Regional Pulmonary Vasoreactivity

with special reference to nitric oxide, prostacyclin and body posture

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For the joy of work and knowledge

To Malin and Felix

Abstract (eng)

The primary aim of the study was to investigate possible mechanisms behind the improved arterial oxygenation in ventilator-treated patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) when turned into prone position. A secondary aim was to discover new knowledge of possible importance for treatment of patients with oxygenation problems in intensive care and during anesthesia.

Previous work have described a dominant blood flow in dorsal lung regions regardless of posture and a better matching of ventilation (V) and perfusion (Q) in prone vs supine posture. Perfusion and also V/Q ratios are more homogeneous in prone than in supine position. The hypothesis of the study was that differences in regional pulmonary vasoreactivity to the endogenous vasodilators nitric oxide (NO) and prostacyclin (PGI_2) may explain the improved gas exchange in severe lung disease during ventilator treatment in prone position.

Regional pulmonary vasoreactivity to NO and PGI_2, as well as effects of posture on distribution of regional pulmonary blood flow, were investigated in isolated human and porcine lung tissue and pulmonary arteries in vitro and in volunteers and patients in vivo.

Presently it was shown that expression of mRNA for endothelial NO synthase (eNOS) was higher in dorsal compared to ventral human lung regions. Ca^{2+}-dependent NOS activity was higher in dorsal than in ventral regions of both human and porcine lungs. Relaxation of porcine pulmonary arteries in vitro by acetylcholine and bradykinin, endothelial-dependent vasodilators, acting via the NO / cGMP pathway was more potent in vessels from dorsal than from ventral lung regions. Furthermore, NOS inhibition by infusion of N^G-monomethyl-L-arginine (L-NMMA) redistributed blood flow from dorsal to ventral lung regions in healthy volunteers in supine position. Altogether these results strongly suggest a role for endogenous NO in regulation of regional pulmonary perfusion.

Inhalation of iloprost, a synthetic PGI_2 analogue, decreased arterial oxygen tension in healthy volunteers. Pulmonary perfusion was redistributed towards dependent lung regions in both supine and prone, whereas ventilation was redirected towards non-dependent lung regions in supine position, indicating increased mismatch of ventilation and perfusion. Prostaglandin synthesis inhibition by the non-selective cyclooxygenase (COX) inhibitor diclofenac did not affect pulmonary perfusion distribution or oxygenation in healthy subjects.

Patients subjected to left side thoracic surgery in lateral decubitus position with one-lung ventilation (OLV) were treated with non-selective COX-inhibition by diclofenac infusion. They had significantly lower shunt and better oxygenation as measured by alveolo-arterial oxygen difference than patients receiving placebo. The results suggest that a COX-related arachidonic acid metabolite attenuates pulmonary hypoxic vasoconstriction.

In conclusion the results strongly suggest a role for endogenous NO in regulation of regional pulmonary perfusion. PGI_2 is likely not involved in regulation of regional pulmonary perfusion during normoxia whereas it may be of importance during hypoxia. The present results may become of importance for patients with oxygenation problems in intensive care as well as during one-lung ventilation for thoracic surgery.

Keywords: Regional pulmonary perfusion, nitric oxide, prostacyclin, NOS-inhibition, COX-inhibition, shunt, one-lung ventilation.

Abstract (swe)

Huvudsyftet med aktuell avhandling var att undersöka vilka mekanismer i lungorna som ligger bakom den förbättrade syresättningen av blodet hos svårt lungsjuka respiratorbehandlade patienter när de vänds i bukläge. Ett andra syfte var att söka efter ny kunskap som skulle kunna vara av betydelse för patienter med syrebrist inom intensivvård och under operationer.

Tidigare forskning har visat att en dominerande del av lungblodflödet går till ryggnära lungdelar oavsett kroppsposition, dvs. både i rygg- och bukläge. Anpassningen mellan luft- och blodflöde har också visats vara mer optimal i bukläge. D.v.s. blodet går i större utsträckning till lungdelar där det finns gott om syre. Hypotesen i studien var att de kroppsegna blodkärlsvidgande ämnena kväve monoxid (NO) och prostacyklin (PGI_2) kunde förklara den förbättrade syresättningen av blodet i lungorna vid svår lungsjukdom under respiratorbehandling i bukläge.

Avhandlingen beskriver studier av reglering av regionalt lungblodflöde av de två ämnena NO och prostacyklin PGI_2, som båda bildas i de celler som täcker insidan av kroppens blodkärl (endotelceller). Effekter på blodflödets fördelning i lungorna beroende på kroppsläge (rygg-, buk- och sidoläge) undersöktes också.

Det visades att genetisk information (mRNA) för proteinet som bildar NO i endotelceller, endotelialt NO syntas (eNOS) fanns i större koncentration i ryggnära lungregioner. Calcium-beroende NO syntas som finns i kärlendotel och nervceller hade högre aktivitet i ryggnära lungdelar än bröstnära hos både människa och gris. Vid hämning av NO-bildning via blockering av NO-syntas omfördelades lungblodflödet i ryggläge hos friska försökspersoner från ryggnära till bröstnära lungregioner. Undersökning av isolerade kärl i så kallade organbad visade även större kärlvidgning i de ryggnära kärlen jämfört med de bröstkorgsnära när de stimulerades med ämnen som verkar via NO. Detta talar för att NO bildat i lungorna har betydelse för regleringen av regionalt lungblodflöde.

Inandning av syntetiskt prostacyklin sänkte syrehalten i blodet på friska försökspersoner. (Hos lungsjuka brukar vanligen blodets syrehalt stiga.) Lungblodflödet omfördelades mot lågt liggande lungregioner både i rygg- och bukläge, d.v.s. effekten av gravitationen förstärktes. Luftflödet omfördelades i ryggläge mot högt liggande lungdelar, dvs. i motsatt riktning mot blodflödet, vilket skulle kunna leda till försämrad matchning av blod och luftflöde och därmed bidra till den sänkta syrehalten i blodet hos lungfriska. Hämning av bildning av kroppsegna prostaglandiner (tex. prostacyklin) med en cyclooxygenashämmare (diklofenak) påverkade inte blodflödesfördelning eller syresättning hos friska.

Patienter som opererades i vänster lunga med ventilation av endast den icke-opererade lungan (enlungs ventilation) fick förbättrad lungblodflödesfördelning vid behandling med diklofenak jämfört med patienter som fick icke verksam behandling (koksalt). Vid avstängning av en lunga uppstår syrebrist i denna och blodet styrs då över till den luftfyllda lungan för att blodets syresättning ska bli så effektiv som möjligt (syrebrist-utlöst kärlsammandragning). Våra resultat tyder på att denna kroppsegna skyddsreflex förstärktes pga hämning av en prostaglandinlik substans (t.ex. prostacyklin) som motverkar den syrebristutlösta kärlsammandragningen.

Sammanfattningsvis tyder resultaten på att kroppseget NO har betydelse för reglering av regionalt lungblodflöde. Kroppseget prostacyklin är sannolikt inte inblandat i reglering av regionalt lungblodflöde hos friska med normal syrehalt i lungorna medan det kan ha betydelse vid syrebrist.
Resultaten kan ge uppslag till nya behandlingsalternativ för patienter med syrebrist inom intensivvård samt vid operationer med enlungs ventilation.

Content

List of Papers

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

I. Rimeika D, Nyrén S, Wiklund NP, Renström Koskela L, Törring A, Gustafsson LE, Larssson SA, Jacobsson H, Lindahl SGE, and Wiklund CU.
Regulation of regional lung perfusion by nitric oxide.
*American Journal of Respiratory and Critical Care Medicine 2004; **170**: 450-455*

II. Rimeika D, Wiklund NP, Lindahl SGE, and Wiklund CU.
Regional differences in nitric oxide-mediated vasorelaxation in porcine pulmonary arteries.
*Acta Anaesthesiologica Scandinavica 2006; **50**: 947-953*

III. Rimeika D, Sanchez-Crespo A, Nyren S, Lindahl SGE and Wiklund CU.
Iloprost inhalation redistributes pulmonary perfusion and decreases arterial oxygenation in healthy volunteers.
*Acta Anaesthesiologica Scandinavica 2009; **53**: 1158-1166*

IV. Rimeika D, Lindahl SGE and Wiklund CU. (2009)
Non-selective cyclooxygenase (COX) inhibition decreases shunt during one lung ventilation for thoracic surgery.
Submitted for publication.

Abbreviation index

ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
CI	cardiac index (=cardiac output/body surface)
CMV	controlled mechanical ventilation
EELV	end-expiratory lung volume (=FRC)
FIO_2	inspired fraction of oxygen
FRC	functional residual capacity
HPV	hypoxic pulmonary vasoconstriction
L-NMMA	N^G-monomethyl-L-arginine
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	non steroidal anti-inflammatory drugs
PASMC	pulmonary arterial smooth muscle cells
P (A-a) O_2	alveolo-arterial oxygen tension difference
PaO_2	arterial oxygen tension
$PaCO_2$	arterial carbon dioxide tension
PaO_2 / FIO_2	arterial oxygen tension/inspired fraction of oxygen
PBF	pulmonary blood flow
PEEP	positive end-expiratory pressure
PGI_2	prostacyclin
PVR	pulmonary vascular resistance
Q	lung perfusion
SPECT	single photon emission computed tomography
SvO_2	mixed venous oxygen saturation
TLC	total lung capacity
V	ventilation
V/Q	ventilation perfusion ratio

Pressure units conversion formula: 101.3 kPa = 760 mm Hg (Torr) = 1 atm.

INTRODUCTION

During the last half-century a striking development has occurred within the medical and technical fields of Intensive Care Medicine. Despite increased possibilities to provide more advanced and gentle mechanical ventilatory support [146], mortality is still high, between 30 and 50%, for patients subjected to ALI or ARDS [19, 76, 86, 91, 121]. The substantial number of patients dying from hypoxia in spite of adequate cardiac function strongly marks the need of increased knowledge in pulmonary pathophysiology. Continued research about mechanisms governing gas exchange is required.
Extracorporeal oxygenation offers an ultimate therapeutic strategy. It is an advanced rescue therapy which is, due to high costs limited to a small number of patients, especially in times of economical strain. However, early initiated position treatment is cost effective and feasible even in smaller Intensive Care Units, and has been shown to improve arterial oxygenation [5, 24, 39, 100], in 65-75 % of the patients turned into prone position [28, 50, 57, 73, 87, 112, 159]. In spite of the improvement in oxygenation no randomized clinical trials have shown improved survival by ventilator treatment in the prone position. Post hoc analysis of subgroups suggest that mortality may be reduced in patients with severe ARDS and there is also a tendency towards lower mortality when prone position treatment is initiated early [50, 87, 137, 160]. Nevertheless, the question still remains concerning the causative mechanisms behind the improved oxygenation. During the last decades there has been a consistency in literature that prone position provides a more homogenous ventilation and perfusion ratio [7, 99, 129]. But why are perfusion and ventilation distributions better matched in the prone position? Suggested mechanisms are increased FRC [113], decreased vertical pleural pressure gradient [102, 147] a more evenly distributed lung tissue due to less compression by the mediastinal and abdominal structures [4, 118, 124], more homogenous regional ventilation/perfusion ratios due to basic vascular anatomical structure [7, 52, 55] and higher vascular conductance in dorsocaudal lung regions [14], recruitment of dorsal atelectatic lung units [81], regional changes in ventilation due to alterations in chest wall mechanics [23, 112] or increased secretion drainage in the prone position. Enhanced understanding, how to optimize the complex ventilation / perfusion matching in the lung, might extend the treatment strategies for the clinicians challenged by severe hypoxic patients.
This thesis will chiefly discuss mechanisms regulating blood flow distribution in the lung, with special reference to vasoreactivity and posture. Distribution of ventilation and V/Q-ratios will not be discussed in detail.

General pulmonary perfusion

The lung possesses a low resistance circulation that must, at all times, accommodate the entire cardiac output. Moreover, to ensure adequate oxygenation of haemoglobin, blood flow must be directed to well-ventilated lung units, i.e., ventilation and perfusion must be matched [48]. One remarkable characteristic of the low pressure pulmonary circuit is its capacity to decrease pulmonary vascular resistance (PVR) as pressure within the system rises. Two mechanisms are responsible for this: recruitment and distension of vessels. Another unique feature of the pulmonary circulation is that vessels are exposed to different distending forces as lung volumes expand and diminish above and below functional residual capacity. As a result, the diameter and length of the vessels are passively influenced by changes in lung volume, at the same time as there are numerous active

neurogenic, chemical and humoral factors governing the vascular diameter [48].Total lung blood flow is also influenced by the cardiac function and changes in pulmonary blood volume.
All interrelated mechanical and chemical factors governing pulmonary blood flow contribute to the complexity of pulmonary physiology and challenge the clinician to make the right decisions to optimize ventilation / perfusion matching. When comparing investigations concerning distribution of pulmonary blood flow, there are many factors in a study design that may influence the results. For instance, spontaneous breathing versus positive pressure ventilation; awake subjects versus anesthetized with muscular relaxation; in vitro versus in vivo conditions will all have great impact on the pulmonary perfusion (and ventilation) distribution [17, 48, 64, 145, 148].

Regional pulmonary perfusion.

The distribution of pulmonary blood flow is chiefly governed by the distensibility of vessels and their transmural distending pressure [48]. It is now apparent that although gravity does have a measurable influence on pulmonary blood flow (PBF) distribution, the anatomic structure of the arterial tree plays a prominent role in the distribution of PBF [49, 52, 55, 66], which will be discussed below. Since the 1960´s, when West introduced the zonal model based on the relationship between pulmonary arterial, alveolar and venous pressures, gravity has been considered the predominant factor for regional pulmonary perfusion and ventilation distribution. Studies using radioactive gases and external scintillation counters on isolated, perfused, animal lungs showed a greater regional blood flow to the dependent (bottom) regions [125, 162]. Later investigations on humans confirmed these findings, and gravity was considered as the most important determinant of lung blood flow distribution [84]. Recent research, however, with high resolution technology using fluorescent microspheres, has shown considerable blood flow heterogeneity within iso-gravitational planes [51, 52, 53, 65, 155], findings that cannot be explained by the zonal model based on the influence of gravity. In animal studies Glenny et al used the redistribution of pulmonary blood flow at a change in posture to demonstrate that the effect of gravity is minor in proportion to overall variation in regional blood flow [53]. Later, SPECT investigations of pulmonary ventilation and perfusion in humans have confirmed that gravity is of less importance in the prone and supine positions, but has greater influence in the upright position [7, 119]. In microgravity environment, i.e. space experiments, the blood flow heterogeneity remains which confirm that gravity is a minor determinant of regional lung blood flow distribution [54, 123]. During hypergravity conditions at 5G, on the other hand, there is a redistribution of blood flow in opposite direction to the gravitational force [117]. Altogether, these results have lead to the challenging assumption that the basic anatomical structure of the pulmonary vascular tree is an important determinant of regional blood flow [66].
In 1986, Beck and Rehder demonstrated regional differences in vascular conductance in isolated dog lungs [14], which raised the postulate that there might exist differences in regional vasoreactivity in the lung. This hypothesis was further strengthened by a study by Pelletier et al., 1998, which showed differences in vasoreactivity to various vasorelaxing agents in isolated dorsal and ventral equine pulmonary arterial rings [111].
In 1946, Euler showed that regional hypoxia could divert pulmonary blood flow away from poorly ventilated lung units with inadequate oxygenation to better ventilated regions [163]. This unique behaviour to hypoxia in the pulmonary vessels, called the hypoxic pulmonary vasoconstriction (HPV), provides an important mechanism for maintaining optimal ventilation / perfusion matching and improves arterial oxygenation [18, 90, 161]. There are numerous local vasoactive substances that modulate HPV [46, 138] and thereby play an indirect role for regulation of regional pulmonary blood flow. Posture may also affect blood flow diversion by HPV [154]. If there exist regional differences in

sensitivity to hypoxia in the lungs has to be further investigated in this work. In this thesis we have focused on vasorelaxing substances acting via the NO /cGMP or the PGI_2 /cAMP pathways.

Pulmonary vasoregulation

The pulmonary circulation is unique in that it must, at all times, accept the entire cardiac output and yet, despite large and variable blood flows, direct the blood flow to well-oxygenated regions. Thus, the regulation of pulmonary vascular tone is complex with a balance between local vasoconstricting factors directing blood flow to well-oxygenated lung units and on the other hand, vasorelaxing factors that must maintain a low resistance in the pulmonary circuit to protect the right ventricle that cannot tolerate a high after-load. The pulmonary vascular resistance (PVR) is normally kept at approximately one-tenth of the systemic vascular resistance.

Table 1. Factors that alter Pulmonary Vascular Resistance

Inreased PVR	**Decreased PVR**
Active	
Sympathetic stimulation	Nitric oxide
Hypoxia	Prostaglandins (PGI_2,PGE_2)
Hypercapnia	Acetylcholin
Acidosis	Bradykinin
Catecholamines	Isoproterenol
Angiotensin II	Theophylline
Endotelin-I	Phosphodiesteras inhibitors
Histamin (H1)	
Prostaglandins (D_2,E_2,F_2,)	
Substance P	
Leukotrienes	
Seretonin	
Passive	
Lung inflation or deflation from FRC	Increased cardiac output
Increased perivascular pressure	Increased P_{pa} and P_{la}
Increased blood viscosity	Increased pulmonary blood volume

Changes in PVR can be caused by active or passive factors [11]. Active changes are accomplished by neurogenic, chemical and humoral factors, while passive changes in PVR imply alteration in vessel calibre in response to factors such as lung mechanics or hemodynamics (where posture, gravity and vascular structure play a role). Some of these factors are listed in (table 1) [48].

Active forces alter PVR and tone, and hence the distribution of pulmonary blood flow, by causing contraction or relaxation of vascular smooth muscle [11]. An increase in tone will occur if any of these factors directly or indirectly increases the cytosolic Ca^{2+}-concentration to more than 10^{-6} mol l^{-1}.

Ca^{2+}-ions then bind to calmodulin (CaCM)

a) which activates myosin light chain kinase (MLCK). The active $4Ca^{2+}$-CM-MLCK complex phosphorylates myosin and enables interaction with actin leading to muscle contraction.

b) bind to caldesmon, which then detaches from the actin tropomyosin complex, thus making it available for filament sliding.

Direct phosphorylation of caldesmon by protein kinase C also seems to induce filament sliding and contraction.

On the other hand, factors causing a decrease in intracellular Ca^{2+}-concentration to less than 10^{-6} mol l^{-1} or activates phophatase induce relaxation. Also, direct activation of protein kinase C stimulates myosin light chain kinase which then phosphorylates myosin regulatory light chain leading to Ca^{2+} -desensitization of myofilament and thereby relaxation of the smooth muscle.

Increased cytosolic levels of cGMP or cAMP is one of the major mechanisms mediating vasodilation under physiologic conditions. cGMP mediates the action of endogenous vasodilators such as NO, carbon monoxide (CO) and natriuretic peptides [151] via inhibition of phosphodiesterases, activation of soluble and membrane bound guanylyl cyclase. An increase in cGMP activates protein kinase G, leading to decrease in cytosolic Ca^{2+}-concentration via uptake in the sarcoplasmatic re-

ticulum and inhibition of voltage-operated calcium channels (VOCC) and thereby causes relaxation. cAMP activates protein kinase A, which inhibits phospolipase C and VOCC, stimulates Ca^{2+} uptake in the sarcoplasmatic reticulum and thereby decrease cytosolic Ca^{2+}-concentration leading to relaxation[95]. Furthermore cAMP and cGMP reduces myofilament Ca^{2+} -sensitivity (Fig.1).

For the most part, vasoactive substances have similar effects on pulmonary and systemic vessels. However, there are some important exceptions; hypoxia, hypercapnia and acidosis produce vasodilatation in the systemic circuit whereas these factors produce vasoconstriction in the pulmonary circulation. One primary physiological determinant of vascular resistance in lung is alveolar pO_2 [61, 98]. The unique behaviour of the pulmonary vascular smooth muscle cells in response to hypoxia is an

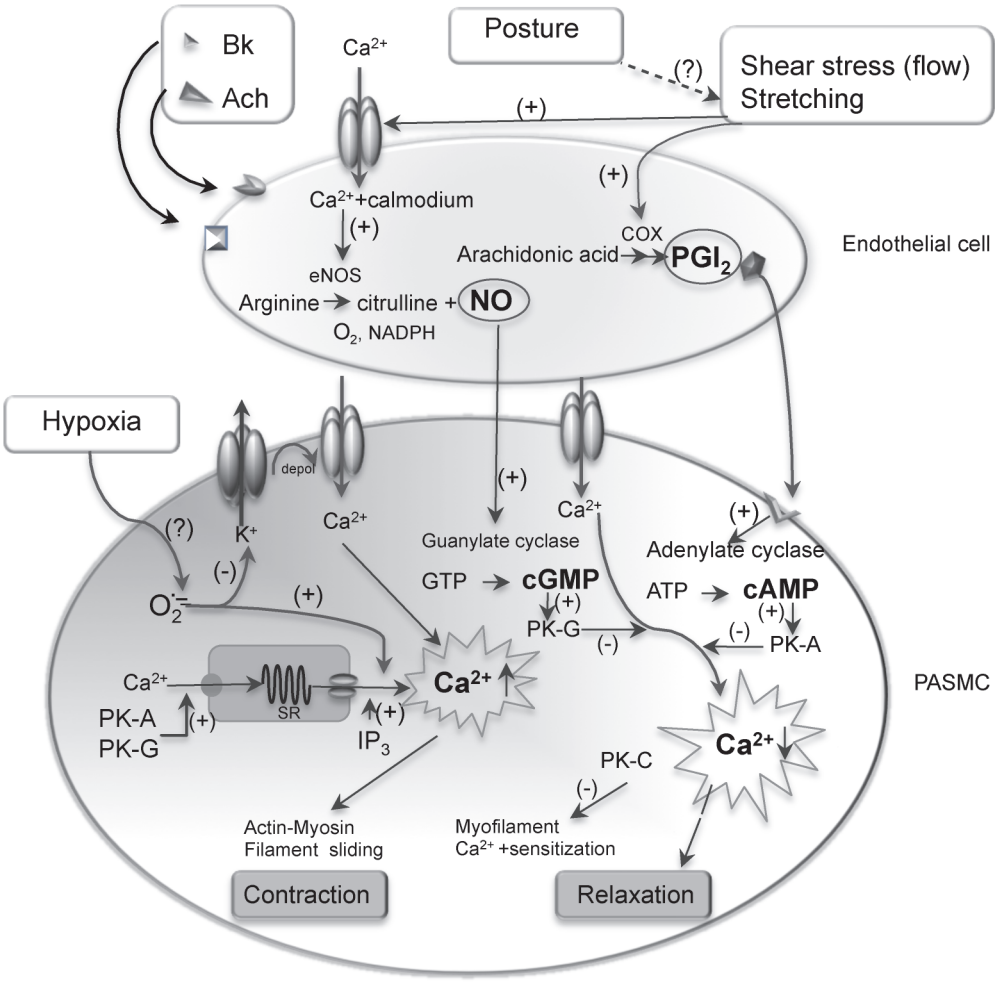


Figure 1 Schematic overview of the proposed mechanisms for a) vasorelaxation mediated by the endothelium dependent eNOS / NO / guanylate cyclase/ cGMP and COX / prostacyclin / adenylate cyclase / cAMP pathways. b) pulmonary vasoconstriction induced by hypoxia.
For details see text page 15. Plus sign = stimulation; minus sign = inhibition, question mark = not established.
Ach = acetylcholine, Bk = bradykinin, NO = nitric oxide, eNOS = endothelial nitric oxide synthase, PGI_2 = prostacyclin, NADPH = dihydro-nicotinamid-adenine-dinucleotide phosphate, PKG = protein kinase G, PKA = protein kinase A, PKC = protein kinase C, SR = sarcoplasmic reticulum, ATP = adenosine 5´-triphosphate, cAMP = cyclic adenosine- 3´, 5´-monophosphate, GTP = guanosine 5´-triphosphate, cGMP = cyclic guanosine 3´, 5´-monophosphate, IP_3= inositol 1´4´5´-triphosphate, PASMC = pulmonary arterial smooth muscle cells

The unique behaviour of the pulmonary vascular smooth muscle cells in response to hypoxia is an adaptive vasomotor response of resistance pulmonary arteries, aimed to divert blood from poorly oxygenated areas to better ventilated lung segments thus improving ventilation-perfusion matching and thereby reducing shunt fraction and optimizing PaO_2 [88, 144, 163]. HPV is intrinsic to the lung and, although modulated by the endothelium, the core mechanism is in the smooth muscle cells. The exact mechanism for HPV is still elusive [161]. The Redox Theory proposes that HPV results from a coordinated action of a redox-based sensor (proximal mitochondrial electron transport chain) which generates a diffusible mediator, a reactive O_2 species (ROS) that regulates effector proteins. Hypoxic modulation of ROS leads to inhibition of the specific O_2-sensitive voltage-gated potassium channels (Kv1.5 and Kv2.1*)*, leading to depolarization of pulmonary artery smooth muscle cells, activation of L-type voltage-gated calcium channels, which increases calcium influx and initiates vasoconstriction [93, 98, 127]. The exact time course of HPV is still under debate in the literature. However, the onset of HPV is immediate (in seconds), seems to be biphasic with a first endothelium independent culmination in 10-15 minutes and then a second endothelium dependent increase after 30-40 minutes [88, 98] with sustained, or even increased effect for hours [72, 142] or until reoxygenation occurs [36]. The sustained response seems to be endothelium dependent and is thus subjected to modulation by endothelium derived vasoactive substances. Vasorelaxing substances like nitric oxide and prostacyclin have been shown to decrease the HPV response via mechanisms not fully known [46, 138].
The principal stimulus for HPV is alveolar hypoxia, whereas decreased mixed venous oxygen tension is only a contributory factor. Marshall and Marshall 1980 showed that the magnitude of blood flow diversion is related to the size of the hypoxic lung segment [88]. If the hypoxic part equals one lung, for instance during one-lung ventilation, numerous clinical studies have found that shunt in the non-ventilated lung is usually 20-25% of the cardiac output as opposed to the 40-60% shunt that might be expected if there was no HPV [17, 18]. In case of global hypoxia, there will be an increase in pulmonary arterial pressure due to general vasoconstriction, instead of PBF diversion. [17, 18, 88, 148]. There has also been demonstrated heterogenicity in HPV response in porcine lungs which partly may explain high-altitude pulmonary oedema [67].

In the present thesis we have focused on the potent pulmonary vasodilators nitric oxide and prostaglandin I_2 to investigate their role in regulation of *regional* pulmonary perfusion, during both normoxia and hypoxia. The influence of posture on regional perfusion distribution has also been a subject of our interest. By altering posture there will be a change in PVR due to influence of passive mechanical factors, as discussed earlier, which might interact with the effects of local vasoactive substances. It is difficult to identify the precise contribution of each of these factors to regulation of regional pulmonary blood flow. They are often interrelated, so a change in one affects a change in another; which adds to the complexity of pulmonary pathophysiological research.

Nitric oxide (NO)

In 1980, Furchgott and Zawadzki observed that intact endothelium was mandatory for induction of arterial relaxation by acetylcholine [47]. This endothelium-dependent vasorelaxation was later shown to be due to production of NO [71]. NO is produced by oxidation of L-arginine to citrulline, catalyzed by the enzyme NO-synthase (NOS) [110]. NO-synthase exists in three isoforms; two Ca^{2+} and calmodulin requiring constitutive enzymes found in neural tissue (nNOS) and in vascular endothelial cells (eNOS), and a Ca^{2+}-independent inducible enzyme (iNOS) isolated from macrophages, vascular smooth muscle cells and neutrophiles [157]. NO can also be produced non-enzymatically by direct reduction of nitrite during acidotic or ischemic conditions [164].

Substances like acetylcholine and bradykinin cause an increase of intracellular free calcium concentration in the endothelium activating NOS and stimulating NO production [62, 85]. NOS is only modestly expressed in the pulmonary vessels in normal lung, suggesting a limited role in maintaining low basal tone [40]. However, it is markedly up regulated in response to hypoxia or inflammation, thus playing a central role in modulating the response to pulmonary vasoconstrictor stimuli such as hypoxia [62] and to local vasoconstrictors like angiotensin-II, endothelin-I or thromboxane A_2 (TXA_2) [115, 136]. Increased vascular shear stress [120] and mechanical factors like stretching [116] are also known to be potent stimuli for endothelial NO synthesis.
NO is a potent pulmonary vasodilator, relaxing precapillary resistance vessels to a greater degree than the large capacitance vessels [44]. It diffuses freely over the cell membrane, activates guanylyl cyclase leading to increased levels of cGMP and thereby a decrease in intracellular free calcium concentration in the pulmonary vascular smooth muscle cell and thus vasorelaxation [34, 96, 149]. NO is short-lived with a biological half-life of 2 – 30 seconds. It rapidly and avidly binds to haemoglobin and forms nitrosylhemoglobin and methemoglobin [32]. NO binds to and is inactivated by haemoglobin before it can reach the systemic circulation, which makes it suitable as a selective pulmonary vasodilator when inhaled [46]. NO synthesis can be blocked by using competitive inhibitors to the substrate L-arginine which binds to a specific site on the activated NO-synthase [96, 68].
Besides regulation of vascular tone, NO is involved in a variety of biological processes. In the lung, NOS is expressed in airway epithelial cells, in macrophages, mastcells, neutrophiles, smooth muscle cells, fibroblasts and platelets [56, 141].

Prostaglandin I_2 (PGI_2)

Prostacyclin is one among a number of prostaglandins synthesised in the lung. Prostaglandins, a group of the eicosanoid family, have an important role in regulation of pulmonary vascular and bronchial tone. They also have important anti-inflammatory actions and modify platelet aggregation [63, 75]. The prostaglandins are derived from the cyclooxygenase (COX) metabolism of arachidonic acid, a component of membrane phospholipids [156]. There are two isoforms of the enzyme cyclooxygenase, COX-I being constitutively expressed, and COX-2, which is induced in inflammatory processes [63, 132]. The various prostaglandins have potent, often opposing actions in the pulmonary vasculature and airways, for instance PGD_2, PGE_2 and $PGF_{2\alpha}$ as well as TXA_2 elicit vasoconstriction, while PGI_2 (prostacyclin) and PGE_1 cause vasodilation [75]. The most important pulmonary cell type responsible for the synthesis of cyclooxygenase products is the vascular endothelial cell, and the preferentially synthesised product is prostacyclin. Prostacyclin is a potent pulmonary vasodilator with a half-life of 4 minutes, and biological effect of 20-30 minutes. It acts via stimulation of the adenylyl cyclase / cAMP pathway, thus decreasing the intra-cellular calcium concentration and thereby initiating vasorelaxation. Prostacyclin is metabolised by beta-oxidation in the liver [63, 132].
PGI_2 production can be up regulated via increases in blood flow, shear stress [45, 131] and hypoxia [60].
Aspirin and non-steroidal anti inflammatory drugs (NSAIDs) are analgesic and anti-inflammatory by virtue of inhibition of the cyclooxygenase mediated biosynthesis of prostaglandins. The efficacy and selectivity of the COX-inhibiting effect depends on dose and drug properties. Among the non-selective COX-inhibitors, indomethacin and ipobrufen inhibit both the synthesis of prostacyclin and TXA_2, while diclofenac seems to have less effect on the TXA_2 synthesis [27].
As compared with nitric oxide, prostacyclin has a variety of effects in the lung. Besides modulation of vascular tone, prostacyclin inhibits platelet aggregation and smooth muscle cell proliferation, effects that can be of importance in treatment of pulmonary hypertension [105, 107,69].

Posture

During the latest two decades many investigations have demonstrated better oxygenation in hypoxic and ventilator treated patients, when treated in the prone position. [24, 28, 39, 50, 57, 73, 87, 100, 112, 159].
Several investigations have shown more homogenous ventilation / perfusion ratios in prone compared to supine position during mechanically ventilation [7, 99, 129]. Perfusion and ventilation are more uniform in prone compared to supine position most likely depending on several factors discussed above. In the upright lung, Wests zonal model explains the gravity-influenced relationship, and therefore posture, between the intravascular hydrostatic pressures and the vertical height of the lung. In zone 1 the regional pulmonary arterial pressure (P_{pa}) is lower then the alveolar pressure (P_{alv}) which results in no blood flow in the capillaries surrounding the alveoli. In zone 2 Ppa is above but pulmonary venous pressure (P_v) below P_{alv} which results in an increasing vertical gradient in blood flow down this zone. In zone 3 $P_{pa} > P_v > P_{alv}$ resulting in maintained blood flow but decreased alveolar size [162]. Several investigators in recent studies with high resolution techniques have confirmed an increased blood flow to dependent regions, due to the vertical pressure gradient, in upright position [70, 84, 119]. Glenny showed 1999 that gravity explained 25% of the blood flow heterogeneity in upright baboons, but only 7% and 5% in the supine and prone postures respectively [51]. It should be kept in mind that in supine position, left atrial pressure is 10 cm H_2O in healthy subjects, resulting in an extension of zone 3 to 10 cm above the center of the left atrium, which includes most of the lung height in prone and supine position. However, positive pressure ventilation might induce zone 2 conditions also in horizontal posture. Therefore the posture has limited effect on ventral-to-dorsal distribution of blood flow in prone and supine postures [118]. When analysing results from investigations of regional pulmonary perfusion distribution indifferent postures, it is important to relate alterations in perfusion distribution to changes in tissue redistribution [118, 124].
During the last decades the Seattle group has shown convincing data that the anatomical structure with fractal branching of the vascular and bronchial tree is the most important determinant of distribution of ventilation and perfusion [52, 55, 66]. The structure of the vascular tree is suggested to explain 64% of the more homogenous PBF distribution in the prone position [7]. These findings do not exclude coexisting differences in regional vasoreactivity of importance for regulation of pulmonary perfusion.
Could the optimized ventilation/perfusion matching in prone position reflect an adaptive evolutionary mechanism from the quadruped stage? Structure as well as regional vasoreactivity in the lung might have developed during millions of years to counteract the effect of gravity on ventilation/perfusion distribution.

Aims

General aims

The majority of ventilator treated patients in intensive care units, suffering from Acute Lung Injury, ALI, improve gas exchange when turned prone. The overall purpose of this thesis was to reach new knowledge of importance for better understanding of mechanisms behind the improved gas exchange in prone position. The final goal was to explore new knowledge of possible use to improve treatment of patients with life-threatening lung disease in intensive care.

Specific aims were to find out if:

- expression of mRNA for endothelial nitric oxide synthase (eNOS) is different in ventral and dorsal lung regions in humans (**I**)
- Ca^{2+}-dependent and Ca^{2+}-independent NOS activity is different in ventral and dorsal lung regions in humans (**I**)
- lung perfusion in ventral and dorsal lung regions is influenced by inhibition of NO synthesis (**I**)
- production of NO, in pigs, is different in ventral and dorsal lung regions (**II**)
- endothelium dependent mediators like acetylcholine and bradykinin act differently on ventral and dorsal pulmonary arteries in pigs (**II**)
- eicosanoids are involved in regulation of regional pulmonary perfusion in healthy humans (**III)**
- inhibition of prostaglandin synthesis decrease shunt fraction in the lungs during one-lung ventilation for thoracic surgery (**IV**)

Subjects, Materials and Methods

The methods have been described in detail in paper I-IV. Below follows a description of the methods more in general.

Table 2. Methods, total number of patients, volunteers and animals are given. Types of investigational methods are shown

Paper			No.of	No.of	No.of
No	Methods	Investigation	Patients	Volunteers	Animals
I	SPECT	Effect of NOS-inhibition on pulmonary perfusion in different body postures in healthy humans		9	
I	PCR	eNOS mRNA	13		
I, II	Citrulline assay	Ca^{2+}-dependent NOS-activity	21		18
II	Experimental organ bath	Vasoreactivity			20
III	SPECT	Effect of prostacyclin inhalation and COX-inhibitionon pulmonary perfusion in different body postures in healthy humans		19	
IV	Clinical trial	Effect of COX-inhibition on pulmonary perfusion during hypoxia	32		

Subjects

Patients

Altogether 53 patients were studied. In **paper I** there were 9 males and 12 females with mean age 69 years (range 41-92) subjected to lung biopsies. All 21 were investigated by analysis of NOS activity, 13 of those were also investigated by analysis of eNOS mRNA. In **paper IV** there were 11 men and 21 females with mean age 65 (range 36-81). They were all scheduled for lung surgery because of diagnosed or suspected pulmonary malignancy. Exclusion criterias were occurrence of other pulmonary disease or use of corticosteroids, acetyl salicylic acid or non-steroidal anti-inflammatory substances.

Volunteers

Altogether 28 non-smoking healthy volunteers participated. In **paper I** there were 5 males and 4 females, mean age 31 years (range 23-47) and in **paper III** there were 9 males and 10 females, mean age 29 years (range 21- 45) studied.

Animals

In **paper II** twenty pigs specially breaded for research, weighing 18.5-22.5 kg were studied. They were 18-22 weeks old.

Investigational methods

Real time polymerase chain reaction (PCR) for mRNA eNOS quantification (paper I)
Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and quantified by spectrophotometry. Two micrograms of total RNA were used for cDNA synthesis. Approximately 50 ng of cDNA was amplified by real time polymerase chain reaction using primers and probes as described in (Table 3). Samples were analyzed in duplicate, and polymerase chain reaction amplification was correlated against a standard curve. eNOS mRNA expression was quantified in relationship to mRNA expression of β-actin, selected as a housekeeping gene.

Table 3 Target primers and probes used for real-time polymerase chain reaction

Target Gene	Sequences (5'–3')
eNOS	Primer forward GCAACGCTACCACGAAGACAT
	Primer reverse CGGCTTGTCACCTCCTGG
	Probe TCGGGCTCACGCTGCGCA
β-Actin	Primer forward CTGGCTGCTGACCGAGG
	Primer reverse GAAGGTCTCAAACATGATCTGGGT
	Probe CCTGAACCCCAAGGCCAACCG

Definition of abbreviation: eNOS = endothelial nitric oxide synthase.

Citrulline assay for NOS-activity (paper I and II)
The conversion of l-(U-14C) arginine to l-(U-14C) citrulline was used for measurement of NOS activity as previously described **(paper I, II)** [97, 133]. The analysis was performed in the presence or absence of the calcium chelator ethyleneglycol-*bis*-(aminoethyl ether)-*N*, *N*-tetra acetic acid (EGTA) to differentiate between Ca^{2+} -dependent and Ca^{2+} -independent NOS activity.

Organ bath experiments for vasoreactivity studies in isolated pulmonary arterial ring preparations (paper II)
During anesthesia with spontaneous breathing the porcine heart and lungs were harvested, immediately chilled in ice-cold physiological salt solution (PSS) and dissected on ice. Preparations of vascular rings from ventral and dorsal porcine pulmonary arteries were mounted vertically between two stainless steel hooks. The hooks were attached by silk sutures and arranged vertically between a stationary hook and a force transducer in 25 ml organ bath in PSS at +4°C. The organ bath was continuously aerated with 5% CO_2 in oxygen. The isometric tension generated in the vessel rings was continuously recorded on a multi channel Grass polygraph. Ring preparations were given an initial isometric load of 5 mN. The PSS temperature was raised to +37°C over 60 minutes and the rings were then equilibrated for another 60 minutes before start of the experiment.
Sub maximal contraction was achieved with norepinephrine 10^{-6} M and cumulative dose-response curves for bradykinin, acetylcholine and nitroprusside were studied. NO-mediated relaxations were inhibited with L-NMMA, while diclofenac was used to inhibit possible prostacyclin mediated relaxations. The antagonism by L-NMMA or diclofenac was reversed by L-arginine and prostacyclin respectively.

SPECT-single photon emission computed tomography for pulmonary perfusion or ventilation studies (paper I and III)

Regional lung blood flow was investigated using macro aggregates of albumin labelled with radioactive technetium ^{99m}Tc [134]. Regional ventilation was marked using Technegas, a dispersion of ultra fine carbon particles labelled with ^{99m}Tc [25]. All SPECT images were obtained with a three-headed gamma camera equipped with high resolution low-energy collimators. SPECT scans were performed in 90 projections during 360° rotation with an examination time of 20 minutes. A 128x128 matrix was used, giving a spatial resolution of 3.5 mm/pixel, for data acquisition and reconstruction.

SPECT data analysis:

3D images representing perfusion distribution were reconstructed from every acquisition performed. To be able to distinguish the effects of an intervention between two acquisitions a subtraction procedure was done to "eliminate" the disturbing activity from the former image. Corrections for attenuation were performed [29].

In **paper I** perfusion distribution was analysed using rectangular volumes of interest (VOI)-width =18 mm and length = lung dimension in ventral–dorsal direction. Volumes were defined on the interface between the apical and mid and the mid and basal third of the right lung (Fig 2B). They were divided in two equal parts (ventral/dorsal) and their perfusion was expressed in percent of that in the entire VOI.

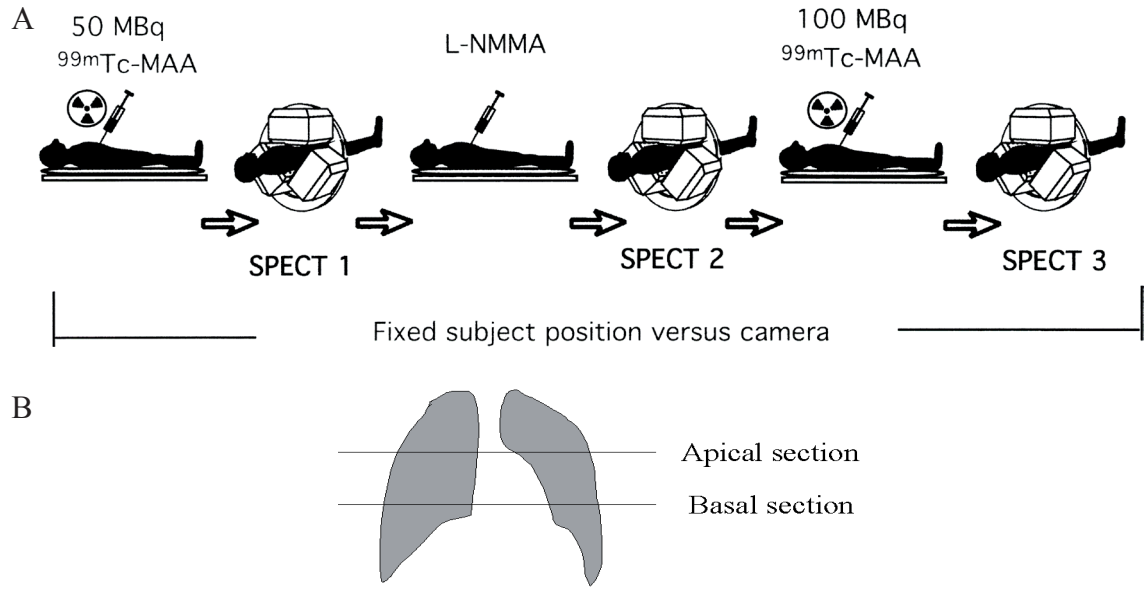


Figure 2. A) Design of study for SPECT analysis of pulmonary perfusion. Tracer administration and NO synthase inhibitor administration are indicated.
B) Frontal view of apical and basal sections of SPECT image planes. Rectangular volumes of interest (VOI) were defined on the interface of the apical and mid third (apical section), and the mid and basal third (basal section) of the right lung. The VOI has a width of 18 mm and a length corresponding to the depth of the right lung. The VOI was divided in two equal parts (ventral/dorsal) and activity in each part is presented as a percent of the entire VOI.

In **paper III** perfusion distribution was reconstructed in coronal sections from a 128x128 matrix, giving a spatial resolution of 3.5mm/pixel (Fig 3B).The average activity deposition from ventral to dorsal in both supine and prone position was calculated. Activity data were collected by dividing the area under activity distribution curve in 20 sections, each 5% of the ventro-dorsal length. In order to perform inter-individual comparisons the average activity distribution was normalized to the length of the lung from ventral to dorsal.

SPECT 1 after an intravenous injection of macro aggregates (50 MBq) illustrates perfusion under basal conditions. In paper I, perfusion distribution during NOS inhibition was obtained by subtracting SPECT 2 data from SPECT 3, which was performed after the second injection of macro aggregates (100 MBq). SPECT 2 was used to exclude any change in shape of basal activity. SPECT 1 and SPECT 2 were not significantly different, which made subtraction analysis possible (Fig. 2A) In paper III, SPECT I illustrates basal conditions and SPECT 2 conditions after intervention, i.e. inhalation of iloprost or infusion of diclofenac (Fig. 3A).

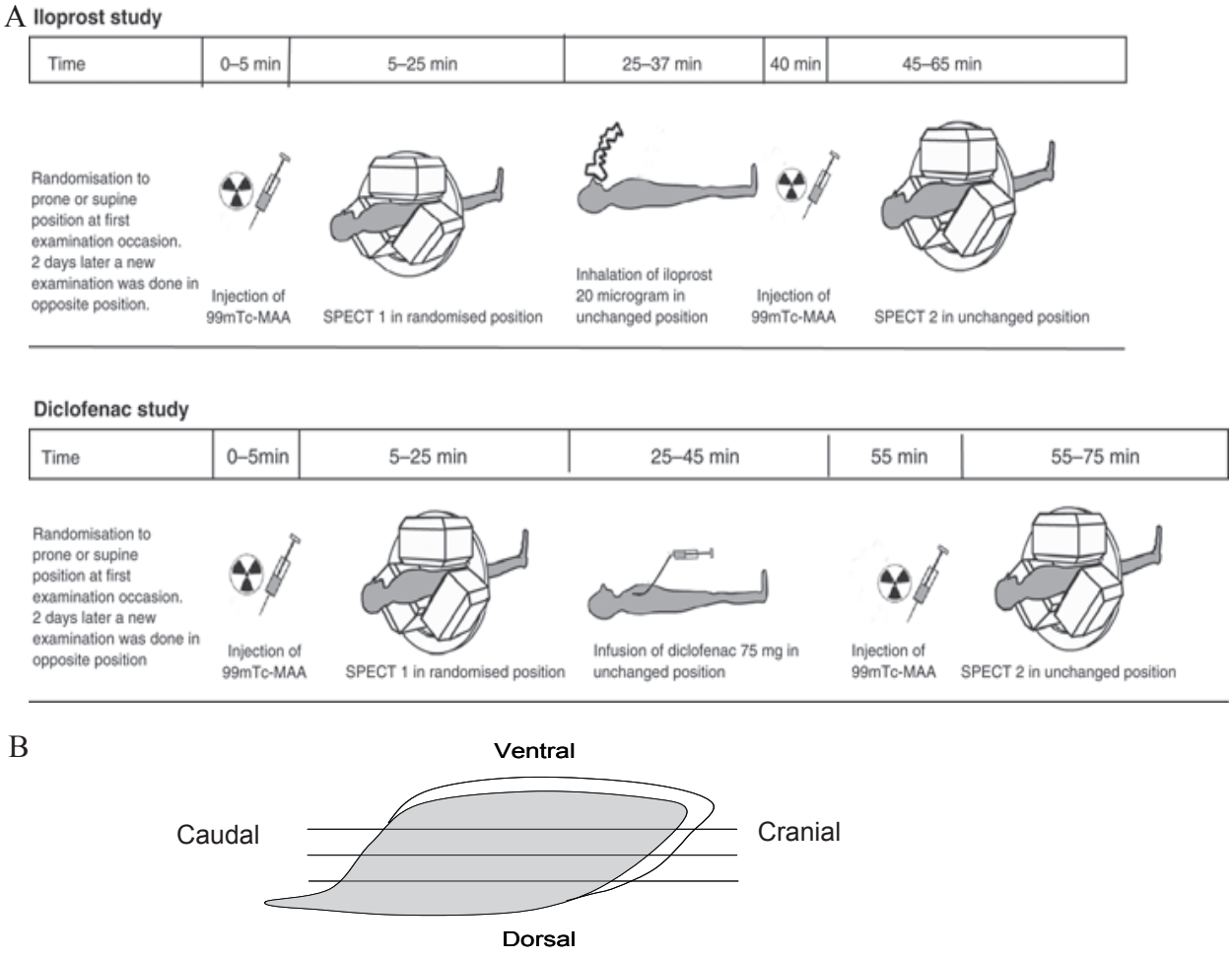


Figure 3. A) Protocol outline for the iloprost inhalation experiment (upper panel) and the diclofenac infusion experiment (lower panel). Blood samples for arterial bloodgases were taken every 10 min. Blood pressure and heart rate were registered every 5 min. For the ventilation SPECT procedure, see the upper panel. Instead of using an injection of 99mTc-labelled macro-aggregated human albumin (MAA), the volunteers inhaled a gas mix containing 99mTc-labelled ultra-fine carbonaceous particles for 1 min.
B) Lateral view with coronal sections of SPECT image planes.

Clinical investigation with shunt calculations during unilateral hypoxia ***(paper IV)***
During thoracic surgery with one-lung ventilation unilateral alveolar hypoxia appears followed by a right to left shunt. The shunt fraction was investigated during pharmaceutical intervention. For sampling of mixed venous blood a thermo dilution catheter was placed in a pulmonary artery via the right internal jugular vein. Arterial blood gases were collected via a radial arterial line. Hemodynamic variables as continuous cardiac output, invasive systemic and pulmonary blood pressures, heart rate and gas exchange data were registered.

Shunt fraction was calculated using the formula proposed by Riley et al. [130]. $Qs/Qt = (Cc´O_2\text{-}CaO_2) / (Cc´O_2\text{-}CvO_2)$. The $Cc´O_2$ is approximated with CAO_2 calculated according to the following formula $CAO_2 = 1.39 \times Hb + PAO_2 \times 0.23$ where $PAO_2 = FIO_2 (PB\text{-}PH_2O) - PACO_2 (FIO_2 + (1- FIO_2) / RQ$. (RQ = respiratory quotient. For a mixed diet assumed to be 0.8)

Study protocols

The study designs for the SPECT investigations are shown in (**paper I and III**) (Fig 2, Fig 3).

Pharmaceutical interventions

NO synthesis inhibition was achieved with N^G-monomethyl-L-arginine (L- NMMA) and the effect was verified by measurements of exhaled NO by chemiluminescence's according to American Thoracic Society guidelines [9] (**Paper I)**
Prostaglandin synthesis inhibition was obtained with the non-selective cyclooxygenase inhibitor diclofenac [152] (**Paper III).** The inhibiton of prostacyclin synthesis was controlled with plasma analyses of 6-keto-prostaglandin1Fα, a stable metabolite of prostacyclin [105].
Inhalation of a prostacyclin analogue Iloprost (Ilomedin®) was performed with a micro processor controlled ultrasonic nebulizer (Optineb®-ir).The systemic uptake was controlled with plasma analyses of 6-keto-prostaglandin1Fα, a stable metabolite of prostacyclin.

Anesthesia

Patients

All patients scheduled for lung surgery were premedicated with morphine intramuscularly (**paper I**) or diazepam 5-10 mg orally (**paper II**) and anesthetized with propofol 1-2 mg kg^{-1}, fentanyl 0.1 mg, glycopyrron 0.2 mg and midazolam 1-2 mg after application of a thoracic epidural catheter at level $Th_{5\text{-}6}$. A radial catheter was placed for continuous blood pressure measurements and arterial blood samples. Neuromuscular block was achieved with atracurium 0.5 mg kg^{-1} followed by endobronchial intubation with a left-sided double lumen tube, size 37 CH for women and 39 CH for men. The correct position of the tube was confirmed by bronchoscopy, initially in supine and later in lateral decubitus position. Anaesthesia was maintained with propofol infusion 5-6 mg kg^{-1} h^{-1}. An epidural infusion with bupivacain 5 mg /ml (**paper I**)/chirocaine 2.5 mg/ml (**paper II**) + sufentanyl 1 microgram/ml of 4-6 ml/h was started after a test dose of 4 ml bupivacaine 5 mg/ml with adrenalin 5 microgram/ml was evaluated. Mean arterial pressure (MAP) was maintained within 30% of baseline values by administration of crystalloids and if necessary intermittent injections of 2-4 micrograms norepinephrine.
Mechanical ventilation was performed by using volume controlled ventilation with a Servo Ventilator (**paper I**) or a Draeger Julian Ventilator (**paper IV**) at FIO_2 of 0.6 or higher if deemed. Arte-

rial blood gases were sampled from a radial catheter and the ventilator was set to achieve normal $PaCO_2$ values. The I:E ratio was set to 1:2. In **paper I** there was no PEEP applied, in **paper IV** PEEP of 5 cm H_2O was applied.

Volunteers
There were no sedatives used in the studies **(paper I and III)**. The volunteers were awake and spontaneously breathing.

Animals
After pre-medication with ketamine hydrochloride 500 mg intramuscularly anesthesia with pentobarbital sodium 25-30 mg kg^{-1} was induced. When there was no reaction to painful stimuli, biopsies were taken from ventral and dorsal lung regions during spontaneous breathing, frozen within 1 minute in liquid nitrogen and stored in – 80°C. Then the heart and lungs were removed and the animals were exsanguinated.

Statistics

Data in the text and tables are presented as means ± SD or median and 25^{th} and 75^{th} quartiles. Statistical analysis was performed using SPSS for Windows. Statistical significance was tested according to Student's *t*-test (**paper I, III**), Wilcoxon rank sum test (**paper III**) or Mann-Whitney non parametric U-test (**paper IV**) for paired and unpaired data. Two way analysis of variance for repeated measures was used to compare related data (**paper I, paper III**). Linear regression analysis was used to investigate correlations between different factors (**paper IV**). Chi-square and Fisher's exact tests were used to analyse differences in number of subjects needing increased F_IO_2 in the different groups (**paper IV**). *P*-values < 0.05 were considered significant. In paper III, differences with 95% confidence intervals not intersecting with zero were considered significant.

Ethical considerations

All studies were approved by ethical committees and in **paper I and III** approvals by radiation protection committee were obtained. Written consent was obtained from all subjects in **paper I, III and IV**.

Results

The results have been presented in detail in paper I-IV, and below follows a description of the findings more in general.

Differences in vasoreactivity between ventral and dorsal lung regions with reference to nitric oxide. (Paper I, II)

Humans: mRNA expression for eNOS was twice as high in dorsal compared to ventral lung tissue in humans. P< 0.05 (Fig. 4A).

The Ca^{2+}-dependent NOS activity was significantly higher (1.5 times) in dorsal compared to ventral human lung tissue (Fig. 4B).

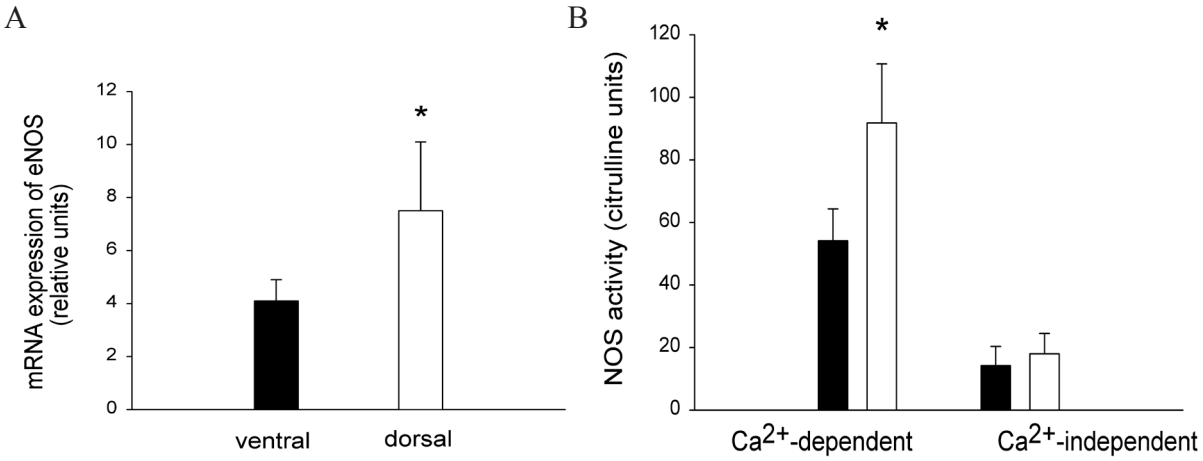
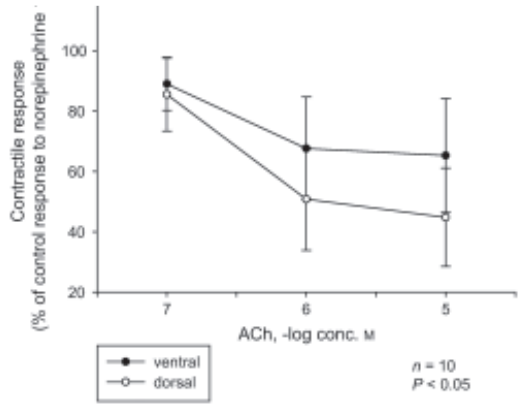


Figure 4 A) Expression of mRNA for eNOS in ventral and dorsal lung tissue. Human lung eNOS mRNA levels are illustrated as relative units between the expression of eNOS mRNA and the housekeeping gene β-actin. Differences between the eNOS expression levels in dorsal and ventral parts of the lung were evaluated by Wilcoxon rank sum test for paired groups. (=P<0.05, n=13).*
B) NOS activity in ventral and dorsal lung tissue. Activity of nitric oxide synthase in human lung specimens obtained at surgery. The activity was determined in ventral (filled bars) and dorsal (open bars) specimens from the lower lobe in the presence and absence of 1.8 mM Ca^{2+}. (=P<0.05, n=21).*

Animals: The relaxing potency of acetylcholine and bradykinin, both endothelium dependent vasorelaxants acting via NO-release from vascular endothelium, was higher in isolated sub-maximally contracted porcine pulmonary arterial ring preparations from dorsal compared to ventral lung regions. Sodium nitroprusside, an endothelium independent NO-donor, was equally potent in relaxing dorsal and ventral isolated pulmonary arterial rings (Fig. 5).

The Ca^{2+}-dependent NOS activity was 1.4 times higher in dorsal compared to ventral porcine lung tissue. P< 0.05 (Fig. 6).



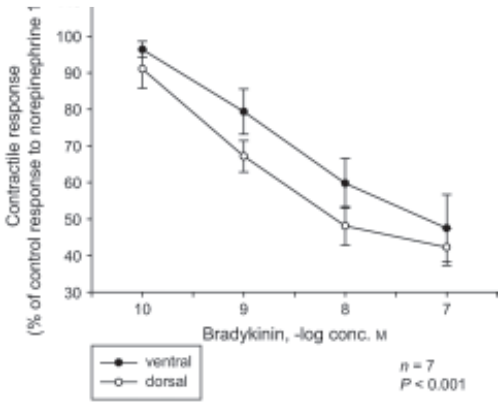
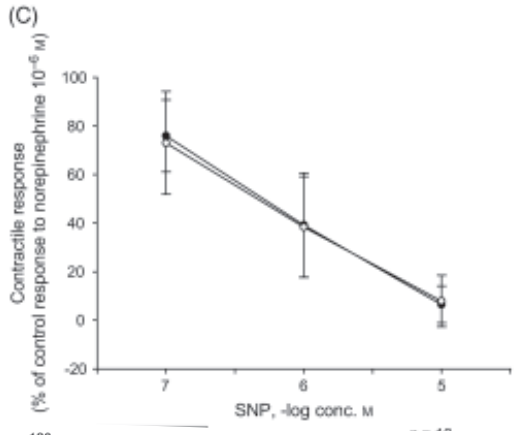


Figure 5. Porcine pulmonary artery. Cumulative dose–response curves for vasorelaxing agents on submaximal contractions to norepinephrine (10^{-6} M) in dorsally (open symbols) and ventrally (filled symbols) located porcine pulmonary arteries. There were significantly greater relaxation responses in dorsal than ventral pulmonary arteries to the endothelium-dependent vasodilators acetylcholine (10^{-7}–10^{-5} M) ($P < 0.05$) (A) and bradykinin (10^{-10}–10^{-7} M) ($P < 0.001$) (B). There were no significant differences in relaxation to the endothelium-independent vasodilator nitroprusside (10^{-7}–10^{-5} M) (C). n = number of different animals.



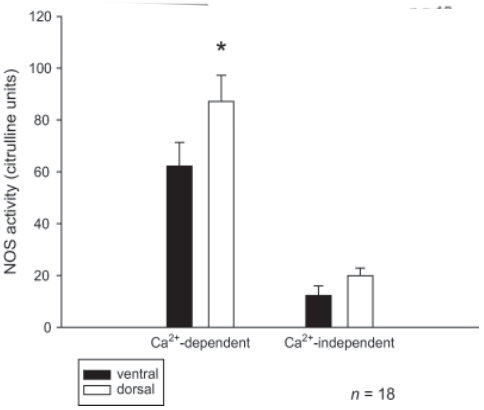


Figure 6. Porcine lung. Nitric oxide synthase (NOS) activity determined by citrulline assay in ventral *and dorsal lung tissue. The activity was determined in ventral (filled bars) and dorsal (open bars) biopsies from the lower parts of the lung in the presence and absence of 1.8 mM Ca^{2+}. *$P < 0.05$, n = 18.*

NOS-inhibition, regional pulmonary perfusion and arterial oxygenation in vivo, healthy humans (Paper I)

Volunteers: In the supine position, NOS-inhibition with L-NMMA redistributed regional pulmonary perfusion from dorsal dependent lung regions to ventral pulmonary parts (Fig.7). Dorsal perfusion decreased from 56 to 52% and ventral perfusion increased correspondingly from 44 to 48%. In the prone position, however, there was no redistribution of regional pulmonary blood flow detected after NOS-inhibition (Fig. 8).

There were no differences in arterial oxygenation after NOS-inhibition by L-NMMA in neither supine nor prone positions in healthy humans.

There was a well-tolerated 10% increase in systemic arterial pressure and 19% decrease in heart rate during NOS-inhibition with L-NMMA. There were no significant differences in heart rate or blood pressure during the NOS-blockade due to posture (Fig. 9).

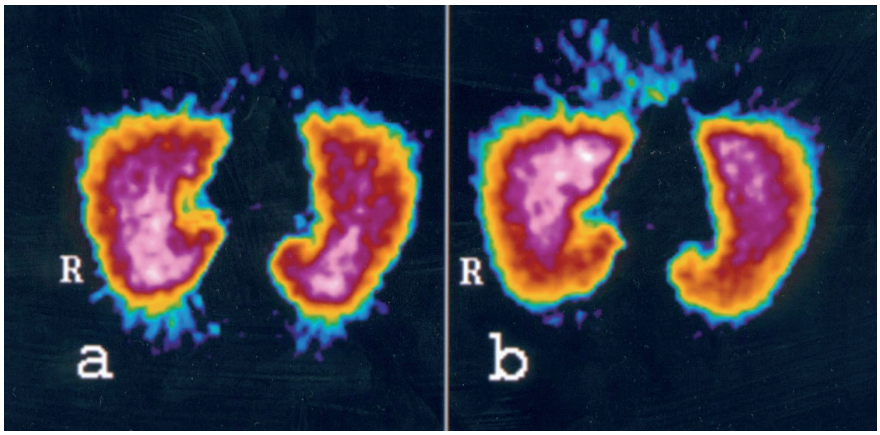


Figure 7. SPECT image of pulmonary perfusion before and after NOS inhibition. SPECT acquisition in a healthy human volunteer before (a) and after (b) administration of the NO synthase inhibitor L-NMMA iv. A horizontal projection is shown with the ventral lung regions facing upwards and the dorsal downwards. The subject was placed in the supine position throughout the procedure, and the basal parts of the lungs are shown. R=right lung.

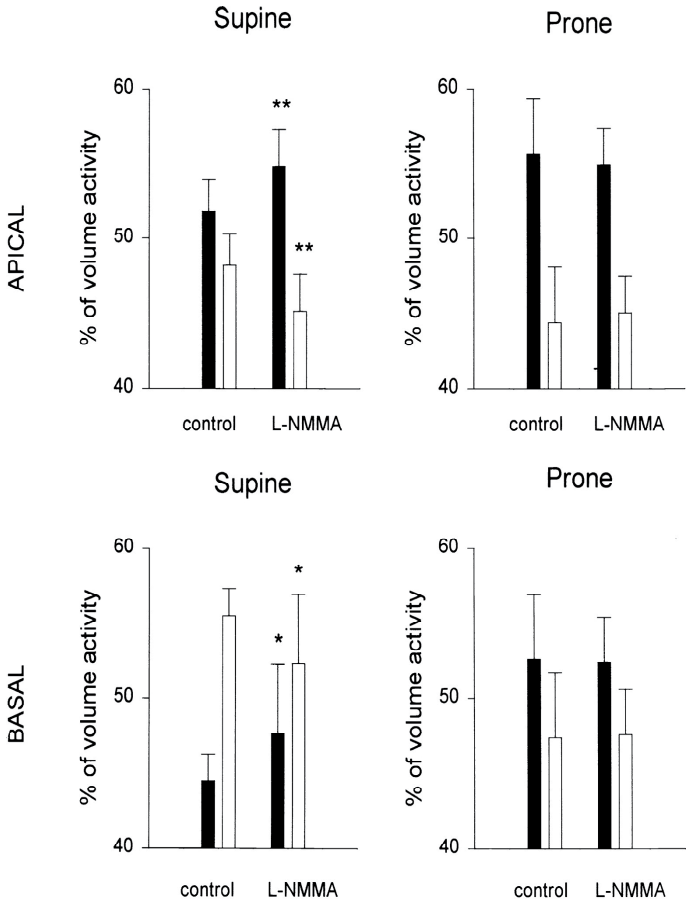


*Figure 4. Regional pulmonary perfusion in supine and prone positions. Distribution of pulmonary blood flow is determined by SPECT in healthy human volunteers, studied at two different occasions in prone (n=8) and supine positions (n = 9), respectively. The relative distribution was determined for the ventral (filled bars) and dorsal (open bars) regions of the lungs before and during intravenous infusion of the NO synthase inhibitor L-NMMA. At the first row, relative blood flow distribution in apical sections is shown and at the second row in basal sections. In the left column, data from supine positions are demonstrated, and in the right column, data from prone positions are shown. (Statistical significance in the figure is indicated for differences between control conditions and in the presence of L-NMMA for ventral and dorsal VOIs, respectively; *p < 0.05, **p < 0.01.)*

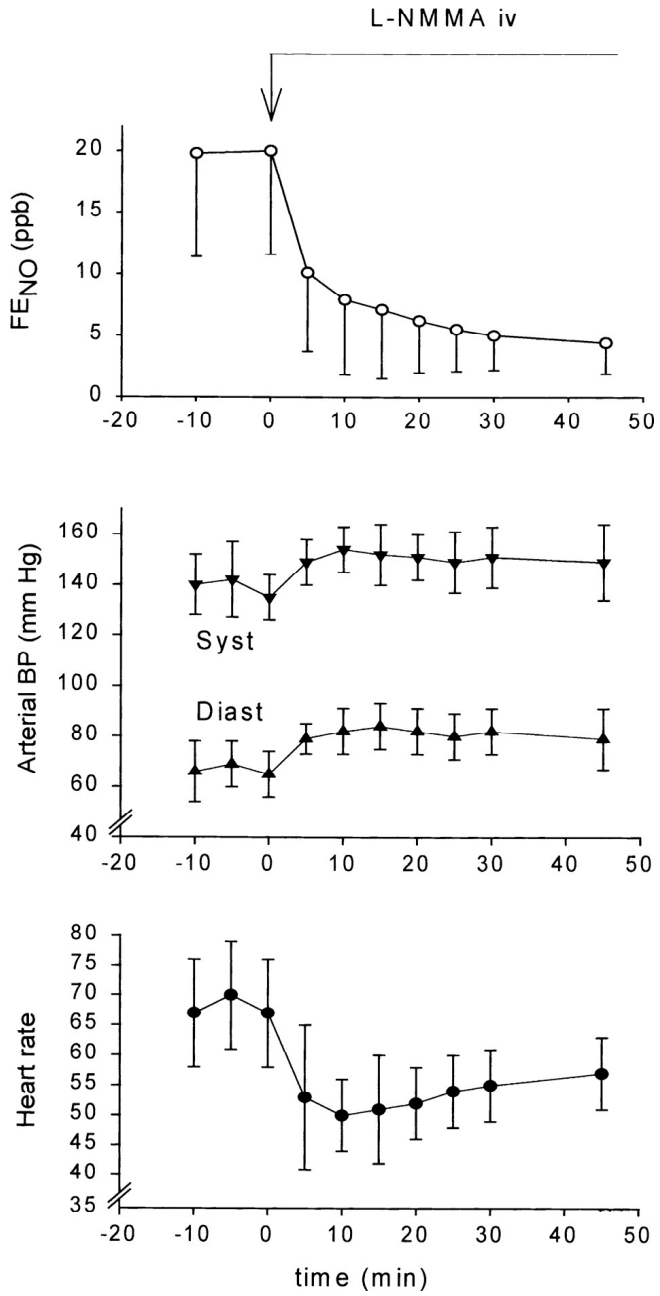


Figure 9. Effects by L-NMMA on exhaled NO, blood pressure, and pulse rate (healthy human volunteers in prone and supine positions). Summary of effects of intravenous infusion of the NO synthase inhibitor L-NMMA on exhaled nitric oxide (FE_{NO}), arterial systolic (Syst) and diastolic (Diast) blood pressures, and heart rate (n = 17).

Differences in regional pulmonary perfusion and arterial oxygenation with reference to prostaglandins in healthy humans. (Paper III)

Decreased prostacyclin levels:
In healthy humans there were no significant differences in regional pulmonary perfusion in ventro-dorsal directions measured with SPECT after non-selective COX-inhibition (Fig.10). Neither arterial oxygenation, heart rate nor blood pressure differed between control and diclofenac groups.

Increased prostacyclin levels:
Arterial oxygenation: After inhalation of the synthetic prostacyclin analogue iloprost there was a decrease in arterial oxygenation which was of the same degree in both prone and supine positions; from 13.7 ± 1.4 kPa to 10.9 ± 2.1 kPa ($P < 0.01$) in supine and from 14.2 ± 0.5 kPa to 11.7 ± 1.7 kPa in prone position ($P < 0.01$, Fig 2 pekIII).
Changes in PaO_2 were normalized 30 minutes after termination of the iloprost inhalation (Fig.11).

Perfusion distribution: Iloprost inhalation induced an augmentation of blood flow in the dependent parts in both supine and prone position with a significant decrease in arterial oxygenation (Fig.12).

Ventilation distribution: After iloprost inhalation in the supine position, there was a significant change in the ventilation distribution from dependent to non-dependent lung regions.

Hemodynamic parameters: There was a transient increase in heart rate and decrease in arterial pressure during the iloprost-inhalation, normalized 8 min after termination of the inhalation. At the time when pulmonary perfusion distribution was measured by SPECT and the PaO_2 was diminished, heart rate and blood pressure were completely normalized. The concentration of 6-keto-prostaglandin F1α was not altered at the time the radioactive tracer was injected and trapped in the pulmonary capillaries.

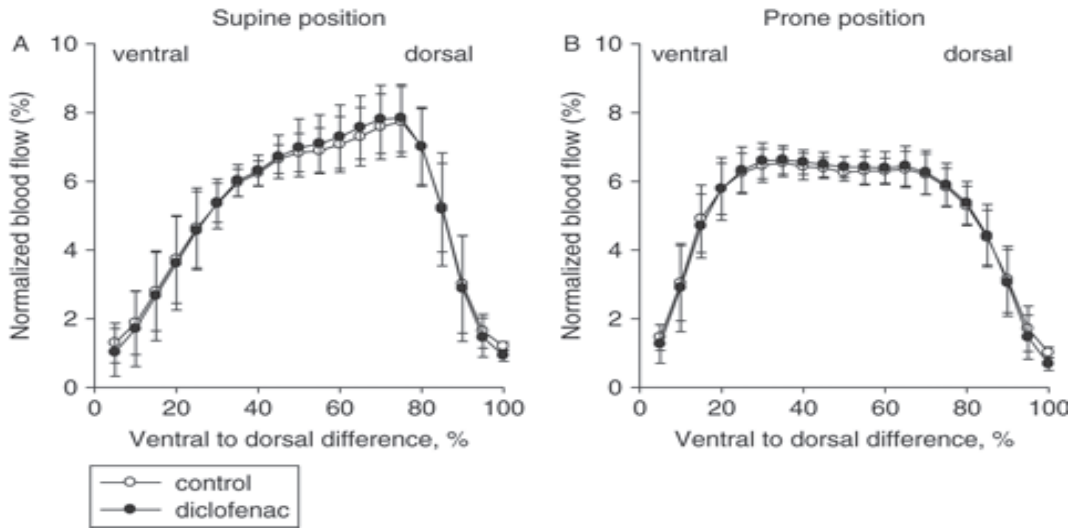


Figure 10. Blood flow as a function of % ventral-to-dorsal distance in supine and prone positions before and after infusion of 75 mg diclofenac. The plots demonstrate the normalized mean values±SD for the vertical lung sections, each 5% of the total distance along a ventral-to-dorsal axis, n=10. A) Perfusion distribution in the supine position, control (open circles) and after diclofenac infusion (filled circles). B) Perfusion distribution in the prone position, control (open circles) and after diclofenac infusion (filled circles). The edge effect (underestimation of radiotracer concentration at the lung periphery) causes blood flow per section to be underestimated at the extremes of the ventral-to-dorsal distance.

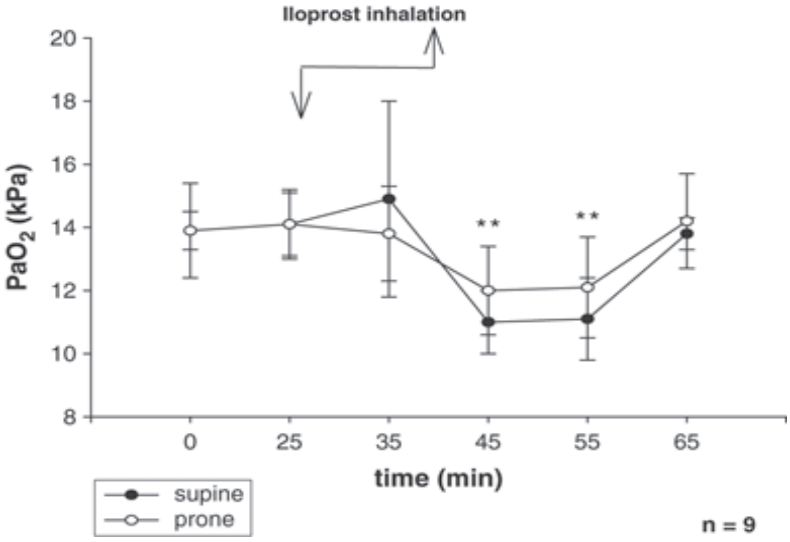


Figure.11. Effects by iloprost inhalation on PaO_2 in healthy volunteers in prone (open circles) and supine (filled circles) positions. PaO_2 decreased during inhalation of iloprost in both supine and prone positions ($P<0.01$). There was no significant difference between the effect of iloprost in the supine and the prone position.

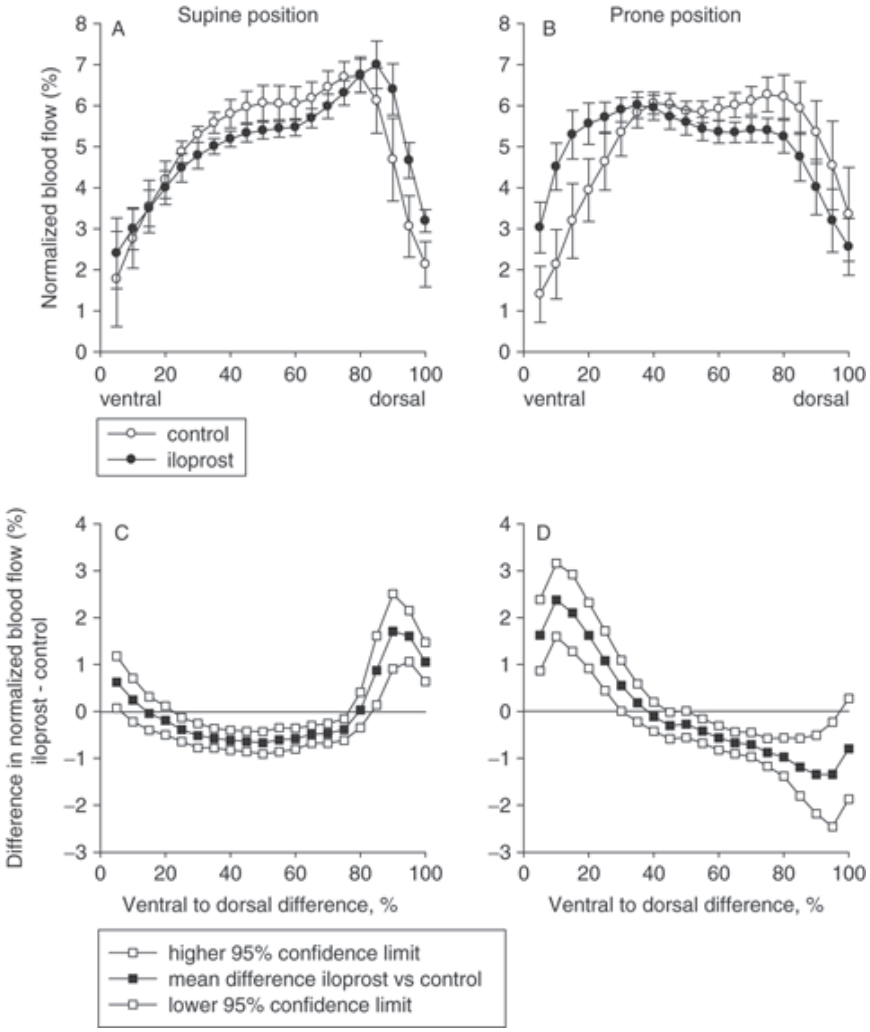


Figure 12. Blood flow as a function of % ventral-to-dorsal distance in supine and prone positions before and after inhalation of 20 μg iloprost. The top row: the plots demonstrate normalized mean values±SD for the vertical lung sections, each 5% of the total distance along a ventro-dorsal axis, n=9. The first column shows the perfusion distribution in the supine position (A) control (open circles) and after iloprost inhalation (filled circles). The second column shows the perfusion distribution in the prone position (B) control (open circles) and after iloprost inhalation (filled circles). The edge effect (underestimation of radiotracer concentration at the lung periphery) causes blood flow per section to be underestimated at the extremes of the ventral-to-dorsal distance. The bottom row displays the differences section by section between the regional perfusion distribution along the ventro-dorsal axis before and after iloprost inhalation in supine (C) and prone (D) positions. The lines show mean differences (filled squares) between blood flow with iloprost and control, low and high 95% confidence intervals (open squares), respectively. Confidence intervals that do not intersect with zero indicate a significant difference between drug and control. Perfusion increased in dependent regions and decreased in non-dependent regions in both the supine and the prone position. Note the different pattern in differences between drug and control in the supine as compared with the prone position.

Differences in pulmonary perfusion with reference to prostaglandins during regional hypoxia in vivo. (Paper IV)

In regional pulmonary hypoxia during one-lung ventilation (OLV) for thoracic surgery, the diclofenac treated group showed a significant reduction of the shunt fraction compared to the placebo group. After 15 minutes of OLV the shunt increased 3.1 times compared to base line measurements in the placebo group, while the increase was just 2.2 times in the diclofenac group ($P > 0.043$, Fig.13).

There were no differences between groups in patient characteristics or preoperative conditions (Table 4). During investigations there were no significant differences in peroperative bleeding, serum creatinine, respiratory or hemodynamic variables between the groups (Table 5, Table 6). Shunt fraction in the placebo group increased from 12 (10, 14) % (median (25^{th},75^{th} percentiles)) during TLV to 39 (24, 44) % and 38 (31, 46) % after 15 and 30 minutes after OLV respectively. In the diclofenac group shunt fraction increased from 12 (12, 14) % at TLV to 28 (16, 36) % and and 30 (18, 35) % after 15 and 30 minutes of OLV respectively. Shunt fraction was significantly improved in the diclofenac group compared with the placebo group after 15 minutes of OLV ($P = 0.043$), but not after 30 minutes of OLV ($P = 0.068$) (Fig.13). This lack of statistical significance might have been due to the smaller number of patients (n = 20) evaluated due to prior initiation of surgical vessel ligation at this time point. During left-sided surgery (LSS), shunt fraction was significantly improved at both 15 ($P = 0.020$) and 30 min ($P = 0.036$) of OLV (Fig.14 A). During right-sided surgery (RSS) reductions of shunt fractions due to diclofenac were not prominent and did not reach statistical significance (Fig.14 B).

Plasma concentrations of 6-keto-prostaglandin $F_{1\alpha}$ were [228 (164, 469 pg/ml)] in the placebo group and [73 (47, 94)] in the diclofenac group ($P = 0.003$) 60 minutes after start of the diclofenac infusion, i.e. at OLV 2.

In order to maintain SpO_2 above 90% FIO_2 had to be increased above 0.6 in 10 patients (4 RSS and 6 LSS) in the placebo group and in 5 patients (all RSS) in the diclofenac group ($P = 0.077$). Preoperative PO_2 and PCO_2 values were similar in patients requiring increased FIO_2 compared with those who did not. In the entire patient material PaO_2/FiO_2 ratios and P (A-a) O_2 differences were not changed after diclofenac treatment (Table 5). In patients subjected to left-sided lung surgery, however, P (A-a) O_2 values in the diclofenac treated patients at 15 minutes of OLV were significantly lower than in the placebo group ($P < 0.029$, Fig. 15).

Table 4. Patient demographics.

	placebo (n=16)	diclofenac (n=16)	*P*
Age (yr)	65 (28-76)	71 (36-81)	0.10
Females	9	12	
BMI (kg m^{-2})	24.2 (20, 25)	26.7 (22.9, 28.3)	0.11
Hemoglobin (g l^{-1})	128 (120, 144)	137 (128, 143)	0.48
Albumin (g l^{-1})	38 (35, 40)	38 (37, 41)	0.35
Creatinine (g l^{-1})	72 (60, 93)	65 (58, 84)	0.30
PaO_2 kPa	10.4 (10.1, 10.8)	10.4 (8.9, 12.8)	0.96
$PaCO_2$ kPa	5.4 (5.1, 6.0)	5.1 (4.9, 5.6)	0.10
FEV_1 (liters s^{-1})	2.6 (1.9, 3.0)	2.1 (1.8, 2.7)	0.16
% of predicted FEV_1	84	85	
VC (liters)	2.9 (2.5, 4.1)	2.8 (2.6, 3.5)	0.8
% of predicted FVC	82	87	

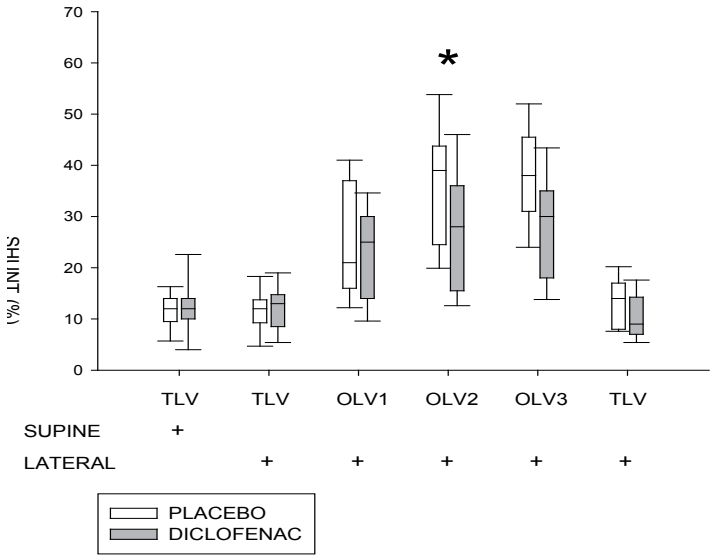


Figure 13. Shunt fraction (%) in all patients, left and right thoracic surgery, pooled data for the placebo (unfilled) and diclofenac groups (filled boxes). Measurements were performed after induction of anesthesia (supine position), in lateral decubitus position during two-lung (TLV) and one-lung ventilation (OLV) and finally after restoration of TLV. Measurements were registered at 15 min intervals. The shunt was significantly larger in the placebo group at OLV2 (P = 0.043; n = 16). Data are presented as median, quartiles and 5th and 95th percentiles.

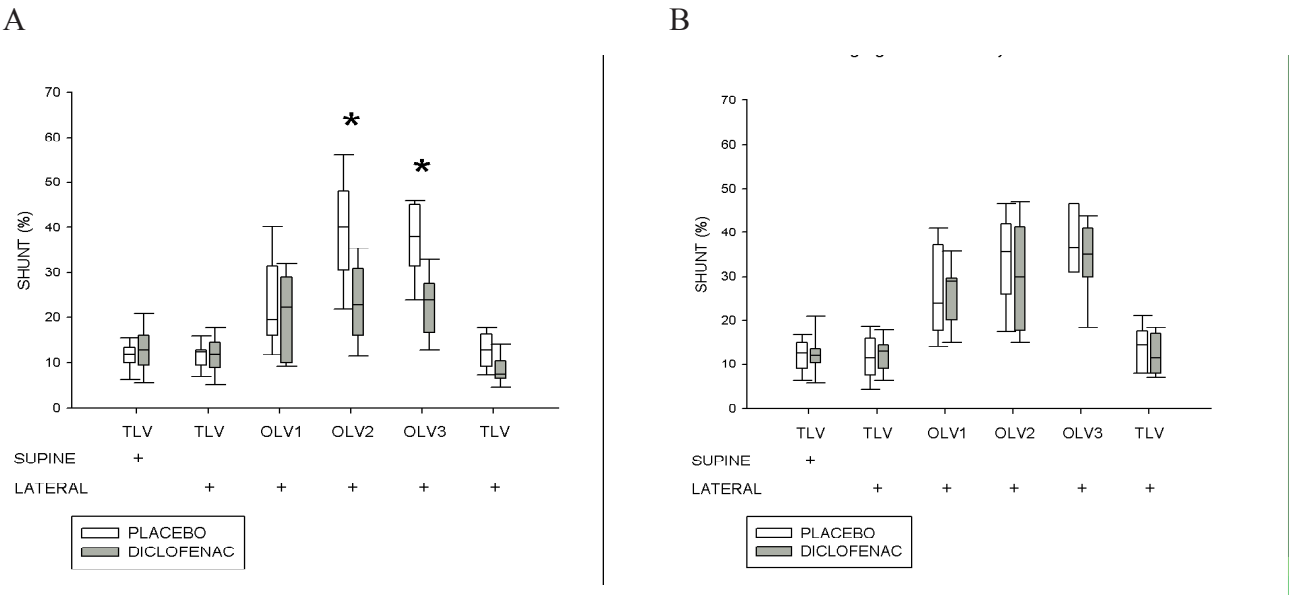


Figure 14 A) Shunt fraction (%) in patients subjected to left-sided thoracic surgery, placebo (unfilled) and diclofenac groups (filled boxes). Measurements were performed after induction of anesthesia (supine position) and in lateral decubitus position during two-lung (TLV) and one-lung ventilation (OLV) and finally after restoration of TLV. Measurements were registered at 15 min intervals. The shunt was significantly larger in the placebo group at OLV2 (P = 0.020) and at OLV3 (P = 0.036); n = 8). Data are presented as median, quartiles and 5th and 95th percentiles.

B) Shunt fraction (%) in patients, subjected to right-sided thoracic surgery, placebo (unfilled) and diclofenac groups (filled boxes). Measurements were performed after induction of anesthesia (supine position) and in lateral decubitus position during two-lung (TLV) and one-lung ventilation (OLV) and finally after restoration of TLV. Measurements were registered at 15 min intervals. The shunt was not significantly different between placebo and diclofenac (P = 0.642) at OLV2 and (P = 0.667) at OLV3; n = 8). Data are presented as median, quartiles and 5th and 95th percentiles.

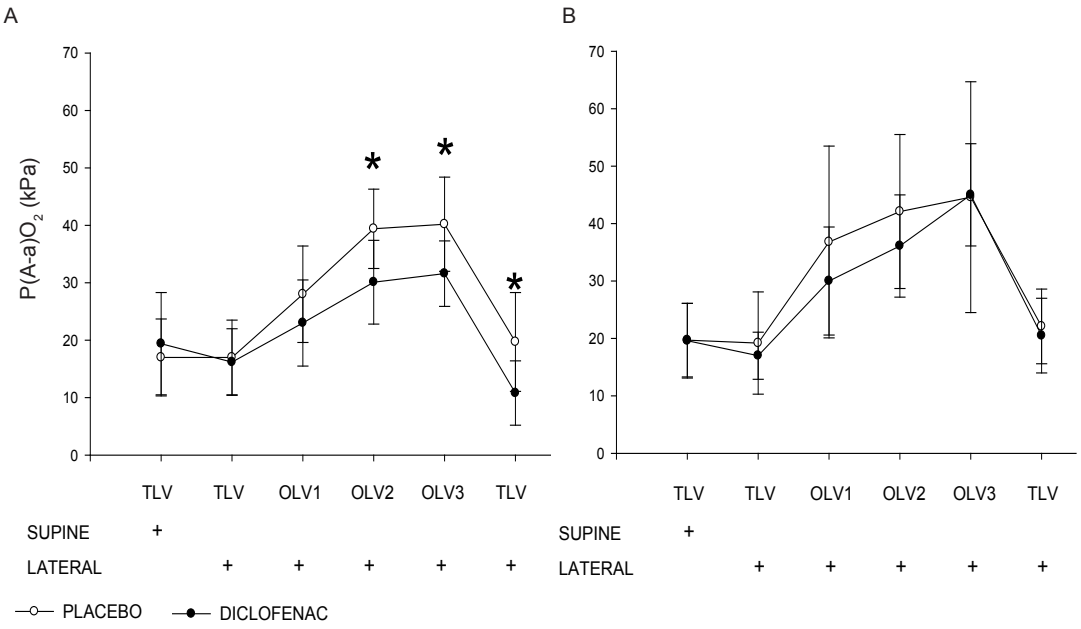


Figure 15. $P(A\text{-}a)O_2$ during left-sided (A) and right-sided (B) thoracotomy in placebo (unfilled symbols) and diclofenac treated patients (filled symbols). Measurements were performed after induction of anaesthesia (supine position) and in lateral decubitus position during two lung (TLV) and one-lung ventilation (OLV) and finally after restoration of TLV. Measurements were registered at 15 min intervals. During left thoracotomy $P(A\text{-}a)O_2$ values were significantly different between placebo and diclofenac treated at OLV2 (P = 0.024), OLV3 (P = 0.027) and after restoration of TLV (P = 0.032; n = 8).

Table 5. Gas exchange data, pooled material

	PvO_2		PaO_2		$PaCO_2$		P (A-a) O_2		PaO_2/FIO_2		% Shunt	
TLV supine		p		p		p		p		p		p
placebo	5.8 (5.4, 6.0)		25.7 (19.4, 33.7)		5.4 (5.2, 5.6)		18.5 (13.0 ,23.8)		49.5 (40.0, 61.2)		12 (10, 14)	
diclofenac	5.8 (5.5, 6.1)	ns	25.2 (18.8 ,34.4)	ns	5.0 (4.5, 5.4)	ns	22.0 (13.0, 24.0)	ns	46.5 (38.0, 61.2)	ns	12 (10, 14)	0.850
lateral												
placebo	5.8 (5.4, 6.5)		24.4 (21.2, 30.7)		5.4 (5.2, 5.8)		19.0 (11.2, 24.5)		45.5 (39.5, 60.2)		12 (9, 14)	
diclofenac	6.0 (5.9, 6.3)	ns	26.3 (21.0 , 34.5)	ns	5.3 (5.0, 5.8)	ns	17.0 (12.0, 19.0)	ns	50.5 (43.0, 62.8)	ns	13 (8, 15)	0.636
OLV 5 min												
placebo	5.5 (5.3, 5.8)		15.6 (10.2, 24.2)		5.6 (5.0, 5.9)		28.0 (24.2, 35.5)		30.0 (16.2 ,41.5)		21 (16, 37)	
diclofenac	5.9 (5.4, 6.3)	ns	15.8 (12.5, 26.6)	ns	5.2 (4.8, 5.4)	ns	27.0 (18.2, 31.5)	ns	29.0 (24.0, 48.2)	ns	25 (14, 30)	0.854
15 min												
placebo	5.0 (4.8, 5.5)		9.0 (8.3, 10.0)		5.3 (4.9, 5.9)		39.0 (31.0, 51.0)		16.0 (12.0, 22.0)		39 (24, 44)	
diclofenac	5.1 (4.9, 5.8)	ns	9.3 (8.5, 22.8)	ns	5.0 (4.8, 5.2)	ns	32.0 (27.0 ,41.0)	ns	17.0 (15.0, 31.0)	ns	28 (16, 36)	0.043
30 min												
placebo	5.0 (4.8, 5.5)		9.3 (8.6, 9.9)		5.4 (5.0, 5.9)		37.0 (34.0, 58.0)		15.0 (12.0, 17.0)		38 (31, 46)	
diclofenac	5.1 (5.0, 6.1)	ns	10.4 (8.4, 19.2)	ns	5.1 (4.7, 5.2)	ns	36.0 (31.0, 48.0)	ns	16.0 (13.0, 35.0)	ns	30 (18, 35)	0.068
after lig.												
placebo	5.0 (4.8, 5.5)		11.2 (9.8, 15.2)		5.4 (4.8, 5.7)		39.0 (30.8, 53.2)		19.0 (17.0, 22.8)		26 (21, 32)	
diclofenac	5.5 (5.1, 5.8)	ns	17.5 (10.7, 23.8)	ns	4.9 (4.6, 5.1)	ns	28.0 (21.8, 39.0)	ns	28.5 (16.8, 42.8)	ns	22 (14, 29)	0.198
TLV lateral												
placebo	5.5 (5.2, 6.0)		25.0 (20.8, 32.4)		5.3 (4.9, 5.7)		23 (14.0, 27.0)		48.0 (38.0, 58.0)		14 (8, 17)	
diclofenac	5.6 (5.4, 6.1)	ns	27.2 (24.2, 31.6)	ns	5.2 (4.8, 5.5)	ns	15.0 (9.2, 21.2)	ns	51.0 (47.2, 64.2)	ns	9 (7, 14)	0.092

Table 6. Hemodynamic data, pooled material

	HR		MAP		CVP		MPAP		CI		% Shunt	
TLV supine		p		p		p		p		p		p
placebo	72 (56, 75)		74 (61, 90)		15 (12, 17)		24 (18, 27)		2.2 (1.8, 2.4)		12 (10, 14)	
diclofenac	62 (56, 73)	ns	66 (60, 81)	ns	16 (12, 18)	ns	24 (19, 26)	ns	2.2 (1.4, 3.2)	ns	12 (10, 14)	0.850
lateral												
placebo	68 (55, 74)		72 (64, 89)		16 (13, 18)		23 (21, 27)		2.3 (2.0, 2.6)		12 (9, 14)	
diclofenac	67 (59, 75)	ns	72 (68, 92)	ns	16 (13, 20)	ns	26 (19, 31)	ns	2.6 (2.1, 3.0)	ns	13 (8, 15)	0.636
OLV 5 min												
placebo	72 (68, 81)		74 (65, 90)		14 (13, 17)		24 (23, 27)		2.4 (2.0, 2.8)		21 (16, 37)	
diclofenac	73 (64, 78)	ns	77 (72, 88)	ns	15 (12, 19)	ns	24 (20, 30)	ns	2.5 (2.1, 2.8)	ns	25 (14, 30)	0.854
15 min												
placebo	73 (66, 80)		70 (62, 78)		17 (14, 18)		26 (23, 27)		2.4 (2.1, 2.6)		39 (24, 44)	
diclofenac	73 (62, 75)	ns	70 (64, 83)	ns	15 (12, 16)	ns	24 (22, 29)	ns	2.6 (2.3, 2.8)	ns	28 (16, 36)	0.043
30 min												
placebo	76 (71, 86)		67 (66, 90)		16 (14, 19)		25 (24, 29)		2.7 (2.1, 3.1)		38 (31, 46)	
diclofenac	70 (65, 82)	ns	82 (64, 95)	ns	15 (13, 16)	ns	25 (21, 29)	ns	2.8 (2.3, 3.1)	ns	30 (18, 35)	0.068
after lig.												
placebo	71 (64, 82)		72 (67, 81)		16 (13, 17)		26 (23, 28)		2.5 (2.2, 2.8)		26 (21, 32)	
diclofenac	69 (61, 82)	ns	86 (78, 92)	ns	15 (13, 16)	ns	26 (21, 30)	ns	2.5 (2.1, 2.9)	ns	22 (14, 29)	0.198
TLV lateral												
placebo	70 (60, 75)		72 (70, 88)		15 (11, 18)		26 (21, 29)		2.6 (2.0, 2.8)		14 (8, 17)	
diclofenac	67 (62, 82)	ns	78 (65, 86)	ns	14 (11, 17)	ns	24 (22, 29)	ns	2.4 (2.1, 2.9)	ns	9 (7, 14)	0.092

Discussion

The highlights of this thesis are that endogenous NO synthesis is involved in regional regulation of pulmonary vascular tone and that NO production is higher in dorsal compared with ventral lung regions in humans. SPECT images in volunteers, before and after NOS inhibition, verify the functional importance of these findings.
The higher degree of NO production in the dorsal parts of the lungs provide the final link in searching for an answer to questions raised by a study 1986, where Beck and Rehder [14] demonstrated higher vascular conductance in dorsocaudal compared to ventrocephaled regions in isolated dog lungs. The results contribute to better understanding of the more uniform perfusion distribution [65, 80, 104, 147, 155] and V/Q ratios [7, 99, 129] with improvement in oxygenation demonstrated when patients with ALI and ARDS are turned into prone position [24, 39, 100].

Are there differences in regional pulmonary vasoreactivity to nitric oxide?

An important determinant for regional distribution of pulmonary blood flow has, during the recent decades, been suggested to be the fractal anatomy of the vascular tree [49, 52, 55, 66, 129]. However, vascular trees are not passive structures. Regional differences in smooth muscle vascular tone may modify the influence of an "intrinsic structure", as we have demonstrated in these series. First, we have shown differences in vasoreactivity between isolated porcine arterial rings from dorsal and ventral pulmonary arteries to the endothelial-dependent vasorelaxants, acethylcholine and bradykinin, acting via nitric oxide release (**paper II**). Second, the mRNA expression of eNOS is higher in dorsal compared with ventral human lung tissue. Ca^{2+}-dependent NOS-activity, as measured by citrulline assay is higher in dorsal lung regions in two different species, humans and pigs. Finally, NOS-inhibition by L-NMMA in vivo redirected blood from dorsal to ventral lung regions in the supine position **(paper I). In paper II**, we could also define the endothelium as the site of interest for the differences in vasoreactivity in the pulmonary arteries, by investigating the effect of an endothelium-independent NO-donor, sodium nitroprusside. Sodium nitroprusside, acting directly on the smooth muscle cell, showed no differences in vasorelaxing potency between dorsal and ventral porcine arterial rings, in contrast to the endothelium-dependent vasodilator acetylcholine.
Our findings are consistent with earlier *in vitro* studies. Beck and Rehder demonstrated in 1986 a higher vascular conductance in dorsocaudal than in ventrocephalad regions of isolated perfused dog lungs [14]. Later, Pelletier et al. showed a more pronounced endothelium-dependent vascular relaxation in dorsal compared to ventral isolated equine pulmonary arterial rings to a number of substances acting via the cGMP / NO pathway [111].
Whether the increased NOS activity in dorsal lung regions, found in our study, is a primary or secondary phenomenon cannot be fully answered by this study. NO production *in vivo* is regulated by different factors. Shear stress caused by high blood flow may result in enhanced NO synthesis in dorsal lung regions [120]. This might indicate that the observed higher Ca^{2+}-dependent NOS activity described in this study is a secondary phenomenon and not the cause of higher blood flow in dorsal regions in the supine position. NOS activity was analyzed *in vitro* under standardized conditions, for example, equal amounts of enzyme substrate, L-arginine, indicating that constitutional enzyme activity was higher in dorsal lung regions. The NOS activity analysed in our study was not influenced by shear stress because the analysis was made *in vitro*, not in blood vessels *in vivo*. Ca^{2+}-independent, inducible NOS activity did not differ between ventral and dorsal tissue samples. Furthermore, expression of mRNA for eNOS was higher in dorsal regions. Taken together, our

results strongly suggest a difference in eNOS mRNA expression and Ca^{2+}-dependent NOS activity under basal conditions, leading to differences in vasoreactivity in dorsal and ventral lung regions. To summarise, the findings in paper I and II add evidence to a regulatory role for nitric oxide in regulation of regional pulmonary blood flow distribution.

Can differences in regional pulmonary vasoreactivity play a role in regional lung blood flow distribution with reference to posture?

A dominant dorsal lung perfusion in the supine position [8, 77] and more uniform perfusion in the prone position has been shown in previous studies in humans and animals during spontaneous ventilation [65, 101, 104, 155]. These earlier findings were obtained with the use of various techniques such as radiospirometry [13] and SPECT in humans [108] and microspheres in animals [65, 155]. The agreement between different species using a variety of techniques indicate a true description of physiologic conditions, which is further strengthened in this study. It should be kept in mind that the present SPECT method does not measure perfusion per se but the image produced by radioactive microspheres trapped in capillaries. Also, it is important to keep in mind the confounding effects by the compressible nature of lung parenchyma when evaluating perfusion redistribution caused by changes in posture. Normalisation for perfusion / alveolus or density instead of unit volume must be considered [118, 123]. The tracer technique is, on the other hand, well established [13, 92] and allows for relative comparisons, as conditions are stable between the acquisitions. NOS inhibition by L-NMMA in the supine position redirected blood from dorsal to ventral lung regions (Fig.7, Fig. 8). Most probably this is caused by a relatively larger change in the vascular tone of dorsal pulmonary vessels as compared with ventral vessels while supine. However, it is surprising that NOS inhibition while prone did not result in a parallel redirection of pulmonary blood flow. Hence, body position may affect the response to L-NMMA. It is not likely that this is due to technical factors concerned with the SPECT technique or the infusion of the NOS inhibitor.

This observation may invoke the assumption that the mechanical change, supine to prone, causes a different stretching of the pulmonary vasculature that changes the response to L-NMMA. Increased exhalation of NO during ventilation with positive end expiratory pressure in rabbits has been suggested to be caused by a stretch-dependent regulation of pulmonary NO formation in animals [1, 116]. The greater compression of dependent lung regions in supine [4, 124] results in regional hypoxia [7] which may induce HPV in dorsal regions. The higher NO production in dorsocaudal regions, found in this study, will attenuate HPV [46]. Hence, inhibition of NO production by L-NMMA would enhance HPV leading to redistribution of pulmonary blood flow. A weaker regional HPV due to less tissue compression in prone position may explain the lack of effect by L-NMMA in prone posture. However, the difference in response to NOS inhibition between supine and prone position could not be fully explained in this study, and further investigations need to confirm and clarify this point.

The increased vascular conductance in dorsal lung regions, demonstrated by Beck and Rehder, could be explained by anatomical differences such as number of vessels and branching differences. Fractal vascular trees and perfusion heterogeneity in iso-gravitational planes suggest an anatomic structural basis for regulation of regional pulmonary blood flow [49, 55, 66, 129]. The Seattle group [7] demonstrated in 2004, by in vitro microsphere technique in anesthetized, mechanically ventilated pigs, that pulmonary structure was responsible for around 65% of total blood flow heterogeneity and around 75% of total ventilation heterogeneity. There was a posture-mediated relatively small redistribution of blood flow primarily oriented along the ventrodorsal axis, with increased blood flow to the dependent regions, more pronounced in the supine position. Hence, gravity contributes to regulation of regional pulmonary perfusion, but has to be considered as a secondary determinant [51, 53].

However, these observations do not exclude differences in regional pulmonary vasoreactivity to nitric oxide, found in this thesis, as an important contributor to the increased vascular conductance in dorsal lung regions and more uniform pulmonary perfusion distribution seen in the prone position.

Are there differences in regional pulmonary vasoreactivity to prostacyclin?

Regional pulmonary vasoregulatory differences could include other signal systems than those mediated by NO / guanylylcyclase / cGMP pathway **(paper I, II).** The prostaglandin system, for instance, involves substances acting via the adenylyl cyclase / cAMP pathway. Inhalation of the synthetic prostacyclin analogue iloprost has been shown to have selective pulmonary vasorelaxing effects [12, 105, 107]. In this thesis, we could not confirm any regional differences in effects on regional pulmonary basal vascular tone to an exogenous prostacyclin analogue. Iloprost inhalation in both supine and prone positions increased perfusion in dependent lung regions, i.e., enhanced the effect of gravity (Fig.12). Non-selective COX-inhibition did not redistribute blood flow in either prone or supine position, which further supported the suggestion that the eicosanoid system is not involved in regulation of regional pulmonary perfusion in normal lungs (Fig.10). However, the limited number of observations cannot exclude an action by endogenous eicosanoids.
The observed effects in this study might be explained by an uneven gravity dependent drug distribution. The iloprost particle size used in the present study, should guarantee a drug deposition in peripheral airways [135, 150]. Ventilation distribution was also studied in a subset of the volunteers in supine position. Iloprost, which is known yo have bronchorelaxant effects [143], redistributed ventilation towards ventral, that is, non-dependent lung regions. This argues against that an uneven, preferentially dependent distribution of inhaled iloprost would explain the perfusion redistribution towards dependent lung regions in both supine and prone positions.
Surprisingly, iloprost inhalation in these healthy volunteers caused a decrease in arterial oxygenation in both the supine and prone position (Fig.11). The PBF redistribution towards dependent regions regardless of body position, and the gas redistribution in opposite direction in supine position, contributed to increased V/Q mismatch with impaired arterial oxygenation. Other possible mechanisms behind the changed oxygenation might be altered cardiac output by systemic drug effects. However, the administration method and dose of iloprost presently used has been shown to selectively dilate pulmonary vasculature [105, 107]. Although heart rate and blood pressure increased during the iloprost inhalation, they were completely normalized when pulmonary perfusion distribution was measured by SPECT and at the same time PaO_2 was diminished. Furthermore, the fact that the concentration of 6-ketoprostaglandinF1α was not altered at the time the radioactive tracer was injected and trapped in the pulmonary capillaries strongly indicates that the effect by iloprost on pulmonary perfusion distribution was mediated via local receptors in the lungs and not indirectly via systemic effects.

Vasoactive drugs, hypoxic pulmonary vasoconstriction and regional pulmonary perfusion.

The effects of iloprost inhalation in healthy humans are not consistent with the findings in several clinical studies where patients with ALI and ARDS have been treated with inhalations of prostacyclin or its analogues and have shown improved oxygenation [35, 153]. In experimental lung injury models enhanced pulmonary vascular resistance has been demonstrated due to either damaged endothelial function or hypoxic vasoconstriction or a combination of the two [33], which might explain the favourable effects of prostacyclin treatment in severe lung disease. These observations confirm that underlying pathophysiology, the clinical conditions or the presence of positive pressure ventilation with concomitant effects on pre-existing vascular tone are of crucial importance for the effects of vasoactive substances [58].

In the present studies, COX-inhibition on healthy humans **(paper III)** did not influence regional blood flow distribution, while in the hypoxic situation **(paper IV)** during one lung ventilation for thoracic surgery, there was a marked reduction of shunt fraction due to redistributed blood flow after administration of a non-selective COX-inhibitor during left-sided thoracic surgery. Other factors like anesthetic agents [15, 16, 20, 78, 79, 89, 109, 126], cardiac output [42], hypercapnia [38, 43] and pulmonary blood flow [37] known to interact with HPV and shunt fraction were similar in the placebo and diclofenac groups. These factors were therefore less likely to be responsible for the observed differences between the groups.

It is well documented in animal and human studies that HPV is modulated by endothelial derived vasoactive substances [46, 138]. When lung pathology and hypoxia is at hand, the effect of COX-inhibition might be different compared to normal physiological conditions [35, 58, 69, 105, 153, 165]. During one-lung ventilation the low alveolar oxygen tension activates HPV in the non-ventilated lung with a redistribution of 40-50 % of the blood flow to the ventilated lung [18]. Our findings in humans are consistent with many in vitro and in vivo animal studies, showing decreased shunt fraction and improved arterial oxygenation after administration of non-selective COX-inhibitors when regional hypoxia is induced [6, 59, 138].

Interestingly, there was a posture related difference in the response to prostaglandin-inhibition during one lung ventilation in the lateral decubitus position. Alterations of lung perfusion are also known to be influenced by changes in body position [7, 30, 104, 119, 129]. However, the smaller effect on the shunt fraction by COX inhibition in patients with right-sided surgery was interesting and difficult to explain. In the lateral decubitus position the dependent lung has a reduced lung volume due to anesthesia, muscular paralysis and compression by the mediastinal and abdominal contents [103], all of which contribute to atelectasis [145] and a decrease in compliance [82]. Hence, both pulmonary perfusion and ventilation will be compromised, more marked when the smaller left lung is dependent [30]. This leads to a lower regional Po_2 and therefore a higher grade of HPV in the ventilated lung when the left lung is dependent [30], which might counteract the blood flow redistribution from the non-ventilated lung. In addition, HPV is more effective in diverting blood flow and reducing hypoxemia when the hypoxic region is a small fraction of the total lung [88]. Thus, it is possible that the effect of COX inhibition on blood flow redistribution due to HPV is more effective when the larger right lung is dependent. Hence, body position cannot be ruled out as contributory factor to the observed difference in effect by COX-inhibition between left and right thoracotomy.

What are the clinical implications of the findings in this thesis?

It is of great importance to understand the causative mechanisms to impaired gas exchange in patients with lung pathology to be able to find new treatment strategies. There exists an extensive amount of research concerning the many passive factors determining PBF distribution like gravity and posture [119, 125, 162], vascular structure [49, 52, 55, 66] and lung mechanics [103, 114]. Some of these factors can be used in clinic practice by changing body posture and by optimizing ventilation characteristics.

In this thesis we have investigated the role of the vasoactive endothelial-derived factors, NO and prostacyclin, in regulation of regional PBF during normal and hypoxic conditions. The findings in the present thesis, that there are differences in vasoreactivity between dorsal and ventral pulmonary regions due to higher endothelial production of NO in dorsal pulmonary arteries might have clinical implications. Selective inhibition of eNOS activity in order to redistribute PBF from the dependent dorsal regions in the supine position may become a valuable tool in the treatment of patients with ALI or ARDS. The blood flow redistribution may lead to a more homogeneous ventral-to-dorsal perfusion distribution which in turn may improve arterial oxygenation due to optimised ventilation/perfusion matching.

The findings in paper IV that the use of a non-selective COX-inhibitor during one-lung ventilation decreases shunt during left-sided thoracic surgery has interesting clinical implications. First, the patients subjected to thoracic surgery are often elderly with inherent lung diseases and thus might benefit from preoperative COX-inhibition to reduce the risks for severe perioperative hypoxia during one-lung ventilation. Especially in minimally invasive thoracic procedures, requiring a well-collapsed lung, where CPAP to the non-ventilated lung is inapplicable, COX-inhibition may be advantageous [22, 26]. Second, non-selective COX-inhibition may offer an easy way to minimize the exposure to high F_IO_2, minimizing absorption atelectasis [41] and decreasing the risk of oxygen toxicity and inflammatory activation in the ventilated lung [74, 83, 122].

Conclusions

Based on the results in this thesis it was concluded that:

- expression of mRNA for endothelial nitric oxide synthase (eNOS) in humans is higher in dorsal than ventral lung regions (**I**).
- Ca^{2+}-dependent NOS activity is higher in dorsal compared with ventral human lung regions (**I**).
- in pigs Ca^{2+}-dependent NOS activity is higher in dorsal than in ventral lung regions (**II).**
- relaxation of porcine pulmonary arteries by endothelium dependent vasoactive mediators such as acetylcholine and bradykinin is more potent in dorsal than in ventral arteries (**II**).
- the endothelium independent NO donor sodium nitroprusside relaxes, in pigs, ventral and dorsal pulmonary arteries equally (**II).**
- in supine volunteers, pulmonary blood flow is redirected from dorsal to ventral lung regions during NOS inhibition (**I).**
- iloprost inhalation redistributes pulmonary blood flow to dependent lung regions regardless of posture while ventilation is redirected towards non-dependent lung regions in supine position leading to decreased arterial oxygen tension in healthy humans (**III).**
- non-selective inhibition of cyclooxygenase (COX) by diclofenac does not affect pulmonary perfusion distribution in healthy humans (**III**)
- non-selective inhibition of cyclooxygenase (COX) by diclofenac reduces shunt fraction during one-lung ventilation for left sided thoracic surgery, accompanied with improved gas exchange, showed by a decrease in alveolo – arterial oxygen tension difference. (**IV)**.

General Conclusions

Based on the results in this thesis it was concluded that there exist differences in pulmonary vasoreactivity between ventral and dorsal lung regions for the endothelial derived mediator nitric oxide, at different levels such as gene transcription and enzyme activity, adding new knowledge contributing to logical explanations to variations in gas exchange between supine and prone positions.
The eicosanoid system is involved in regional perfusion distribution during regional hypoxia.

This knowledge may improve treatment strategies for patients with acute lung injury and acute respiratory distress syndrome and for patients subjected to one-lung ventilation during thoracic surgery.

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