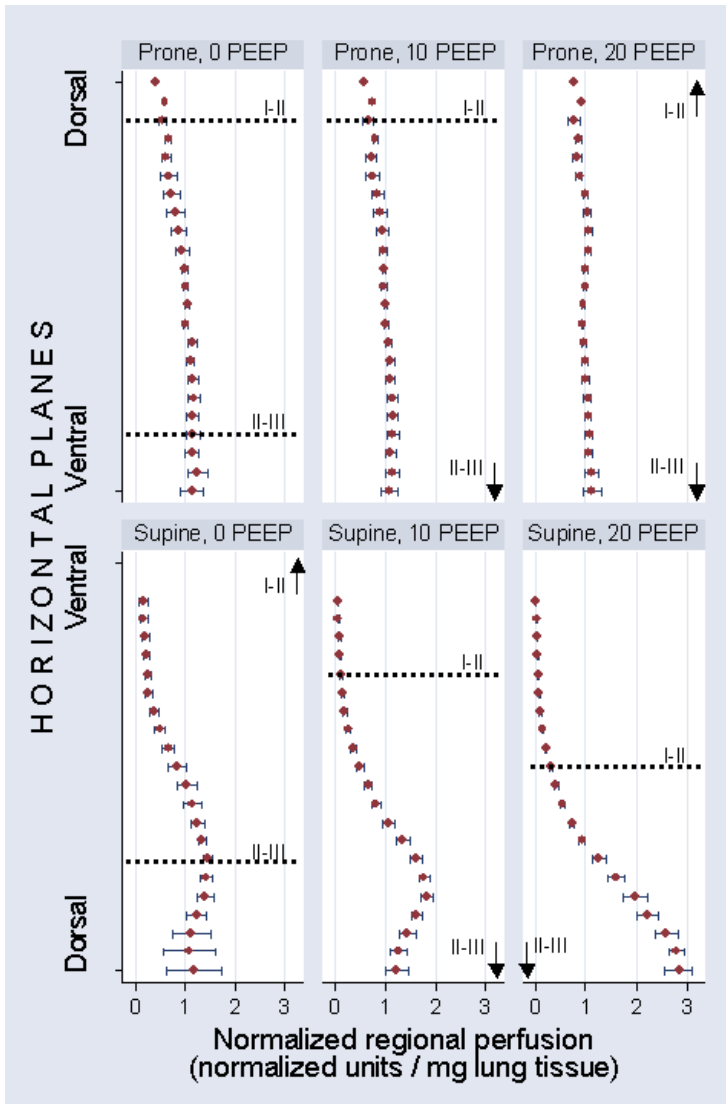


Figure 7. Normalized regional perfusion per unit weight for each horizontal slice in supine and prone sheep (Walther et al., 2005).

The distribution of Q in the supine position was different from in the prone position in sheep. Here Q was distributed with a greater vertical gradient from non-dependent ventral to dependent dorsal regions. When 10 and 20 cmH<sub>2</sub>O PEEP were applied, the vertical gradient

was aggravated resulting in further redistribution of Q dorsally. The linear gradient was a poor descriptor of the distribution of Q, the gradient was unimodal with its modality redistributed further dorsally with PEEP. The redistribution in the supine position was in accordance with the classical zonal model describing a vertical gradient of increasing blood flow from non-dependent to dependent lung. Theoretically, when  $P_A$  increases with 10 and 20 cmH<sub>2</sub>O PEEP in the zonal model, the vertical Q gradient increases since lung perfusion in Zones I and II decreases. This was confirmed in the supine position in **Paper II**. When Q heterogeneity was studied using the coefficient of variation (CV), increased perfusion heterogeneity due to increased vertical gradient with increasing PEEP levels was, as expected, unveiled. However, Q heterogeneity in the horizontal planes, i.e. iso-gravitational heterogeneity, was unchanged with increasing PEEP in this position.

Other research groups have reported comparable results that conflict with classical thinking. Examining a single cross-section of dog lung under constant airway pressure with positron emission tomography (PET), Treppo *et al.* (Treppo *et al.*, 1997) found a larger vertical perfusion gradient and higher perfusion heterogeneity in the supine than in prone position. The spatial resolution of Q in their lung section was approximately the same as in **Paper II**. In a more recent study using magnetic resonance imaging (MRI) in awake, spontaneously breathing humans, Henderson *et al.* (Henderson *et al.*, 2013) also found a significantly greater vertical perfusion gradient in the supine position. They measured Q in one sagittal slice and the relationship between the average values for voxels lying within the same 1 cm high horizontal plane in the supine and prone positions. Due to spatial smoothing and removal of signals from large vessels, the voxel size was approximately 1.8 cm<sup>3</sup>. Petersson *et al.* (Petersson *et al.*, 2010) studied Q in healthy anaesthetized humans mechanically ventilated with and without 10 cmH<sub>2</sub>O PEEP with SPECT. They also found an increasing vertical gradient from non-dependent (ventral) to dependent (dorsal) regions in the

supine position with and without PEEP. PEEP caused a redistribution of Q from non-dependent to dependent regions in their study, in accordance with the zonal model.

In contrast to the findings presented in **Paper II**, Petersson *et al.* (Petersson *et al.*, 2010) detected a unimodal vertical Q gradient in the prone position also with Q increasing from non-dependent (dorsal) to dependent (ventral) lung. They reported, moreover, a redistribution of Q from non-dependent dorsal to dependent ventral regions in the prone position when PEEP was applied. These data are in contrast to the findings in **Paper II** where such redistribution was non-existent. In the study by Petersson *et al.* (Petersson *et al.*, 2010), redistribution of Q in both positions was in line with the zonal model, with increased blood flow in dependent lung regions when  $P_A$  was increased by PEEP. The heterogeneity of Q distribution was not given numerically, but illustrations in the paper suggest a more heterogeneous distribution in both prone and supine positions with 10 cmH<sub>2</sub>O PEEP.

The differences in Q distributions between **Paper II** in sheep and the recent study in humans could be both methodological and/or anatomical. There are differences in chest configuration between sheep and humans, already discussed in section 4.3, where the transversal/sagittal diameter ratio is greater in the human than in the sheep thorax. The sheep were positioned in the sphinx position on a v-shaped semi-soft bedding thereby mimicking the position of the diaphragm in a standing sheep as much as possible. The humans were in a comfortable prone position without any further description as in other studies from the same group (Nyrén *et al.*, 2010). It is likely that Q measurements were influenced by differences in the position and mobility of the diaphragm in the human and sheep experiments. Altemeier *et al.* (Altemeier *et al.*, 2004) showed in pigs that in the supine position Q to dorso-caudal regions near the diaphragm was decreased when turning from the prone to supine position. These regions are influenced by the hydrostatic pressure of the lung and abdominal content through relocation of the diaphragm (Froese and Bryan, 1974) and from the heart.

To summarize, pulmonary blood flow was distributed from non-dependent to dependent regions with a significantly smaller gradient in the prone than in the supine position. When PEEP was applied this gradient was abolished in the prone and aggravated in the supine position in mechanically ventilated sheep. The findings in the prone position were in contrast to the classical zonal model.

## 7. Gas exchange

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The principal function of the lung is gas exchange requiring adequate levels of ventilation and perfusion at the level of the alveoli. The efficiency of the gas exchange process can be divided into two main components: diffusion and matching of ventilation and perfusion ( $V/Q$ ).

### 7.1 Diffusion

Diffusion of a gas is the process where net transfer of molecules takes place from a region where the gas exerts a high partial pressure to another where it exerts a lower partial pressure. In the lung, diffusion is the movement of  $O_2$  from the minor airways to the alveolus, over the alveolar/capillary membrane, through the plasma, and into the red blood cell, as well as the movement of  $CO_2$  in the opposite direction. Diffusion of nitrogen, the third major component of air, does not occur due to complete equilibration across the airways. These processes do not include active biological transport or mass movement of gas in response to a total difference in pressure. In the alveolus the mean partial pressure of  $O_2$  in capillary blood,  $P_{bO_2}$ , is determined by the following: the mixed venous  $PO_2$ ; the amount of pulmonary blood flow; the time each red blood cell spends in the capillary (transit time); the concentration of haemoglobin; the number of erythrocytes in the capillary blood; and the rate of  $O_2$  uptake from air. In the healthy lung diffusion is not a limiting factor for gas exchange.

## 7.2 Ventilation – perfusion matching

Efficient gas exchange requires a close matching of regional V and Q. This is a finely-tuned process since air and blood are spread thinly over a large surface area and must come together in close contact. This means that matching V and Q is dependent on the homogeneity of distribution of both of V and Q, as well as the correlation between them. The process of V/Q matching was theoretical analysed by Wilson and Beck (Wilson and Beck, 1992), and they determined that V/Q heterogeneity, expressed as the variance in the V/Q distribution,  $\sigma_{\log V/Q}^2$ , can be related to V and Q by the equation:

$$\sigma_{\log (V/Q)}^2 = \sigma_{\log V}^2 + \sigma_{\log Q}^2 - 2\rho\sigma_{\log V}\sigma_{\log Q}$$

where  $\sigma_V$  and  $\sigma_Q$  are the standard deviations of ventilation and perfusion distributions, respectively, and  $\rho$  is the correlation between regional ventilation and perfusion in the log domains. This means that regardless of the heterogeneity of V or Q, tight coupling of V and Q leads to minimal V/Q heterogeneity. If matching is not accurate the result will be hypoxemia and wasted ventilation at the macro level. Gas exchange, oxygenation and wasted ventilation can be assessed in the clinical situation by measuring the alveolar-arterial O<sub>2</sub> tension differences ((A-a) O<sub>2</sub>), PaO<sub>2</sub>, SaO<sub>2</sub>, SvO<sub>2</sub> and the difference between end-tidal PCO<sub>2</sub> (EtCO<sub>2</sub>) and PaCO<sub>2</sub>.

## 7.3 Ventilation/perfusion heterogeneity

During the late 1940s Rahn (Rahn, 1949), Riley and Cournard (Riley and Cournard, 1949) assumed the existence of unequal pulmonary blood flow as well as unequal pulmonary ventilation. Before it was possible to separately measure ventilation and perfusion with higher spatial and regional resolution, Martin *et al.* (Martin *et al.*, 1953) demonstrated variations in V/Q across the lung by identifying differences in lobar oxygen concentrations in man. Gas samples were taken from the right upper and lower lobes in the supine, Trendelenburg (head

down on a inclined table) and upright positions. End-tidal  $P_{O_2}$  was higher and  $P_{CO_2}$  was lower in the upper lobe than the lower lobe in both the upright and supine positions. They concluded that  $V/Q$  was not as greatly altered in the supine as it was in the upright position since the differences were higher in the upright position. In the Trendelenburg position there was no difference between upper and lower gas concentrations. Later Kaneko *et al.* (Kaneko *et al.*, 1966) used inhaled and intravenous administration of  $^{133}\text{Xe}$ , and measured emissions with eight to twelve scintillation counters to estimate  $V/Q$  distributions in humans in the supine, prone and lateral decubitus positions. They concluded that the regional differences in  $V/Q$  ratio were less in all subjects and positions than those found in upright man, indicating that  $V$  and  $Q$  are generally better matched in the other positions. This is in accordance with observations that the alveolar-arterial  $O_2$  tension differences ( $A - a$ ) $O_2$  were smaller in the supine than in upright position (Riley *et al.*, 1959). Since the 1960s distribution of perfusion and ventilation has been explained by the effect of gravity on regional perfusion pressure and regional lung compliance (West and Dollery, 1960). In this gravitational model, regional pulmonary blood flow and regional ventilation per unit volume increase from the apex in erect man, or non-dependent lung in other positions, to the base or dependent lung. Since the differences in  $V$  per unit lung volume were less marked than the differences in  $Q$  between the highest and the lowest parts of the lung, there was a progressive fall in  $V/Q$  down the lung (West, 1962). However, with increasing knowledge about the heterogeneous distributions of both  $V$  and  $Q$  this gravitational paradigm has been questioned.

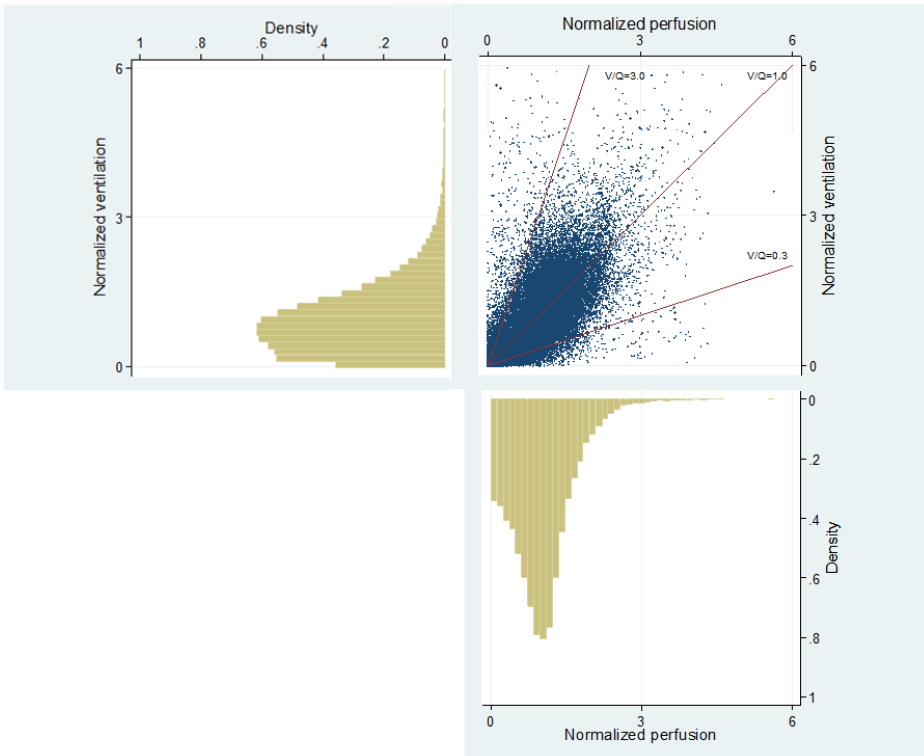
Analysing  $V/Q$  distributions and heterogeneity requires independent methods that measure  $V$  and  $Q$  simultaneously. The MIGET was developed in the 1970s (Wagner *et al.*, 1974). Its principle is based on the observation that the retention or excretion of any gas is dependent on the solubility of that gas and the  $V/Q$  distribution. The method uses six gases with different solubility, ranging from very soluble (acetone) to very insoluble (sulphur

hexafluoride). Saline is equilibrated with these gases and infused at a constant rate. When a steady state is reached, arterial blood and mixed expired gas are collected. The levels of the tracer gases are then measured by gas chromatography. Retention and elimination is then calculated for each tracer in blood passing through the lung and exhaled. Since retention and elimination are related to the gas's solubility coefficient, it is possible by numerical analysis to compute a theoretical distribution of Q and V, respectively, in relation to a spectrum of V/Q ratios of typically 50 compartments. Albeit this technique has provided us with many important insights into lung function, it cannot provide spatial information on the distribution of V/Q heterogeneity. The spatial resolution for measurements of regional V/Q was low, even with as many as twelve measurement positions with external scintillation counters (Kaneko *et al.*, 1966). With the introduction of the microsphere technique for the measurement of Q, however, the resolution of blood flow measurements increased considerably (Reed and Wood, 1970). When this method was combined with inhalation of a wet  $^{99m}\text{Tc}$  aerosol or Technegas, an independent method for the simultaneous measurement of regional V and Q became possible (Melsom *et al.*, 1997) and high resolution data were obtained. Melsom *et al.* (Melsom *et al.*, 1997) who pioneered this technique found, in spontaneously breathing awake goats, that both V and Q were vertically homogeneously distributed resulting in small vertical variations in V/Q within the lungs.

In parallel to the work by Melsom *et al.* (Melsom *et al.*, 1997), Robertson *et al.* (Robertson *et al.*, 1997) developed a method for simultaneous measurement of V and Q by delivering a dry aerosol of 1.0  $\mu\text{m}$  FMS at the same time as an infusion of 15  $\mu\text{m}$  FMS in pigs. They concluded that, the sensitivity of a combination of aerosol deposition and intravascular microsphere-infusion for estimating regional V and Q appears adequate to describe the range of V/Q heterogeneity observed in normal lungs when using MIGET. The most striking observation in their study, apart from the feasibility aspect, was that if a region

was well perfused it was predictable, with high accuracy, that the same region was well ventilated also as a sign of good matching.

Data on  $V$  and  $Q$  distributions obtained with the microsphere-based method for determining  $V/Q$  heterogeneity can be analysed in many ways. One approach when analysing the  $V/Q$  distribution is to plot  $V$  and  $Q$  on the y- and x-axis respectively and include isopleths of  $V/Q$  ratios (an isopleth is a line with the same  $V/Q$ ), **Figure 8**.



*Figure 8. Scattergram of regional ventilation and perfusion.  $V$  and  $Q$  are shown for each region in one animal. The heterogeneity of  $V$  and  $Q$  can be studied by collapsing the data as frequency density distributions on y- and x-axis respectively.*

The heterogeneity of  $V$  and  $Q$  can be estimated by compressing ventilation data to the vertical and horizontal axis, respectively, and then viewing them as a frequency density

distribution. Most regions in normal lung are clustered along the  $V/Q = 1$  isopleth. The regions below the isopleth  $V/Q = 0.3$  will increase  $(A-a)O_2$  and lower  $PaO_2$ . Regions above and to the left of isopleth  $V/Q = 3.0$  will decrease  $CO_2$  elimination and increase the difference between  $EtCO_2$  and  $PaCO_2$ .

#### **7.4 Ventilation/perfusion distribution and body position**

Turning the patient from the supine to the prone position and application of PEEP is sometimes used to improve gas exchange in the clinical situation. Bryan (Bryan, 1974) proposed that turning patients to the prone position, especially when PEEP was applied, was the only feasible way to expand and ventilate dependent dorsal portions of the supine lung, since the position of the diaphragm due to abdominal content otherwise hindered this expansion. In the prone position the correlation between  $V$  and  $Q$  was closer (Mure *et al.*, 2000, Altemeier *et al.*, 2004) and distribution of  $V$  and  $Q$  was more homogeneous (Johansson *et al.*, 2004, Walther *et al.*, 2005) which may result in improved gas exchange compared to the supine position. However, if spatial distributions differ between  $V$  and  $Q$  thereby altering the  $V/Q$  correlation this would worsen gas exchange. Differences in  $V/Q$  heterogeneity and mismatch between  $V$  and  $Q$  caused by change in position of mechanically ventilated animals were analysed by Altemeier *et al.* in pigs (Altemeier *et al.*, 2004). They found that changes in  $V$  and  $Q$  brought about by repositioning were largely confined to juxta-diaphragmatic regions while cranial lung regions remained undisturbed.

When turning from the prone to the supine position,  $Q$  was redistributed from regions close to the diaphragm to dorsal-caudal regions consistent with an effect of hydrostatic pressure. In contrast,  $V$  redistributed away from dorsal-caudal regions, presumably due to reduced regional compliance. These effects of uncorrelated redistributions of  $V$  and  $Q$  in the dorsal-caudal regions may be magnified in clinical respiratory insufficiency.

### 7.5 Ventilation/perfusion distribution and PEEP

Application of PEEP is thought to improve gas exchange by increasing FRC and keeping recruited alveoli distended thereby reducing regions with low V/Q. Optimal PEEP correlates with the highest total respiratory compliance, the highest mixed venous oxygen tension, and the lowest dead space ventilation (Suter *et al.*, 1975). With PEEP exceeding this level, arterial oxygen tension could increase and intrapulmonary shunt could decrease, but at the expense of decreased oxygen transport due to decreased cardiac output. High airway pressures, including PEEP, could also injure the lungs. In the ARDS network study, the investigators concluded that ventilation with a tidal volume of 6 ml/kg of predicted body weight and limited the plateau pressure to 30 cmH<sub>2</sub>O was better than higher tidal volumes (The Acute Respiratory Distress Syndrome Network, 2000). Normal lungs tolerate relatively large tidal volumes delivered at relatively low pressures as long as the stress and strains applied are below an injurious threshold (Futier *et al.*, 2013). There is still an ongoing debate on which level of PEEP is the most optimal for patients with compromised lung function.

## 8. PEEP, position and V/Q distribution

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**PAPER III:** *Minimal redistribution of regional ventilation-perfusion ratios by 10 and 20 cm H<sub>2</sub>O positive end-expiratory pressure in prone sheep.*

The application of PEEP together with mechanical ventilation in humans during intensive care and general anaesthesia for surgical procedures is part of day-to-day hospital care. PEEP is used to increase FRC and prevent recruited lung parenchyma from collapsing at the end of expiration. In patients with severe respiratory failure, repositioning in the prone position and the application of PEEP are both used to improve gas exchange. Prone positioning has been used in intensive care units since the 1970s (Piehl and Brown, 1976 and Douglas *et al.*, 1977) and has recently gained in popularity since new clinical studies have shown improved survival in patients with acute respiratory distress syndrome (ARDS) (Guerin *et al.*, 2013 and Beitler *et al.*, 2014). An additional benefit is that lower levels of PEEP are required in the prone position (Gainnier *et al.*, 2003). The mechanisms behind this improvement are not fully understood. Although gas exchange depends on diffusion and the matching of regional ventilation and perfusion (V/Q), mechanisms and structures involved in the matching of regional V and Q with PEEP and changes in body position have not been fully explored in normal lungs, and certainly not in diseased lungs.

### 8.1. Effects of PEEP and position

Mean V/Q was almost equal in all horizontal planes in the prone position at all PEEP levels in **Paper III**. Matching was good since mean V/Q was close to 1 with low V/Q heterogeneity in

all planes, **Figure 9**. This was in concert with a low  $(A-a)O_2$  in prone when 0 and 10 cmH<sub>2</sub>O PEEP was applied. When 20 cmH<sub>2</sub>O PEEP was applied in prone heterogeneity remained low but  $(A-a)O_2$  increased compared to 0 and 10 cmH<sub>2</sub>O.

In the supine position mean V/Q per horizontal plane was more heterogeneously distributed, with low mean V/Q in the most dependent and high mean V/Q in the most non-dependent horizontal planes with both 0 and 10 cmH<sub>2</sub>O PEEP. When 20 cmH<sub>2</sub>O PEEP was applied in the supine position, regions with low mean V/Q in the most dependent parts were recruited. Regions with high V/Q were seen with 20 cmH<sub>2</sub>O PEEP in non-dependent lung in the supine but was almost absent in the prone position. V/Q heterogeneity was significantly larger in the supine positions at all PEEP levels compared to the prone position, **Table 1**.

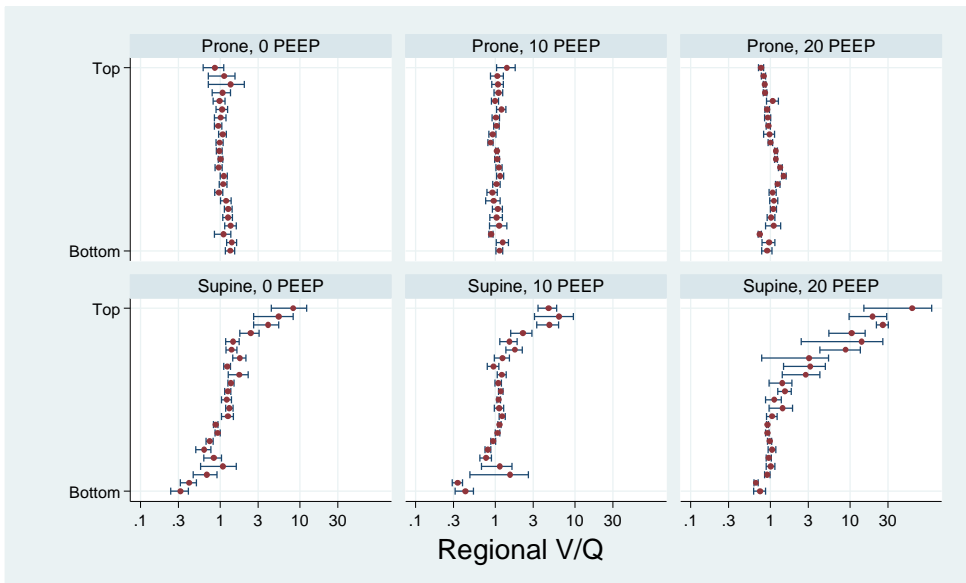


Figure 9. Regional ventilation-perfusion ratios (V/Q) plotted per horizontal plane (note logarithmic x-axis). Circles and horizontal bars denote mean and SEM.

The effect on gas exchange can be explained by analysing the distributions of V and Q and the correlation between them in the way suggested by Wilson and Beck (Wilson and Beck,

1992). A close correlation of V and Q maintains gas exchange when V and Q are heterogeneously distributed. This is illustrated by the heterogeneous distributions of V and Q in the supine position which were offset by a close correlation of V and Q in the supine resulting in maintained gas exchange, **Table 1**. The role of a closer correlation was prominent when comparing the effect of 10 and 20 cmH<sub>2</sub>O PEEP in the supine and prone positions. Correlations between V and Q were lower in the prone position but gas exchange was good since V and Q distributions were homogeneous.

	Supine 0 PEEP	Supine 10 PEEP	Supine 20 PEEP	Prone 0 PEEP	Prone 10 PEEP	Prone 20 PEEP
<b>Vcv</b>	0.83 (0.10)*	0.89 (0.10)*	0.92(0.17)*	0.58 (0.09)	0.56 (0.29)	0.49 (0.09)
<b>Qcv</b>	0.62 (0.10)*	0.69 (0.10)*	0.90 (0.10)*	0.39 (0.11)	0.32 (0.07)	0.31 (0.08)*
<b>Correlation</b>	0.69 (0.26)	0.77 (0.20)*	0.89 (0.03)*	0.66 (0.16)	0.56 (0.13)	0.51 (0.13)
<b>SDlog(V/Q)</b>	0.37 (0.07)*	0.32 (0.07)*	0.39 (0.09)*	0.22 (0.05)	0.21 (0.09)	0.19 (0.04)
<b>(A-a)O<sub>2</sub> mmHg</b>	31.1 (10.3)*	27.9 (12.3)	27.6 (9.0)	13.9 (6.7)	18.0 (14.7)	26.9 (11.0)

*Table 1. The distribution between V and Q and the correlation between them, values are means (± SD). Vcv and Qcv are coefficient of variation for V and Q respectively. (A-a)O<sub>2</sub> are alveolar oxygen tension difference. \* P<0.05 compared to prone same PEEP level*

High V/Q heterogeneity is a hallmark of mismatch, others are an increased (A-a)O<sub>2</sub> difference, increased difference between EtCO<sub>2</sub> and PaCO<sub>2</sub>, and a lower PaO<sub>2</sub>. In **Paper III** there was a significant difference in V/Q heterogeneity between the supine and prone positions expressed as a significantly larger SDlog(V/Q), larger (A-a)O<sub>2</sub> difference, and lower PaO<sub>2</sub> in the supine position when ventilated without PEEP. This is in accordance with measurements of V/Q heterogeneity in dogs using PET (Treppo *et al.*, 1997).

The pattern described by West (West, 1962) where vertical V/Q distribution, with high V/Q in non-dependent and low V/Q in dependent lung regions, was thought to be due to increases in V and Q, though V somewhat less than Q, from non-dependent to dependent lung regions, is similar to the relationship between V/Q and vertical height in supine sheep reported in **Paper III**. These distribution patterns were also comparable to a recent study in man using SPECT (Petersson *et al.*, 2010). The application of 10 cmH<sub>2</sub>O PEEP in the supine position unveiled only small differences in V/Q distribution between humans and sheep.

The effect of 20 cmH<sub>2</sub>O PEEP in **Paper III** was similar to that found by Hedenstierna and co-workers (Hedenstierna *et al.*, 1979a and 1979b) in supine dogs when the effect of applying 20 cmH<sub>2</sub>O PEEP was examined with MIGET and microspheres. However, they found that approximately one-third of alveolar ventilation was located in regions with high V/Q in non-dependent parts, whereas only approximately 5 % of ventilation was in regions with a V/Q > 3.0 in **Paper III**. This difference could be explained by static overexpansion and no ventilation of ventral lung regions shown in more recent studies using non-invasive imaging techniques in lung-injured experimental animals (Gattinoni *et al.*, 1988 and Viera *et al.*, 1998). We found that dependent regions with a V/Q less than 0.3 were recruited when 20 cmH<sub>2</sub>O PEEP was applied in the supine position. This may be explained by the increase in P<sub>A</sub> causing a shift of the border between Zones II and III to more dependent lung regions.

The absence of a vertical V/Q gradient in the prone animals without PEEP reported in **Paper III** was in close agreement with other animal studies (Melsom *et al.*, 1997 and Mure *et al.*, 2000). This pattern, however, was not present in two studies in anaesthetised mechanically ventilated humans where a vertical V/Q gradient with increasing V/Q from non-dependent to dependent lung was found (Nyrén *et al.*, 2010 and Petersson *et al.*, 2010). 10 and 20 cmH<sub>2</sub>O PEEP, applied in the prone position in **Paper III**, preserved the homogeneous distribution of V/Q without creating non-dependent regions with a high V/Q (here V/Q > 3.0).

Application of 10 cmH<sub>2</sub>O PEEP in the prone position unveiled differences in V/Q distribution between mechanically ventilated humans and sheep. Petersson *et al.* (Petersson *et al.*, 2010) showed an increased vertical V/Q gradient when PEEP was applied in the prone position compared to zero PEEP. This was in contrast to the observations in **Paper III**. These differences are probably due to species-related differences, in particular the configuration of the thorax as well as methodological differences.

## 9. Matching of ventilation and perfusion

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**M**atching of regional ventilation and perfusion is crucial for the exchange of  $O_2$  and  $CO_2$  in the lung. The optimal scenario would be if the mechanisms regulating the matching of  $V$  and  $Q$  were exceptionally robust and at the same time highly adaptable. These mechanisms must adjust to the subject's change in body position and alterations in physical activity without convoluted feedback mechanisms, and still keep  $PaO_2$  and  $PaCO_2$  within the normal range.

The mechanisms that regulate the matching of  $V$  and  $Q$  can be divided into passive and active. The shared effects of gravity on  $V$  and  $Q$  distributions, the anatomical geometry where the airways and vascular tree follow each other, and the peripheral homogenising effects on gas flow and capillary perfusion, are denoted passive mechanisms. Active mechanisms that are known are hypoxic pulmonary vasoconstriction (HPV), inhalation of nitric oxide (NO) from the sinuses, regional NO production in the lung, and vasculature and airway reactions to changes in  $P_ACO_2$ .

### 9.1 Passive mechanisms

In normal healthy lungs the passive mechanisms are thought to be most important, but active mechanisms may play a role. In the diseased lung active mechanisms exert a more prominent part. The common effect of gravity on the distributions of  $V$  and  $Q$  that enforces a spatial correlation of  $V$  and  $Q$  is one passive mechanism (West and Dollery, 1960) This was embodied in a model where both  $V$  and  $Q$  increase down the lung, but  $V$  to a lesser degree,

creating an exponential increase in V/Q ratios from dependent to non-dependent lung (West, 1962). However, this model has been questioned following elegant experiments by Glenny *et al.* (Glenny *et al.*, 1991 and Glenny *et al.*, 1999) where they showed that gravity was a minor determinant of pulmonary blood flow in dogs and baboons. In baboons, the estimated gravity-induced Q heterogeneity was 7 % in the supine, 5 % in the prone, and 25 % in the upright position (Glenny *et al.*, 1999).

The most straightforward explanation for efficient gas exchange, that enables humans and other mammals to transport O<sub>2</sub> from inhaled air and deliver it to the blood, and to exhale CO<sub>2</sub> in volumes that make it possible for hard physical work, is that the lungs are anatomically constructed to match V and Q. Airways and pulmonary arteries follow each other through many bifurcations (Weibel *et al.*, 2005). In an earlier study Weibel (Weibel, 1991) concluded that the branching airway pattern is basically the same at all levels, from the large airways to the small peripheral bronchioles. As each airway divide into two branches, the length and diameter of the daughter branches are reduced by a constant factor; a feature called self-similar branching, one of the hallmarks of fractal trees (Mandelbrot, 1983). Blood flow in the lung also exhibit fractal properties (Glenny and Robertson, 1990). Fractal branching has several advantages as shown by West *et al.* (West *et al.*, 1999) and Lefevre (Lefevre, 1983); they create the largest possible surface area for a structure that must be stowed in a given volume, minimise the amount of blood needed to fill a vascular tree, and minimise the work needed to pump blood through the structure. Even if the anatomical structure of the bronchial tree has airways that bifurcate in the same way as the vascular tree, regional ventilation depends on regional compliance that may vary with, for example, body position. This may be explained in the symmorphosis theory developed by Weibel *et al.* (Weibel *et al.*, 1991) where structural design matches functional demands. This structure of branched airways and vasculature trees can explain the isogravitational heterogeneity of V

and Q when examined in horizontal planes. A theoretical study (Ross and Farhi, 1960) as well as an experimental study in dogs (Tsukimoto *et al.*, 1990) suggests that rebreathing improves gas exchange by homogenising the peripheral gas mixture. A red blood cell passes several alveoli during its passage through the pulmonary capillary bed (Staub and Schultz, 1968), and this, together with rebreathing of dead space gas, creates buffering properties for gas exchange in the peripheral lung.

### 9.2 Active mechanisms

One active mechanism involved in local V/Q matching is vasodilatation caused by NO. NO is formed in the normal lung by two of three different isoforms of nitric oxide synthases (NOS); endothelial nitric oxide synthase (eNOS) and neural nitric oxide synthase (nNOS). Local production of NO with subsequent local vasodilatation could increase Q to well ventilated regions and thereby improve local V/Q matching.

Small pulmonary arteries constrict at moderate levels of alveolar hypoxia in contrast to arteries in the systemic circulation that dilate in response to hypoxia. This constricting mechanism is called hypoxic pulmonary vasoconstriction (HPV). HPV is an adaptive mechanism for preserving arterial blood oxygenation through the reduction of V/Q mismatch due to localised alveolar hypoxia that occurs for example in pneumonia. Blood that is shunted through the diseased area will be oxygenated again in other regions, but this will result in regions with lower V/Q ratio since the ventilation in these regions remains unchanged. The onset of HPV is rapid and reverses promptly when local oxygenation improves.

Increasing PACO<sub>2</sub> by either increasing CO<sub>2</sub> in the inspired air or decreasing ventilation causes pulmonary vasoconstriction probably through both hypercapnic acidotic pulmonary vasoconstriction and enhancement of HPV. This improves arterial oxygenation by reducing V/Q heterogeneity (Swenson *et al.*, 1994). Acidosis and hypercapnoea also causes relaxation

of airway smooth muscles, potentially improving V/Q matching (Elguindi *et al.*, 1984). In contrast, hypocapnic hyperventilation leads to bronchoconstriction (Laffey and Kavanagh, 1999). To separate systemic and local airway effects of hypercapnia Brogan *et al.* (Brogan *et al.*, 2004) added CO<sub>2</sub> during the second half of the inspiration phase, thus limiting added CO<sub>2</sub> to the conducting airways. In this way they were able to improve arterial oxygenation, reduce (A-a) O<sub>2</sub> difference, and cause a decrease in V/Q heterogeneity without the development of systemic acidosis. They reported that the effect of inspired CO<sub>2</sub> was largely due to conversion of units with high V/Q to normal mid-range values.

## 10. Endogenous NO and V/Q matching

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**PAPER IV:** *Inhibition of constitutive nitric oxide synthase does not influence ventilation-perfusion matching in prone adult sheep with mechanical ventilation.*

**N**itric oxide (NO) is produced in many tissues in the mammalian body. In the lung NO is formed in both the airway and in the vasculature. Beck and Rehder (Beck and Rehder, 1986) showed that vascular conductance was higher in dorsal lung in dogs in the supine, lateral and head-up positions, but the prone position was not examined. Evidence for uneven formation of NO in the lung was provided by *in vitro* studies showing greater eNOS activity in dorsal than in ventral lung regions in animals and humans, and similar regional differences in NO-mediated vasorelaxation in equine and porcine pulmonary arteries (Pelletier *et al.*, 1998, Rimeika 2004 *et al.*, Rimeika *et al.*, 2006). Rimeika *et al.* (Rimeika *et al.*, 2004) tried to confirm this by examining humans with SPECT in the prone and supine positions after inhibition of NO production. They found a small redistribution of Q to non-dependent ventral lung in the supine position after inhibition of nitric oxide synthase (NOS), but in the prone position there was no redistribution.

### 10.1 Synthesis of nitric oxide

Furchgott and Zawadzki (Furchgott and Zawadzki, 1980) demonstrated that endothelium-derived relaxing factor (EDRF), later recognised as NO, is a messenger in vasodilatation. NO is produced in mammalian cells by the oxygen-dependent, five-electron oxidation of the terminal guanidine nitrogen in L-arginine. Apart from NO, the reaction yields L-citrulline as a

by-product. A single enzyme nitric oxide synthase (NOS) catalyses all steps in this reaction. NOS exists in three isoforms. In the common nomenclature these are referred to as nNOS (also known as NOS-1) found in neural tissue, iNOS (also known as NOS-2) being the isoform which is inducible in a wide range of cells and tissue, and eNOS (also known as NOS-3) being the isoform that is found in endothelial cells. nNOS and eNOS are constitutive NOS (cNOS) in contrast to iNOS which is inducible. All three isoforms, however, can be induced by various stimuli. They can also be differentiated on the basis of their calcium dependence (eNOS and nNOS) or independence (iNOS) (Alderton *et al.*, 2001).

These three isoforms are the products of different genes, with different localisation, regulation, catalytic properties and inhibition sensitivities. When NO is produced it activates soluble guanylate cyclase thereby stimulating intracellular cyclic guanosine monophosphate (cGMP) accumulation which leads to relaxation of vascular smooth muscle.

### 10.2 Nitric oxide formation in the lung

NO is uniformly and continuously formed by eNOS in the endothelium of the pulmonary vasculature due to the stimulus of tangential shear stresses exerted by viscous drag on the blood vessels (Hampl and Herget, 2000). Circumferential stress on pulmonary vessels induced by mechanical ventilation and the application of PEEP also induce NO production (Persson *et al.*, 1990, Strömberg *et al.*, 1997, Kuebler *et al.*, 2003).

Endogenous NO generation is inhibited by N $\omega$ -nitro-L-arginine methyl ester (L-NAME), a competitive L-arginine analogue (Rees *et al.*, 1990). L-NAME is a pro-drug lacking NOS inhibitory activity unless it is hydrolysed to N $\omega$ -nitro-L-arginine. L-NAME is a non-selective inhibitor inhibiting all three isoforms with almost the same efficiency (somewhat higher doses are required for iNOS). Melsom *et al.* (Melsom *et al.*, 2000) used L-NAME 25 mg/kg for the inhibition of NO-production in awake spontaneous breathing sheep.

To verify that NO production was abolished they infused L-NAME in incremental doses of 5 mg/kg. After 10 mg/kg no NO could be detected in the expired air.

### 10.3 Minimal change in V/Q distributions by L-NAME

Ventilation/perfusion matching was almost perfect in prone sheep being mechanically ventilated with 10 cmH<sub>2</sub>O PEEP (for details see **Paper III**). Regional NO production initiating vasodilatation, thereby improving V/Q matching in these regions, is an attractive hypothesis to explain near perfect gas exchange under these conditions.

Inhibition of NO formation should lead to a worsening of gas exchange if NO is active in local V/Q matching. Furthermore, if NO was formed preferentially in dorsal lung as suggested by prior research (Pelletier *et al.*, 1998 and Rimeika *et al.*, 2004), redistribution of Q to ventral lung should result with blockage of cNOS. However, we found a small but significant redistribution of Q from ventral to dorsal regions, **Figure 10**, contrary to what would be expected (Pelletier *et al.*, 1998, Rimeika *et al.*, 2004, Rimeika *et al.*, 2006). There was no difference in total V/Q heterogeneity as a result of this small redistribution, which was also supported by a preserved PaO<sub>2</sub> and (A-a)O<sub>2</sub> difference. Gravitational Q heterogeneity significantly decreased following dorsal redistribution. These findings were in line with those of Melsom *et al.* (Melsom *et al.*, 2000) where V/Q matching remained unchanged after inhibition of NO production in awake sheep.

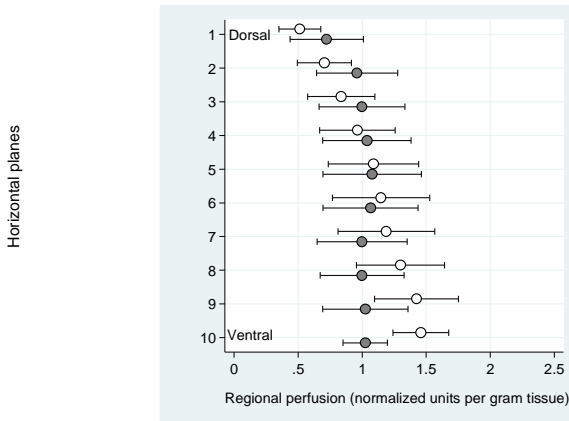


Figure 10. Mean (SD) perfusion per plane at baseline (open circles) and with cNOS blockage (closed circles). Note that normalized perfusion in dorsal planes was greater with cNOS blockage.  $N=7$  per experimental permutation.

Rimeika *et al.* (Rimeika *et al.*, 2004) inhibited NO production with  $N^G$ -monomethyl-L-arginine (L-NMMA) a non-selective NOS inhibitor in spontaneously breathing humans. They found ventral redistribution of pulmonary blood flow in the supine position, but no redistribution in the prone position. Exhaled NO was significantly decreased but not abolished in their subjects. No measurements of mean pulmonary artery pressure (MPAP) were reported.

MPAP increased as expected after NOS inhibition in sheep (**Paper IV**). Independent of topographical localisation, increasing MPAP will increase Q to regions where  $P_A > P_a$ . Since these were most likely located in dorsal lung in the prone position, this agrees with the redistribution of Q which was mainly dorsal. Neumann *et al.* (Neumann *et al.*, 1984) previously observed similar redistribution of Q in a study on mechanically ventilated sheep in the prone position where MPAP was increased either by inflation of a balloon placed in the left atrium of the heart or by stimulating hypoxic vasoconstriction by ventilating the sheep with a low fraction of oxygen.

Theoretically, NO formation due to high local ventilation could improve V/Q matching by increasing local blood flow. Indeed, circumferential stress or local tension in the lung increases NO production (Persson *et al.*, 1990, Strömberg *et al.*, 1997, Kuebler *et al.*, 2003). We examined this idea by measuring perfusion to regions with high ventilation. These regions were spatially located throughout the lung, but preferentially from the centre to the dependent parts of the lung. There was no redistribution of Q away from these regions with cNOS inhibition.

We conclude that the almost ideal matching of V and Q in the prone position in the present experiment was not due to fine tuning of Q by local NO formation.

## 11. Conclusions

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The conclusions based on the data presented in this thesis and associated four papers are the following: When determining regional V, the deposition of a wet aerosol of fluorescent microspheres ( $\approx 1 \mu\text{m}$ ) functions well, as mirrored by the deposition of Technegas.

Redistribution of V following the application of PEEP is different in the supine and prone positions. In the prone position, a linear increase in V from dorsal to ventral regions is eliminated by 10 cmH<sub>2</sub>O PEEP. In the supine position there is a unimodal dorsal distribution of V, which is further augmented by PEEP.

The distribution of Q differs from the classic zonal model. In the prone position most of the lung is in Zone II, both with and without PEEP, furthermore without any vertical gradient. In the supine position Q is redistributed dorsally with PEEP as predicted by the zonal model. A linear vertical gradient is a poor predictor of Q in the supine position because the gradient is unimodal. Q is more heterogeneously distributed in the supine than in the prone position. The difference in heterogeneity between positions is augmented by the application of both 10 and 20 cmH<sub>2</sub>O PEEP.

V/Q mismatch is higher in the supine compared to the prone position at the same level of PEEP. This is also seen as a greater PaO<sub>2</sub> and lower (A-a)O<sub>2</sub> difference in the prone compared to the supine position without PEEP. When 20 cmH<sub>2</sub>O PEEP is applied in the prone position heterogeneity is significantly lower than in the supine at same level of PEEP, but there are no differences in (A-a)O<sub>2</sub> difference and PaO<sub>2</sub>.

Regional vasodilation by nitric oxide, from local formation, was not the cause of the homogeneous distribution of V/Q in adult sheep in the prone position, mechanically ventilated with 10 cmH<sub>2</sub>O PEEP.

# Populärvetenskaplig sammanfattning

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Lungans huvudsakliga uppgift är att ta upp syrgas ( $O_2$ ) och leverera den via blodet till kroppens olika celler och samtidigt transportera koldioxid ( $CO_2$ ) i motsatt riktning. Detta utbyte av gas sker genom att luft med  $O_2$  strömmar ner genom de stora luftvägarna, till de små luftvägarna i den perifera lungan. Denna luftström uppstår när vi andas genom att diafragma spänns och bröstkorgen höjs och därmed bildas ett undertryck som drar in luften. När vi får andningshjälp av en respirator, blåses lungan upp av ett övertryck och därmed strömmar luften ut i lungan. När luften är nere i de perifera luftvägarna sprider sig luften (diffunderar) genom en koncentrationsskillnad mellan de små luftvägarna och lungblåsorna (alveolerna). När luften når alveolerna kommer  $O_2$  diffundera från alveolerna till blodet i kapillärerna eftersom koncentrationen av  $O_2$  är lägre i kapillärerna än i alveolerna.  $CO_2$  transporteras från blodet på samma sätt fast med de omvända mekanismerna, först diffusion sedan med strömning. För att detta utbyte av gas skall ske på ett bra sätt måste transporten av gas (ventilation) till alveolerna möta flödet av blod (perfusion) i kapillärerna. De måste vara matchade till varandra. Detta sker genom att både ventilation och perfusion fördelas lika till olika delar i lungan. Denna fördelning kan ske genom olika mekanismer, som kan delas upp i passiva och aktiva. Lungans påverkan av tyngdkraften (gravitationen) är en sådan passiv mekanism. Lungans egen tyngd (vävnad och blod) gör att lungan kommer att tryckas ihop i de nedre delarna efter en utandning. När sedan en inandning sker kommer dessa delar expandera mer än lungans övriga delar och mer blod och luft kommer till dessa delar. En annan passiv mekanism är lungans anatomi. Luftvägarna och blodkärlen följer varandra inte bara

geografiskt utan även i storlek, dvs. en stor luftväg har ett stort blodkärl precis intill. De aktiva mekanismerna är till för att fördela om blodet då något händer i lungan. Ett exempel är lunginflammation (pneumoni). Vid denna sjukdom kommer bl.a. vita blodkroppar och slem ansamlas i alveolerna som då töms på luft. Blodet kommer då att passera denna del av lungan utan att syresättas och blodet som kommer ut i kroppen bär på mindre O<sub>2</sub> än normalt. Människan och andra däggdjur har utvecklat en mekanism som gör att blodkärlen till dessa sjuka områden i lungan drar ihop sig och blodet flyter till andra bättre ventilerade delar.

I lungan produceras kväveoxid (NO). Detta är en molekyl som gör att blodkärl vidgar sig. Teoretiskt skulle ett område som ökar sin ventilation kunna öka sitt blodflöde dit genom lokal produktion av NO. Detta är också en aktiv mekanism för att matcha ventilation och perfusion. Det råder olika uppfattning om hur ventilation och perfusion fördelas i lungan. Likaledes finns olika uppfattning om hur matchningen mellan dessa sker. Det är heller inte känt om det finns några aktiva mekanismer för att fördela blod och ventilation i den friska lungan.

Syftet med studierna var att försöka bilda klarhet i ovan beskrivna osäkerheter för att kunna förbättra vården av svårt sjuka patienter som vårdas med hjälp av en respirator. I dagens intensivvård används övertrycksandning i respirator på olika sätt. Ett sätt är att åstadkomma ett högre tryck i lungan efter ett andetag och på detta sätt hålla lungan bättre uppblåst. Detta tryck kallas för positivt slut-andnings tryck, på engelska; positive end-expiratory pressure (PEEP). I slutet av 1900-talet upptäckte man att patienter i respirator kan bli bättre syresatta om de ligger på magen. Dessa två åtgärder studerades också i denna avhandling.

I **delarbete I** studerades fördelningen ventilationen hos friska djupt sövda får som vårdas i respirator antingen på rygg eller på mage. Dessutom användes ett positivt utandningstryck på

10 cm vattenpelare (10 cmH<sub>2</sub>O PEEP). För att kunna mäta ventilationen användes små plastkulor (mikrosfärer) märkta med fluorescerande färg. Dessa kulor blandades i saltvatten. Av denna lösning gjordes en dimma som fåren fick andas in. De små kulorna fastnar då alveolerna i proportion till hur mycket ventilation som går dit. Denna metod kontrollerades och jämfördes med ännu mindre radioaktiva kolpartiklar (Technegas®) som en del får fick andas in. Technegas® används på människa för att undersöka lungorna.

Resultatet visade att metoden med en dimma av mikrosfärer var nästan lika bra som Technegas® för att mäta ventilationen i lungan. Fördelningen av ventilationen var mycket jämnare i magläge än i ryggläge. 10 cmH<sub>2</sub>O PEEP gav upphov till olika fördelning beroende på vilket kroppsläge fåren låg i. I magläge blev fördelningen ännu jämnare och ventilation omfördelades mer till områden nära ryggen. I ryggläge omfördelades ventilationen ännu mer mot ryggen.

I **delarbete II** studerades blodfördelningen under samma förutsättningar som i delarbete I. Här lades en ytterligare trycknivå till, 20 cmH<sub>2</sub>O PEEP. Här fanns det en väletablerad metod där blodflödet kunde mätas med lite större radioaktivt märkta mikrosfärer som sprutades in i blodet. Mikrosfärerna fastnade i lungans kapillär i proportion till blodflödet till den regionen. Blodflödet var vertikalt jämnare fördelat i magläge än i ryggläge. Det fanns dock en ojämnhet i fördelningen i det horisontella planet i dessa båda kroppslägen. Denna ojämnhet var relativt större än i det vertikala planet. Dessa fynd överensstämde med tidigare fynd. Det nya här var att PEEP gav upphov till oväntade omfördelningar beroende på kroppsläge. Även här ökade PEEP jämnheten i fördelningen i magläge, men ökade ojämnheten i ryggläge. I ryggläge fördelades mycket blod till ryggnära regioner.

I **delarbete III** studerades hur väl ventilationen och perfusionen matchade varandra och hur fördelningen av detta var i lungan. Här kombinerades metoderna ifrån delarbete I och II. Även här visades en skillnad mellan mag- och ryggläge och mellan de olika PEEP nivåerna. I magläge var matchningen mellan ventilation och perfusion bra och blev något ännu bättre med PEEP. Detta berodde på att ventilationen och perfusionen var jämnt fördelade i sig. I ryggläge var matchningen sämre än i magläge under alla nivåer av PEEP. Vi såg ingen överventilation i de översta delarna av lungan med PEEP i ryggläge till skillnad mot andra studier.

I **delarbete IV** studerades om NO, som ger upphov till en aktiv omfördelningsmekanism, har någon betydelse för fördelningen av ventilation och perfusion i magläge hos friska får som ventileras med PEEP. Här studerades detta hos får i magläge ventilerade med 10 cmH<sub>2</sub>O PEEP eftersom matchningen mellan ventilation och perfusion var bra. Samma metod som i delarbete III användes. Produktionen av NO blockerades med en annan molekyl. När NO blockerats sågs obetydliga förändringar av matchningen mellan ventilation och perfusion. NO spelar därmed ingen roll för fördelningen i frisk normal lunga.

Sammanfattning av studierna:

Metoden att mäta ventilation med en dimma av märkta mikrosfärer fungerar väl. I magläge är fördelningen av ventilation och blodflöde jämnare och matchningen bättre än i ryggläge hos friska djupt sövda får, ventilerade med respirator. I magläge jämnade PEEP ut fördelningarna men i ryggläge skedde en omfördelning till regioner som var ryggnära. Detta kan vara en del av förklaringen till att svårt sjuka patienter som behandlas i respirator får bättre syresättning i magläge. NO är ingen aktiv mekanism som omfördelar blodet i friska lungor.

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