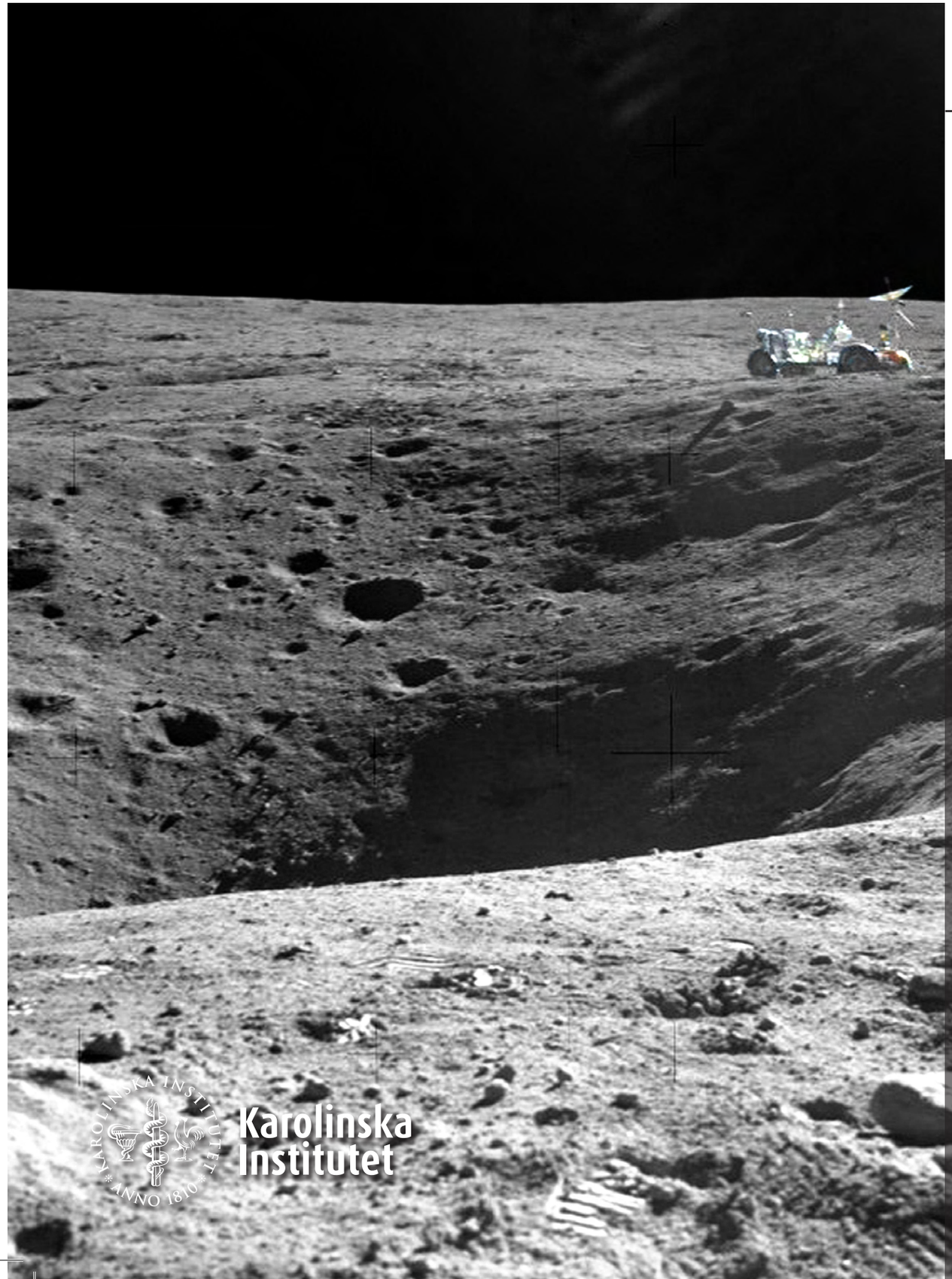

Thesis for doctoral degree (Ph.D.) 2009

Nitric oxide and the lung: effects of spaceflight and hypergravity

Lars Karlsson

Karolinska Institutet

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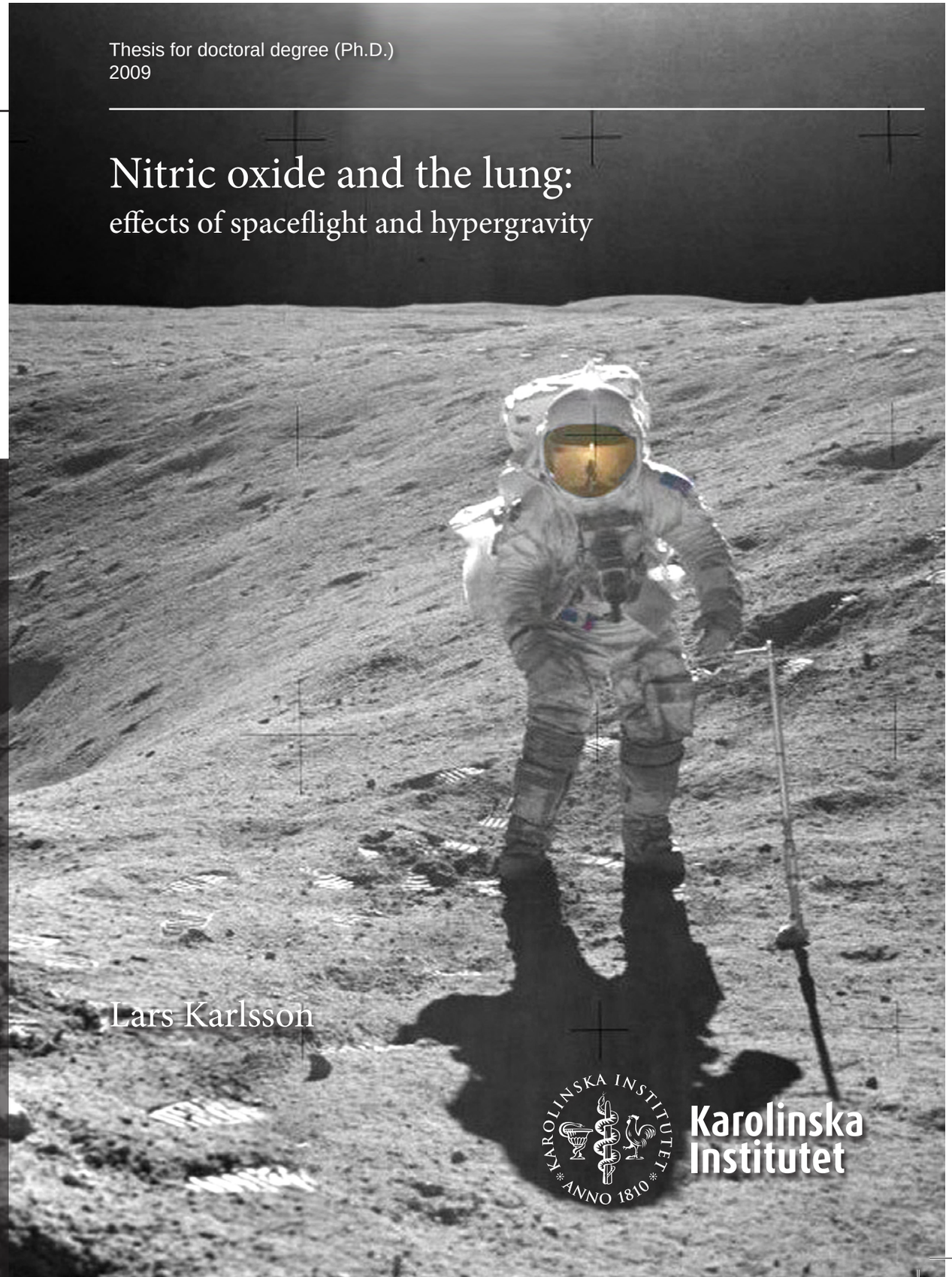

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Cover photo: April 21, 1972. Astronaut Charles M. Duke Jr., Lunar Module pilot of the Apollo 16 mission, is photographed collecting lunar samples during the first Apollo 16 extravehicular activity. The parked Lunar Roving Vehicle can be seen in background. Note that the spacesuit is covered with moondust that may give rise to toxic and/or inflammatory reactions in the airways if inhaled! ©NASA

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Till syster och mor

“Falling in love is not at all the most stupid thing that people do –
but gravitation cannot be held responsible for it. “

Albert Einstein, 1933

ABSTRACT

Nitric oxide (NO) is an important signal molecule in the body, in particular in the cardiovascular system. This highly reactive molecule is difficult to detect in tissues, but in gas-filled cavities such as the airways it can be detected in part per billion amounts. This thesis explores the possible use of NO to monitor or modify lung function when healthy subjects are exposed to reduced and increased gravity, and to reduced ambient pressures.

In the first part of this thesis, the Russian procedure for extravehicular activities (EVA) is studied during ground simulations and aboard the international space station. EVA includes decompression and it was concluded that weightlessness appears to be protective against decompression-related disorders. Therefore, the hypothesis that exhaled NO could be used to detect decompression bubbles in the lung circulation could not be substantiated. It was also concluded that lowered ambient pressure reduces the normal level of exhaled nitric oxide; this is important knowledge if exhaled NO is to be used as a measure of lung health.

In the second and third parts of this thesis the influence of gravity-induced alterations of the distributions of blood, gas and tissue in the lungs on exhaled nitric oxide, was assessed. By exposing healthy subjects to hypergravity and microgravity (weightlessness), it was concluded that hypergravity-induced impaired matching of blood and gas in the lungs slows blood uptake of locally produced nitric oxide, resulting in increased levels of exhaled nitric oxide. Also lung deformation in hypergravity decreases blood uptake and hence increases exhaled levels. In support of the above, it was found that improved matching in microgravity decreases exhaled levels of exhaled nitric oxide. Additionally, in the use of experimental hypergravity data and mathematical simulations, it can be expressed in quantitative terms how the increased levels of exhaled nitric oxide found in hypergravity were caused by decreased contact surface between gas and blood and by narrowing of small peripheral airways.

Exposure of the healthy lungs to hypergravity can simulate key components of acute respiratory distress syndrome, a severe type of lung insufficiency. In the last part of this thesis, the role of the hypoxic pulmonary vasoconstriction in these disorders was assessed by means of hypergravity-induced hypoxemia and pharmacological interventions. No protective role of the vasoconstriction could be seen on hypergravity-induced hypoxia in five times normal gravity. However, recent preliminary data from a follow-up study suggest a protective role at lower hypergravity levels when the lung deformation is less pronounced.

LIST OF PUBLICATIONS

The thesis is based on the following articles, which are referred to in the text by their roman numerals:

I. **Karlsson LL**, Blogg SL, Lindholm P, Gennser M, Hemmingsson T, and Linnarsson D. (2009).
Venous gas emboli and exhaled nitric oxide with simulated and actual extravehicular activity.
Respir Physiol Neurobiol **169S**, S59-62.

II. **Karlsson LL**, Kerckx Y, Gustafsson LE, Hemmingsson TE, and Linnarsson D. (2009)
Microgravity decreases and hypergravity increases exhaled nitric oxide.
J Appl Physiol **107,** 1431-1437.

III. Kerckx Y, **Karlsson LL**, Linnarsson D, and Van Muylem A.
Effect of hypergravity on exhaled and alveolar nitric oxide concentration: a theoretical study.
Submitted, Oct 2009

IV. **Karlsson LL**, Nekludov M, Petersson J, Ax M, Mure M, Linnarsson D, and Rohdin M.
No Protective Role for Hypoxic Pulmonary Vasoconstriction in Severe Hypergravity-Induced Transient Lung Insufficiency.
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ALI	Acute lung injury
ANOVA	Analysis of variance
ARDS	Acute respiratory distress syndrome
BC	Bronchial constriction
Ca^{2+}	Calcium ion
$C_{alv_{NO}}$	Alveolar (acinar airway) nitric oxide concentration
Caw_{NO}	Conductive airway nitric oxide concentration
cGMP	Cyclic guanosine monophosphate
CO	Carbon monoxide
CO_2	Carbon dioxide
DA_{NO}	Alveolar nitric oxide diffusing capacity
Daw_{NO}	Airway nitric oxide diffusing capacity
DCI	Decompression illness
DCS	Decompression sickness
D_L	Lung diffusing capacity
DL_{CO}	Lung diffusing capacity for carbon monoxide
DL_{NO}	Lung diffusing capacity for nitric oxide
Dm_{CO}	Membrane component of lung diffusing capacity for CO
Dm_{NO}	Membrane component of lung diffusing capacity for NO
ECG	Electrocardiogram
eNOS	Endothelial nitric oxide synthase
ESA	European Space Agency
EVA	Extravehicular activity, space walk
FE_{NO}	Fraction of exhaled nitric oxide
FRC	Functional residual capacity
G	Gravity level
HAPE	High-altitude pulmonary oedema
HPV	Hypoxic pulmonary vasoconstriction
HR	Heart rate
iNOS	Inducible nitric oxide synthase
ISS	International Space Station
$J'aw_{NO}$	Conductive airway nitric oxide production
MPP	Mouthpiece pressure
NASA	National Aeronautics and Space Administration
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
PE_{CO_2}	Partial pressure of exhaled carbon dioxide
PE_{NO}	Partial pressure of expired nitric oxide
ppb	Parts per billion
$\dot{Q}$	Perfusion
SD	Standard deviation
SpO_2	(Arterial) Haemoglobin oxygen saturation measured with pulse oximetry
TLC	Total lung capacity
$\dot{V}$	Ventilation
$\dot{V}A_{NO}$	Alveolar nitric oxide production
V_c	Capillary blood volume
VC	Vital capacity
VGE	Venous gas emboli
µ G	Microgravity, weightlessness
1 G	Normal gravity
x G	x times normal gravity

Pressure conversion: 101,3 kPa = 1013 hPa = 1,013 bar = 760 mmHg (Torr) = 1 ata

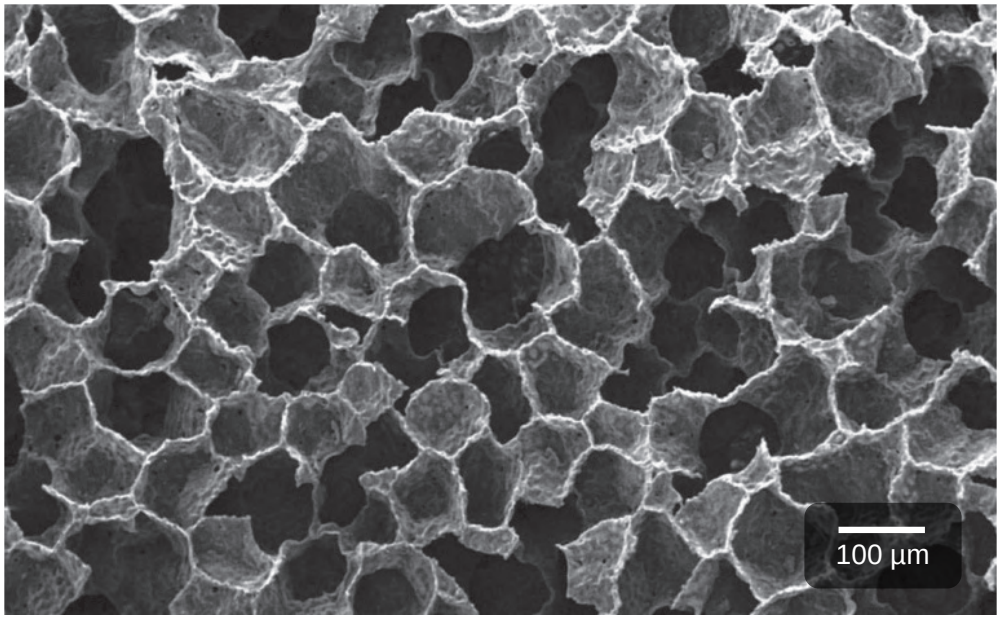


Figure 1. Structure of human alveoli (personal communication Ewald R. Weibel)

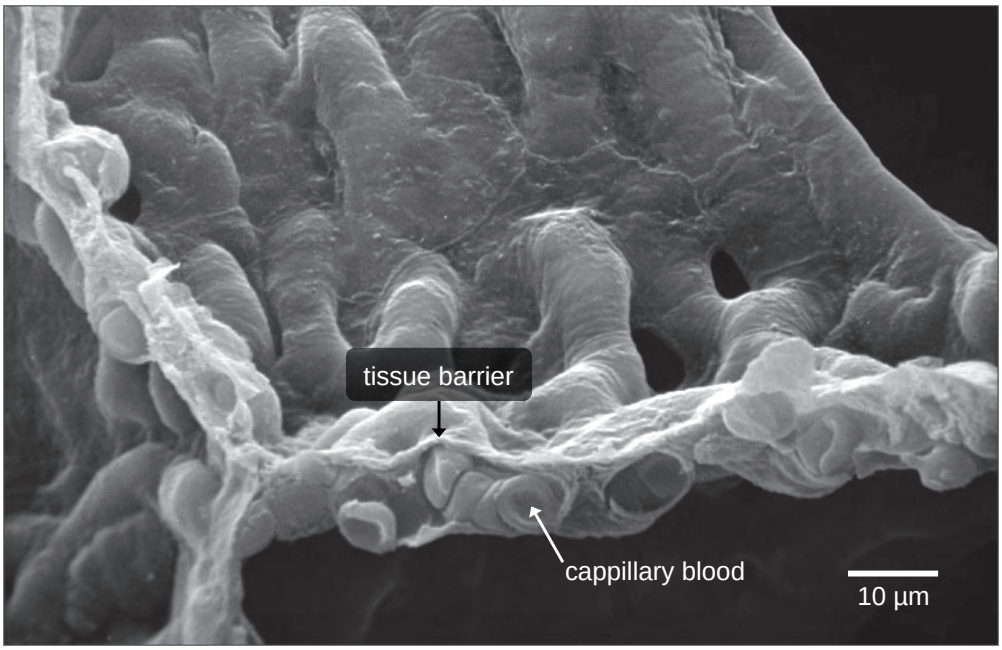


Figure 2. A scanning electron micrograph of the fine structure of the alveolar septum in human lungs. Note the thin tissue barrier (see marker) separating the blood cells from the air. Erythrocytes (see capillary blood marker) have a diameter of 6-8 µm and thickness of 2 µm, whereas the lung capillaries have inner diameters of down to 1.5 µm. Diffusion distance from blood-to-gas is around 1 µm (Weibel, 1963; Weibel *et al.*, 2005).

1 INTRODUCTION

Historically, there has been much musing and theorising about breathing. In the Bible it is stated that God "breathed into Adam's nostrils the breath of life" and then later used Adam's rib (a part of the ventilatory apparatus) to give life to Eve. By the fourth and fifth centuries, the writings of Hippocrates suggested that breathing occurred to cool the heart. Now we know better, but for thousands of years breathing has been synonymous to life.

The lung anatomy is extremely complex and optimized for the vital exchange of gases between blood and alveoli. Up to 450 million alveoli in the lungs create a gas-to-blood interface covering an area of 130 square meters with a diffusion distance from blood-to-gas of around 1 μm (Weibel *et al.*, 2005) (Fig. 1 & 2). The distribution of gas and blood in the lungs are delicately regulated and matched by various mechanisms to further enhance the gas exchange.

In several disorders such as airway inflammation, pulmonary embolism, acute lung injury, and acute respiratory distress syndrome, the essential matching of gas and blood in the lungs is impaired. In this thesis the absence of gravity during spaceflight and the increased gravity during centrifugation in a human centrifuge is used to learn more about the different mechanisms and functions of nitric oxide and the lungs in the described disorders.

2 BACKGROUND

2.1 NITRIC OXIDE IN THE LUNGS

For more than a century, nitroglycerine has been used as a vasodilator (Marsh & Marsh, 2000). In the human body nitroglycerine is converted to nitric oxide (NO) by mitochondrial aldehyde dehydrogenase. In 1992 NO was declared molecule of the year by Science magazine when it had been shown to be formed endogenously as the endothelium-derived relaxing factor. In 1998, Robert F Furchgott, Louis J Ignarro and Ferid Murad were awarded The Nobel Prize in Physiology or Medicine for their discoveries concerning "Nitric oxide as a signalling molecule in the cardiovascular system".

Nitric oxide is a small, short-lived (converted into nitrate and nitrite within seconds), endogenously produced gas molecule with many functions in the human body. It is:

- a signalling molecule in the cardiovascular system
- a signalling molecule in the nervous system
- involved in the natural defence against bacterial and parasitic infections

NO is produced from the amino acid L-arginine and oxygen by nitric oxide synthase (NOS). There are three different known isoforms of NOS; two constitutive and one inducible (iNOS). The constitutive NOS, i.e., neuronal NOS (nNOS) and endothelial NOS (eNOS), are strictly Ca^{2+}-dependent, whereas iNOS is dependent on gene expression regulation.

Enzyme	Location	Function
Neural NOS (nNOS)	Nervous tissue Skeletal muscle	Cell communication
Endothelial NOS (eNOS)	Endothelium	Vasodilatation
Inducible NOS (iNOS)	Immune system Cardiovascular system	Immune defence against pathogens

The potential of NO as a marker of airway disease became apparent when Gustafsson *et al.* (1991) found endogenous NO in the exhalate from both animals and humans, and Alving *et al.* (1993) and Persson *et al.* (1994) found increased levels of exhaled NO in asthmatics with ongoing airway inflammation. Nitric oxide is much more stable in the gas phase than in water (Schedin *et al.*, 1999) and normal exhaled NO levels are in the range of 10–35 parts per billion (ppb) in adults, and around 5–25 in children (Taylor *et al.*, 2006). For asthma patients not adequately treated with anti-inflammatory medication, exhaled NO levels are up to 70–100 ppb and sometimes even higher. Exhaled NO is now used as a diagnostic tool in the monitoring of asthma patients.

Potential sources of exhaled NO include the nasal epithelium (Lundberg *et al.*, 1995a), the airway epithelium (Asano *et al.*, 1994), the alveolar epithelium (Asano *et al.*, 1994), the vascular endothelium (Ignarro *et al.*, 1987), and the blood (Pawloski *et al.*, 2001). NO enters the airway lumen by gas diffusion driven by a concentration gradient. All three isoforms of the NOS enzymes are present in the lung (Ricciardolo *et al.*, 2004), but normal levels of exhaled NO match only the production quantity from iNOS activation (Ialenti *et al.*, 1993; Lane *et al.*, 2004).

The normal physiological role of NO in the lungs has not been completely established but several mechanisms have been proposed:

a) NO may contribute to the balance between vasodilatory and vasoconstrictive agents in the pulmonary vascular bed as it does in the systemic circulation (Ignarro *et al.*, 1987; Persson *et al.*, 1990; Ricciardolo *et al.*, 2004). Such a mechanism is employed when treating patients with pulmonary hypertension with inhaled NO (Frostell *et al.*, 1991; Pepke-Zaba *et al.*, 1991; Frostell *et al.*, 1993). Since NO influences the tone of vascular smooth muscle by means of increasing cyclic guanosine monophosphate (cGMP, Fig. 3, Furchgott & Zawadzki, 1980; Ignarro *et al.*, 1987; Ricciardolo *et al.*, 2004), pulmonary hypertension can also be treated with phosphodiesterase inhibitors such as sildenafil, which suppress the enzymatic elimination of cGMP (Zhao *et al.*, 2001; Kleinsasser & Loeckinger, 2002; Ghofrani *et al.*, 2004; Fesler *et al.*, 2006).

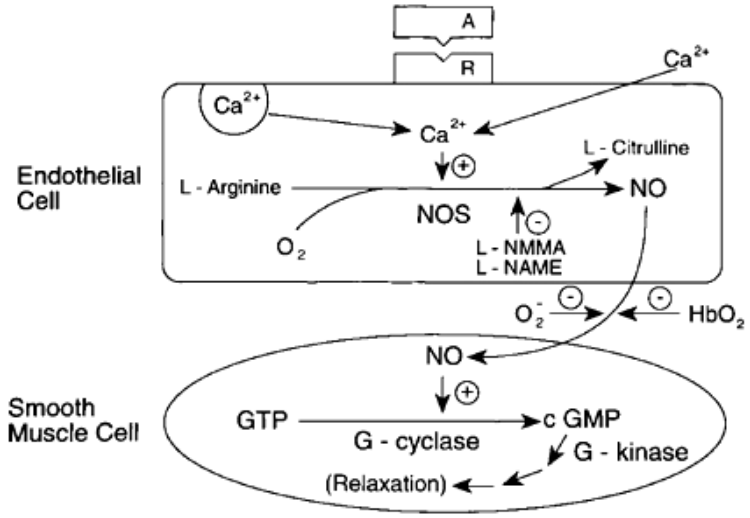
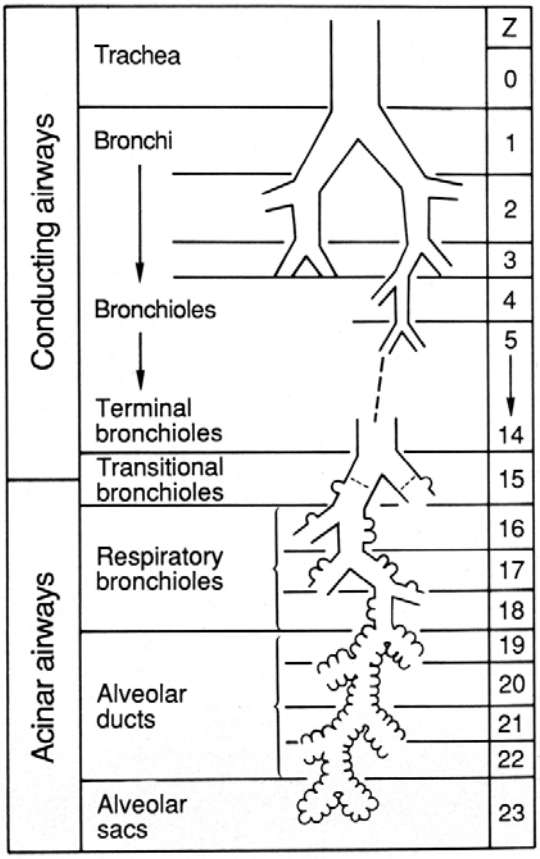


Figure 3. Scheme for endothelium-dependent relaxation. Agent A, acting on receptor (R) of an endothelial cell activates Ca^{2+} influx, with the consequent increase in intracellular Ca^{2+} activating the production of the endothelial nitric oxide synthase (NOS) via calmodulin. NOS is an oxygenase that uses L-arginine and NADPH as co-substrates. NO then diffuses to the smooth muscle cells where it activates guanylyl cyclase, with a resulting increase in cGMP that initiates processes leading to relaxation. L-NMMA and L-NAME are arginine derivatives which inhibit NOS, and O2- and HbO2 are potent scavengers of NO. (Figure. Robert F Furchgott, www.downstate.edu/pharmacology/faculty/furchgott.html)

b) It has been speculated that the physiologically low levels of NO in the air spaces of the lungs can also modify local vascular resistance. Thus Lundberg *et al*. (1995b) have proposed that NO from the upper airways acts via an "aerocrine" mechanism to match perfusion to the lung parts with the best ventilation. Furthermore Strömberg *et al*. (1997) have shown that lung distension causes NO release in the lungs, which also is a potential mechanism for improving the matching between ventilation and perfusion.



The respiratory tract is divided into the upper (sinuses, nasal cavity and pharynx) and lower respiratory tract. Throughout this thesis, the experimental procedures have been designed to minimize the influence of the upper respiratory tract on exhaled NO and hence the focus is on the contribution from the lungs. The lower respiratory tract (the lungs with its airways) consist of conductive airways with mainly convective flow (generation 0 through 15–17) with no respiratory gas exchange and acinar airways with molecular diffusion (generation 16–18 and beyond) with respiratory gas exchange (Weibel *et al.*, 2005) (Fig. 4).

Figure 4. Model of human airway system with symmetric branching from trachea (generation 0) to acinar airways (generations 15-23), ending in alveolar sacs (Weibel *et al.*, 2005).

NO is formed in all lung tissues but the exact origin of the exhaled NO has not been finally established. The source of exhaled NO is clearly the lung with its airways (Gustafsson *et al.*, 1991). Initial studies showed that a majority of the exhaled NO originates from the airways, likely the airway mucosa (Persson *et al.*, 1993). The role of the alveoli and the diffusion in airways and alveoli is more difficult to investigate, and requires flow-dependent measurements and modelling A mathematical two-compartment lung model has been proposed by Tsoukias & George (1998), and further developed by several research groups (Pietropaoli *et al.*, 1999; Hogman *et al.*, 2000; Silkoff *et al.*, 2000; Van Muylem *et al.*, 2003; Condorelli *et al.*, 2007; Kerckx *et al.*, 2008). All proposed models comprise a non-expansile conducting airway compartment, and an expansile acinar airway compartment. The models proposed by Van Muylem *et al.* (2003), Condorelli *et al.* (2007), and by Kerckx *et al.* (2008) include the concept of axial "back-diffusion", i.e., NO from the conductive airways travel by molecular

diffusion to the alveoli (Fig. 5, panel A: Tsoukias & George, Pietropaoli *et al.*, Högman *et al.*, Silkoff *et al.*, and B: Van Muylem *et al.*, Condorelli *et* al., Kerckx *et al.*).

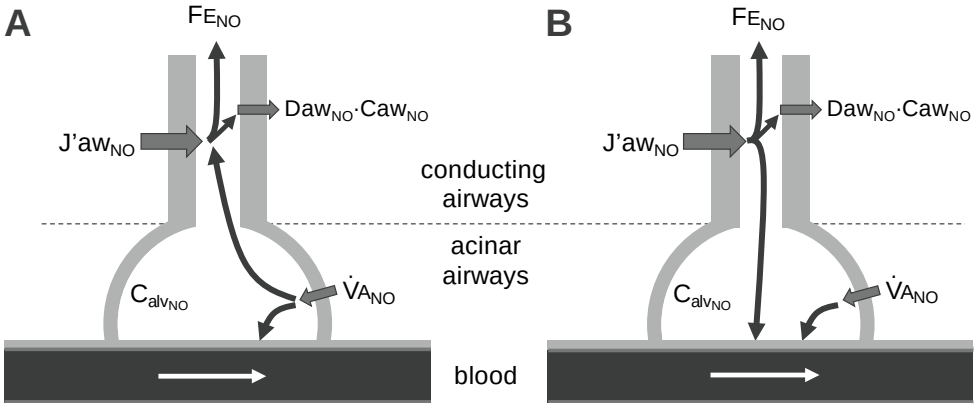


Figure 5. Two-compartment model of the human lung. Panel A: (Tsoukias & George, 1998; Pietropaoli *et al.*, 1999; Hogman *et al.*, 2000; Silkoff *et al.*, 2000), Panel B: (Van Muylem *et al.*, 2003; Condorelli *et al.*, 2007; Kerckx *et al.*, 2008). $C_{alv_{NO}}$, acinar airway NO concentration, $J'aw_{NO}$, conductive airway NO production, $\dot{V}A_{NO}$, acinar airway NO production, Daw_{NO}, conducting airway compartment diffusing capacity of NO, Caw_{NO}, conducting airway NO concentration, FE_{NO}, fraction exhaled NO.

2.2 EXTRAVEHICULAR ACTIVITY AND DECOMPRESSION

2.2.1 EVA procedures

Ever since the Russian cosmonaut Alexei Leonov performed the first space walk (extravehicular activity, EVA) in 1965, EVAs have been an essential activity in space operations. Space walks have been performed both on the moon, from orbital Russian and US space vehicles and from the International Space Station (ISS). In future space operations, including moon and Mars missions, EVAs will be even more important. The environment in space is hostile with dramatic temperature variations, potentially harmful radiation intensities and a near to vacuum ambient pressure. These conditions necessitate that people working outside a space vehicle have to wear special protection, i.e., EVA suits.

The EVA suit is essentially an independent space vehicle with its own life support system. All generations of EVA suits in both the Russian and the US space programs have been equipped with flexible parts covering the extremities to allow movement during, for example, the building of structures on the ISS or walks on the lunar surface. In order to allow flexibility of the joints and in particular those of the hands, the pressure in space suits is substantially lower than the pressure in current space vehicles, including the ISS. The choice of suit pressure is a trade-off between safety (high suit pressure) and flexibility (low suit pressure). The ISS has a “shirt-sleeve environment” with the same ambient pressure as that at sea level on earth (nominally 1013 hPa = 760

mmHg), while the Russian *Orlan* space suit has an internal pressure of 386 hPa. The US *EMU* suit has a somewhat lower internal pressure of 296 hPa (Norfleet & Butler, 2001). These pressures correspond to altitudes of 7440 and 9250 m (24 400 and 30 350 feet) respectively, so the astronauts must breathe pure oxygen during the EVA to avoid hypoxia.

2.2.2 Decompression risks

A critical factor for safe EVA is to avoid decompression illness (DCI), when decompressing from normal to EVA suit pressure. At a given ambient pressure there is a steady-state amount of nitrogen dissolved in the body tissues and fluids. Decompression of the body can result in supersaturation, i.e., at the ambient pressure reached after decompression, there is too much gas dissolved in the tissues and so gas bubbles may form within the tissues and to a certain extent in the venous blood. Bubble formation is not instantaneous and a substantial supersaturation can occur without gas bubble formation. Any bubbles formed in the tissues are usually excreted into small veins and are finally transported to the lungs as venous gas emboli (VGE), where their gas content diffuses out to the exhaled air. The VGE (diameter 50–200 μm) are mainly filtered in lung arterioles with diameters of a similar size as the VGE (Harvey, 1945; Brubakk, 2004).

Venous gas bubbles in tissues or blood can lead to the development of DCI, which may involve serious, or even lethal, cardiopulmonary and/or neurological manifestations. Massive amounts of VGE filtered in the lungs can lead to occlusion of the pulmonary circulation with concomitant right heart failure. If not filtered in the lungs, gas emboli may occlude various tissues, including the brain and nervous system. The most effective treatment of VGE and DCI is recompression and so is less problematic to carry out after altitude exposure than hyperbaric exposure, since the recompression procedure amounts to returning the subject to the sea level and so increasing the pressure to normal ambient pressure. When treating subjects that have had developed VGE or DCI following hyperbaric exposure, the recompression treatment is more laborious, involving hyperbaric pressure chamber treatment and an unavoidable final, additional decompression to normal ambient pressure (Harvey, 1945; Brubakk, 2004).

When exposed to a severe decompression, pulmonary arterial pressure increases due to increased pulmonary vascular resistance. On reaching a pressure threshold, gas emboli may start to bypass the capillary filter of the lung and arterial gas emboli can be generated in the central nervous system, the coronary circulation, and elsewhere in the systemic circulation (Vik *et al.*, 1994).

Since the elimination of nitrogen from tissues is mainly perfusion-limited (Tikuisis & Gerth, 2003), exercise would theoretically be a way to speed up nitrogen elimination if combined with O_2 breathing before altitude decompression. Although this method has

been employed before decompression to reduce the likelihood of DCI occurring, exercise may instead provoke tissue and intravascular bubble formation if performed during and after decompression when tissues are likely to be supersaturated with nitrogen (Harvey, 1945; Jauchem, 1988; Pilmanis *et al.*, 1999).

2.2.3 Previous work on altitude decompression

Pre-breathing pure oxygen is beneficial before EVA exposure as it helps to eliminate much of the body nitrogen stores before decompression. A number of studies have been performed in the United States investigating the occurrence of DCI and VGE when decompressing to pressures relevant for EVA (e.g. Webb *et al.*, 2004; Webb & Pilmanis, 2005). In the open literature there are, to our knowledge, no reports describing the scientific basis for the Russian pre-EVA procedures. This is, of course, not to say that no such basis exists. An especially interesting aspect is that the Russian (Fig. 6) and the US routines to prepare for EVA are strikingly different, with a much more conservative and time-consuming set of procedures in the US routines. To some extent, this difference may be justified by the lower pressure in the US *EMU* suit. Webb and Pilmanis (2005) studied altitude DCI between 6900 and 9100 m in subjects who had pre-breathed 100 % O_2 for 60 min. After 6 h at an altitude of 7620 m (close to the Russian EVA pressure), they found around a 50 % occurrence of both subjective

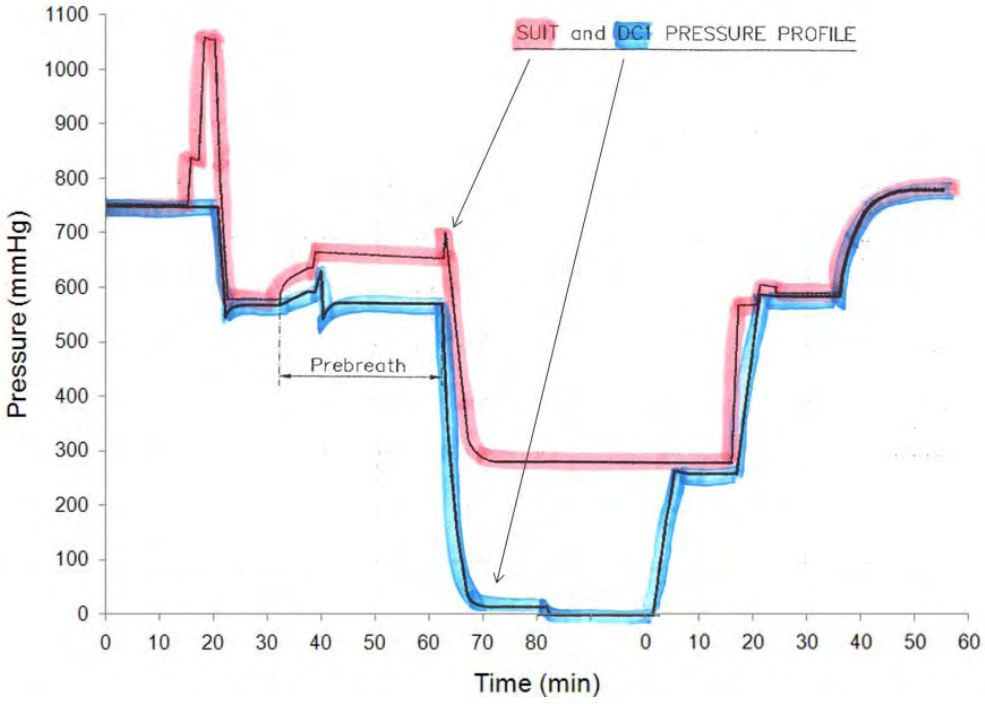


Figure 6. Russian EVA spacesuit (suit) and air-lock (DCI) pressure profile. An initial suit pressure peak is performed to test for leaks and then different locking procedures are performed, before the pressure in the airlock is reduced and the cosmonaut starts the EVA. Note the short oxygen pre-breathe (starts at t = 30 min) and denitrogenation period (Graph: personal communication Christer Fuglesang).

DCI symptoms and VGE, the latter detected using a precordial Doppler ultrasound technique. Corresponding values at 9144 m (appr. the US EVA pressure) were more than 80%. Six hours is a common duration of an EVA session.

Considering these data, it is quite remarkable that no DCI symptoms have so far been reported from any Russian or US space activity. There has been speculation that astronauts under report DCI symptoms (Norfleet & Butler, 2001) so as not to lose their place on the programmes, but it has also been hypothesised that the microgravity environment *per se* may be protective against the generation of VGE and/or DCI symptoms (Balldin *et al.*, 2002; Webb *et al.*, 2005a). Balldin *et al.* (Balldin *et al.*, 2002) observed the same level of DCI symptoms in the supine subjects as in an ambulatory control group, while studying supine subjects as a simulation of microgravity. However, a lower occurrence of VGE was noted in the supine group than in the controls, who, in contrast performed bubble-provoking arm and leg movements. In addition, a large retrospective study comparing the DCI occurrence between ambulatory (49 % DCI) and non-ambulatory (40 % DCI) subjects, showed no differences between the groups (Webb *et al.*, 2005a).

2.2.4 Pulmonary gas embolism and exhaled NO in an animal model

Pulmonary gas embolism is a serious complication not only in decompression, but also after surgery and trauma and the diagnosis is a challenge. Interestingly, Agvald *et al.* (2006) made findings that suggested a novel, simple and non-invasive method to detect VGE in the lungs: these authors injected small amounts of air into central veins of rabbits and found marked elevations of exhaled NO. Potential mechanisms explaining these results are:

a) less scavenging of lung NO due to blocked lung blood capillaries (Rimar & Gillis, 1993)
b) less carbon dioxide (CO_2) inhibition of NO formation (Stromberg *et al.*, 1997; Adding *et al.*, 1999b)

Regardless of mechanism it was considered worthwhile to assess this potential method in a study on altitude decompression, in which the Russian EVA procedure (Fig. 6) was simulated (**Paper I**).

2.2.5 Exhaled NO in hypobaric conditions

Even if determination of exhaled NO is a clinically routine procedure (ATS/ERS, 2005) it could not *à priori* be assumed that this technique would work in the same way and result in the same normal values during an EVA simulation (breathing 100 % oxygen at 38 % of normal ambient pressure) as during standard sea level air breathing.

Unfortunately, previous data on exhaled NO in hypobaric conditions are far from conclusive for the present applications. Beall *et al*. (2001) found increased exhaled NO fractions (F_{ENO}), but not partial pressures (P_{ENO}), in Bolivians and Tibetans living at high altitude. On the other hand, (Brown *et al.*, 2006) showed reduced P_{ENO} levels at high altitude (exhalation flow 350 $ml \cdot s^{-1}$). Hoit *et al*. (2005) showed unchanged P_{ENO} levels at standard exhalation flow of 50 $ml \cdot s^{-1}$ in Tibetan altitude residents compared to sea level controls. Duplain *et al*. (2000) showed that subjects not prone to high-altitude pulmonary oedema (HAPE) had a gradual increase of their exhaled NO output during the first two days of high altitude exposure.

A recent study by Hemmingsson *et al*. (2009) has shown that two commercially available NO monitors showed marked deviations from standard performance at altitudes of 3000–4000 m. These deviations included both the control of expiratory flow and detector sensitivity. Thus, in the design of present study (**Paper I**) special attention has been given to these factors.

2.3 PULMONARY GAS EXCHANGE IN HYPERGRAVITY

2.3.1 Hypergravity research history

Increased gravitational forces, commonly known as hypergravity, are generated when a body is subjected to linear or angular acceleration. In a centrifuge, rotation produces an inertial force on a mass that cannot be distinguished from that of gravity. The acceleration due to the Earth's gravity (*Newton's law of universal gravitation*), termed the gravitational constant and designated 'g', has a value of 9.81 $m \cdot s^{-2}$. The unit of the ratio of an applied acceleration to the gravitational constant is 'G', given by the equation:

$$G = \frac{\text{applied acceleration}}{g}$$

The first hypergravity observations described in the literature were made by the end of the eighteenth century by Charles Darwin's grandfather, Erasmus Darwin (Darwin E, Zoonomia: or, The Laws of Organic Life. London: Printed for J. Johnson, 1794). Darwin describes an interesting way of inducing sleep:

> *Another way of procuring sleep mechanically was related to me by Mr. Bradley, the famous canal engineer, who was brought up to the business of a mill-wright; he told me, that he had more than once seen the experiment of a man extending himself across the large stone of a corn-mill, and that by gradually letting the stone whirl, the man fell asleep before the stone had gained its full velocity, and he supposed would have died without pain by the continuance or increase of the motion. In this case the centrifugal motion of the head and feet must accumulate the blood in both these extremities of the body, and thus compress the brain.*

A large human centrifuge (Fig. 7) was built a few years later in the psychiatric clinic of the Charité University Hospital in Berlin. The centrifuge had a radius of 4 m and was used for the treatment of patients with mental disease. The centrifuge could produce up to five times normal gravity (5 G) at the periphery (at 50 rpm). Marked changes in respiration, heart rate, and blood distribution were apparently observed.

During the nineteenth century the effects of centrifugal forces were studied on animals and humans, but with the development of aircrafts during the World War I and II, investigations of human and animal tolerances to centrifugal forces became more scientific. The main reason for this was that pilots were exposed to great acceleration during quick turns in dogfights and it became crucial to understand how to avoid blackouts, which were caused by blood draining from the head.

When exposed to hypergravity, a number of physiological accommodations based on homeostatic processes take place and with only a limited number of hypergravity exposures, no adaptation occurs. In the work described in this thesis, a human centrifuge was employed to intentionally alter perfusion and ventilation distribution in the lungs and to induce a transient condition similar to acute lung insufficiency in healthy subjects (**Papers II–IV**).

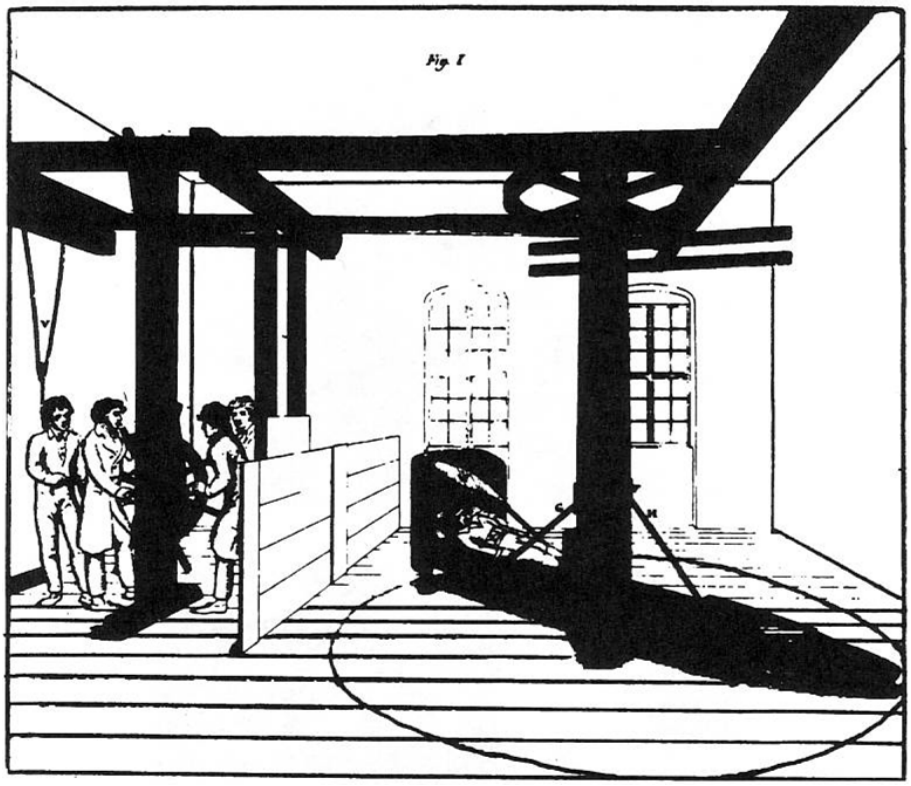


Figure 7. Human centrifuge in the psychiatric clinic of the Charité University Hospital in Berlin, used for treatment of patients with mental disease (Picture: Gauer O. The physiological effects of prolonged acceleration. German Aviation Medicine, World War II. Washington, DC: Department of the Air Force, 1950).

2.3.2 Gravity and lung structure

The human lung is extremely susceptible to changes in the magnitude and the direction of gravitational forces (Glaister, 2001), largely due to the large difference in the densities between air and the blood/tissue and also due to the marked distensibility of the pulmonary tissue. In an upright human, normal gravitational forces results in a pleural pressure gradient which leads to increased ventilation ($\dot{V}$) further down in the lung (Bryan *et al.*, 1966; Milic-Emili *et al.*, 1966). The apical lung parts are more stretched by the weight of the lung than the basal parts (Fig. 8), hence there is greater ventilation in the basal parts (larger possible volume change) in comparison to the apical. Also, the hydrostatic pressure causes a gradient in the apico-basal direction leading to a greater perfusion ($\dot{Q}$) in the basal lung parts (West *et al.*, 1964). Efficient pulmonary gas exchange is dependent on close matching between ventilation and perfusion.

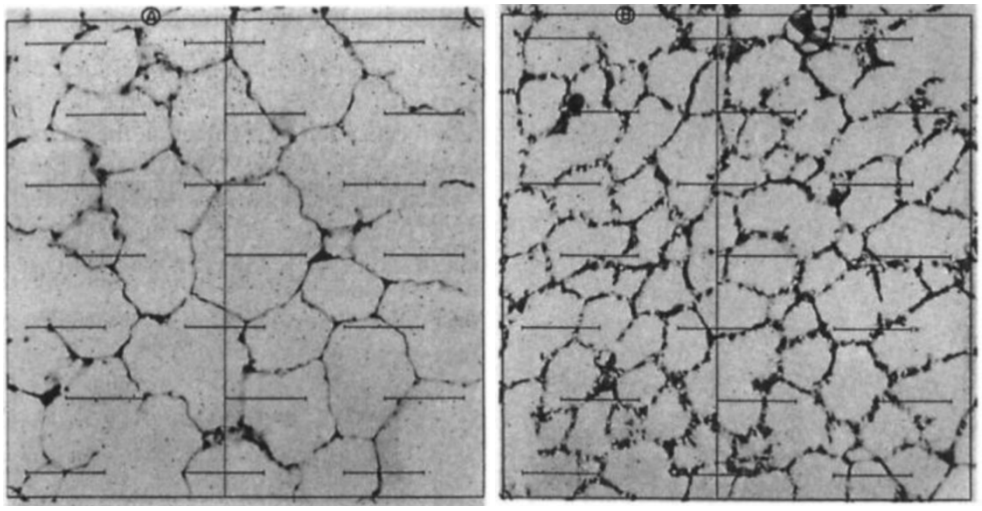


Figure 8. Histological appearance of lung tissue taken from the apex (left panel) and 20 cm lower down (right panel) from the lung of a greyhound frozen in the vertical position. A grid used for determining alveolar size is superimposed on the fields. Each of the test lines has a length of 100 µm (Glazier *et al.*, 1967).

2.3.3 Alveolar-to-blood gas transport in hypergravity

When a human is exposed to an increased gravitational force in the head-to-foot direction (sitting subject), or in the anterior-to-posterior direction (supine subject), the increased weight of the lungs enhances the apico-basal (sitting) / ventral-dorsal (supine) differences in ventilation and perfusion per unit lung volume. This leads to an impaired matching of ventilation and perfusion, and arterial deoxygenation (Glaister, 2001). Several studies have been performed to assess the change in ventilation and perfusion in sitting (Bryan *et al.*, 1966; Rosenhamer, 1967; Glaister, 2001; Rohdin *et al.*, 2004c), supine or prone subjects (Rohdin *et al.*, 2003a; Rohdin *et al.*, 2003b; Rohdin *et al.*, 2004a; Petersson *et al.*, 2006; Petersson *et al.*, 2007).

Rosenhamer (1967) and Rohdin *et al*. (2003a) made similar findings in sitting and supine subjects respectively; the mechanism for arterial desaturation was not alveolar hypoventilation. In fact their subjects showed signs of alveolar hyperventilation. Measurements of arterial PO_2 demonstrated that the alveolar-to-arterial PO_2 difference was markedly widened, indicating that there was an impaired alveolar-to-arterial oxygen transport.

The ability of the lungs to transfer gases between pulmonary blood and lung gas can also be determined as the lung diffusing capacity (D_L). The diffusing capacity for a certain gas X (D_{L_X}) is determined by diffusion over the alveolar-capillary membrane (J'_X, flux of gas molecules) and the partial pressure gradient (Fick's law) between the alveoli (P_{A_X}) and the alveolar capillaries (Pa_X). Both CO and NO are so tightly bound to hemoglobin in the red blood cells that the partial pressures of CO and NO in the capillaries are assumed to be zero.

$$D_{L\,X} = \frac{J'_X}{P_{A\,X} - Pa_X}$$

The diffusion capacity has classically been described as the arrangement of membrane resistance and blood resistance placed in series (Roughton & Forster, 1957). D_{mX} denotes the diffusing capacity for the membrane, θ_X the rate that gas X is taken up by the red cells each minute and for each mmHg of partial pressure, while V_c is the blood volume of the capillary bed.

$$\frac{1}{D_{L\,X}} = \frac{1}{Dm_X} + \frac{1}{\theta_X \cdot V_c}$$

When measuring overall "lung function", $D_{L_{CO}}$ is commonly used, since the variable is influenced by three important components: the surface area of the lung with contact to diffusing alveoli and the thickness of the alveolar-capillary membrane (both affecting Dm_X), and the volume of blood available in the capillary bed of the lung (V_c). For NO, the red cell resistance is almost negligible (Zavorsky *et al.*, 2004), therefore the diffusing capacity for NO ($D_{L_{NO}}$) corresponds to the membrane diffusing capacity for NO Dm_{NO} and is independent of V_c and hemoglobin concentration.

Rohdin & Linnarsson (2002) determined $D_{L_{CO}}$ in sitting subjects at 2 and 3 G. They found that it was decreased by 21 % at 2 G and 34 % at 3 G, and concluded that this could mainly be caused by changes in the Dm component. Therefore, it can be expected that $D_{L_{NO}}$ would be reduced in a similar manner.

2.4 PULMONARY GAS EXCHANGE IN MICROGRAVITY

2.4.1 Microgravity research history

Before the first manned spaceflight was conducted, physiologists were concerned with how the human body would function in the microgravity environment. For example Permutt (1967) predicted that space explorers would suffer generalized interstitial lung oedema. Luckily humans survived the microgravity environment, but with a few non-lung complications (Nicogossian *et al.*, 1988). Current microgravity research continues to include parabolic flights (microgravity duration: 20–25 seconds), sustained microgravity aboard orbital space vehicles and long-term microgravity on the international space station. The initial worries of acute survival have now evolved into concerns regarding radiation protection, cardiovascular and skeletal deconditioning, particle inhalation and mental health during long-term space missions.

2.4.2 Lung diffusing capacity and microgravity

A mere extrapolation from data obtained in hypergravity (see 2.3.3 above) would suggest the fact that the ventilation and perfusion in the lungs would become perfectly matched in microgravity. However, regional differences in ventilation/perfusion matching still exist in microgravity (Prisk *et al.*, 1993; Guy *et al.*, 1994; Verbanck *et al.*, 1997). This likely depends on intrinsic structural inhomogeneities of the lungs in the absence of gravity (Glenny *et al.*, 1991). Nevertheless, diffusing capacity for carbon monoxide ($D_{L_{CO}}$) in microgravity increases by 11-27 % (Prisk *et al.*, 1993; Verbanck *et al.*, 1997). For very short-lasting microgravity, Vaida *et al.* (1997) showed that both diffusing capacity for NO ($D_{L_{NO}}$) and the membrane component of $D_{L_{CO}}$ were increased by > 40 % due to a more homogenous distribution of gas and blood in the lungs.

2.4.3 Gravity and NO in the lungs

Due to the complexity of the lung structure and the many effects of gravity, the over-all effects of gravity on pulmonary NO formation and transport are not easily predicable. It was therefore considered of interest to experimentally determine the effects of a wide range of gravity levels on pulmonary NO.

2.5 ACUTE LUNG INSUFFICIENCY, GRAVITY AND HYPOXIC PULMONARY VASOCONSTRICTION

2.5.1 Hypergravity as a model for acute lung injury

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are serious lung disorders characterized by hypoxemia, non-cardiogenic pulmonary oedema, low

lung compliance and widespread capillary leakage. ALI and ARDS result in high morbidity and mortality (Randolph, 2009). An important mechanism behind the lung insufficiency in ALI is the increased weight of the oedematous lung tissue. The weight of the tissue tends to compress underlying lung tissue layers, resulting in poor aeration of dependent (dorsal in a supine patient) lung parts. Previous studies have shown that hypergravity can induce gas exchange impairment similar in magnitude to that seen in patients with ALI (Rohdin *et al.*, 2003a). Supine healthy subjects developed a severe, but temporary and reversible, lung insufficiency when exposed to 5 G in the anterio-posterior direction. The mechanism is also here an increased weight of the lung tissue. Arterial oxygen saturation was reduced due to an impaired matching of pulmonary ventilation and perfusion (Rohdin *et al.*, 2003b).

2.5.2 Hypoxic pulmonary vasoconstriction - friend or foe?

The phenomenon hypoxic pulmonary vasoconstriction (HPV) was discovered by Euler & Liljestrand (von Euler & Liljestrand, 1946). They showed that regional hypoxia could divert pulmonary blood flow away from poorly ventilated lung units with inadequate oxygenation to better ventilated regions. HPV provides an important mechanism for maintaining optimal ventilation and perfusion matching which improves arterial oxygenation. There are numerous local vasoactive substances that modulate HPV (Frostell *et al.*, 1991) and thereby play an indirect role for regulating regional pulmonary blood flow.

Besides the beneficial HPV effects, there are also situations where HPV induces undesirable effects. At high altitude, the lowered ambient pressure lowers the oxygen partial pressure, resulting in lowered alveolar oxygen pressure. This may lead to a generalized HPV that in turn leads to increased pulmonary vascular resistance, and increased pulmonary artery pressure. The higher afterload for the right ventricle may eventually lead to reduced exercise capacity (Ghofrani *et al.*, 2004) and eventually to right heart failure. HPV is also a key element in the pathogenesis of high-altitude pulmonary oedema (HAPE, Dehnert *et al.*, 2007).

It was reasoned that HPV could be beneficial also in the special case when regional hypoventilation is caused by compression of dependent lung regions during exposure of hypergravity. Petersson *et al*. (2006) using Single-Photon Emission Tomography (SPECT) showed that there were large dependent perfusion defects in the lungs of subjects exposed to hypergravity. This observation suggests that subjects were protected from shunting in hypoxic, dependent regions. This in turn could be a result of passive vascular compression or of an active mechanism such as HPV. It was therefore considered of interest to investigate whether pharmacological suppression of HPV would worsen the hypergravity-induced arterial desaturation in supine subjects.

3 AIMS

The aims for this thesis were to test following hypotheses:

- That the EVA procedures used by astronauts and cosmonauts potentially pose a severe risk for venous gas emboli with a concomitant risk of decompression illness (**Paper I**)

- That venous gas emboli induce elevated levels of exhaled nitric oxide by means of occlusion of pulmonary blood vessels (**Paper I**)

- That reduced ambient pressure reduces exhaled levels of nitric oxide by means of lowered gas density with increased axial back-diffusion and blood uptake of lung nitric oxide (**Paper I**)

- That gravity-induced alterations of perfusion and ventilation distribution changes the diffusing capacity for nitric oxide and hence influence exhaled nitric oxide (**Paper II**)

- That a reduced uptake of pulmonary NO to the blood in hypergravity could be due to slowed back-diffusion caused by compression and/or elongation of small conductive airways (**Paper III**)

- That a reduced uptake of pulmonary NO to the blood in hypergravity could be caused by reduced contact area between the blood and the alveolar gas (**Paper III**)

- That hypoxic pulmonary vasoconstriction could be protective against hypergravity-induced desaturation of arterial blood (**Paper IV**)

4 METHODS

4.1 SUBJECTS

At the time of the experiments all subjects declared themselves to be healthy, non-smokers with no history of airway diseases. For all studies, except the supine hyper-G study, the subjects were on a low nitrite diet and had to refrain from strenuous exercise 24 hours before the experiments (Ricciardolo *et al.*, 2004; Vints *et al.*, 2005). Details are presented in Table 1.

Table 1
Subject data, papers I – IV.

Study	**Simulated spacewalk**		**Actual**	**Hyper-G**	**Micro-**	**Hyper-G supine**	
	A, B	**C**	**spacewalk**	**sitting**	**gravity**	**A**	**B**
Paper(s)	**I**	**I**	**I**	**II & III**	**II & III**	**IV**	**IV**
G-level	1 G	1 G	μ & 1 G	1 – 3 G	μ & 1 G	1 & 5 G	1 & 5 G
n	10	10	4	10	5	12*	12*
Women	4	4	0	3	0	2	3
Men	6	6	4	7	5	10	9
Age	21–35	30–50	34–52	23–42	34–52	20–34	18–36
Height	1.59–1.85	1.65–1.86	1.72–1.82	1.64–1.91	1.72–1.82	1.63–1.85	1.60–1.85
Weight	-	-	68–78	53–87	68–78	51–85	51–86
BMI	19.2–25.9	20.4–31.6	22.0–26.4	-	-	-	-

Gravity exposure (G-level; G), number of subjects (n), gender distribution, age (years), height (m), weight (kg), and BMI ($kg \cdot m^{-2}$) presented for the different studies. * Eight subjects (one female, seven males) participated in both study A and B in the supine hyper-G study.

For the microgravity experiments aboard the ISS, Russian cosmonauts and ESA astronauts participated.

For the simulated spacewalk and the hypergravity experiments, subjects were recruited through contacts and advertising. The simulated spacewalk performed at hypobaric pressures included a decompression profile that potentially could induce venous gas emboli and cause manifest but treatable and reversible decompression illness symptoms. Due to the nature of these experiments, we recruited subjects with extensive diving experience and knowledge of decompression illness and its complications. For the third simulated spacewalk series (Study C), more astronaut-like subjects in terms of age and BMI, compared to study A and B, were recruited. Several studies (Webb *et al.*, 2005b; Foster & Butler, 2009) have shown an increasing susceptibility to bubble formation and DCI with increasing age and BMI.

4.2 INSTRUMENTATION

4.2.1 Hypobaric pressure chamber (Paper I)

The hypobaric pressure chamber at Karolinska Institutet (Fig. 9, upper left panel) was used for the simulated EVAs. The chamber has a volume of 25 m^3 and two airlocks that can be used to move subjects, personnel and objects in and out without changing the chamber pressure.

During the experiments, the subjects wore oro-nasal masks while the chamber attendants wore oxygen hoods and breathed pure oxygen continuously (Fig. 9, upper right panel).

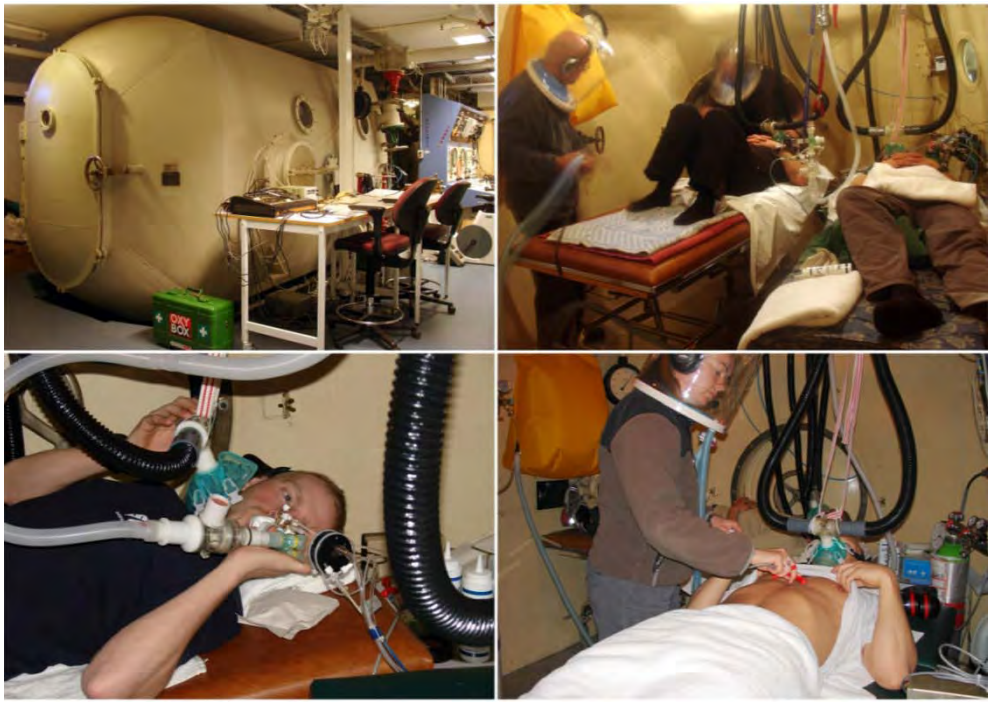


Figure 9. Upper left panel: hypobaric pressure chamber at Karolinska Institutet. Upper right panel: inside view of the hypobaric pressure chamber with two subjects and two attendants. Lower left panel: subject exhaling into equipment to determine exhaled nitric oxide. Lower right panel: Doppler ultrasound monitoring of venous gas emboli passing the subjects heart.

Intermittently, the subjects exhaled into a mouthpiece to measure exhaled partial pressures of nitric oxide and carbon dioxide ($P_{E_{NO}}$, $P_{E_{CO_2}}$) (Fig. 9, lower left panel). Mouthpiece pressure (MPP) and flow was measured by means of differential pressure transducers. $P_{E_{NO}}$ was monitored by means of a chemiluminescense analyser and $P_{E_{CO_2}}$ was monitored by means of an infrared analyser. All signals were digitised and stored on a computer. During the NO and CO_2 measurements subjects were given feedback in terms of MPP from a mechanical manometer with an analogue dial. Subjects exhaled through flow restrictions that had been manufactured to regulate the flow to the target

level of 50 ml·s^{-1} at an MPP of 15 hPa (ATS/ERS, 2005) at each chamber pressure. Pressure and flow were calibrated against physical references and gas analysers against mixtures with known gas concentrations.

At given intervals a pre-cordial Doppler ultrasound monitor was used to screen the subject's heart for VGE (Fig. 9, lower right panel). The individual Doppler sound files were stored on a portable recording device and in duplicate on a desktop computer. The Kisman Masurel (KM) precordial Doppler scoring system (Kisman *et al.*, 1978) was used for real-time quantification of circulating VGE. Briefly, VGE occurrence in the right heart was judged on a scale that ranged from no acoustic bubble echoes to continuous, high-intensity bubble-echoes throughout the cardiac cycle.

4.2.2 International Space Station (Papers I and II)

The opportunity arose to make actual space microgravity measurements aboard the International Space Station (ISS) (Fig. 10). The subjects were cosmonauts and ESA astronauts.

Figure 10. The International Space Station (ISS), September 2009. © NASA

Handheld NO analysers were used for both **Paper I and II**. The analyzer used an electrochemical sensor to detect the normally low levels (parts per billion, ppb) of exhaled nitric oxide. The results were displayed on a built-in screen. Additionally, the results were also stored on personal smartcards for later offline assessment. The results were also down-linked periodically to the ground control.

The analyser is commonly used in clinical practice, but the units used aboard the ISS underwent extensive "space use evaluations and modifications" to meet the strict requirements for space use. The modifications included a new power supply and shielding against electromagnetic radiation. In Fig. 11, cosmonaut Valery Tokarev performs a F_{ENO} manoeuvre in microgravity aboard the space station.

Figure 11. Cosmonaut Valery Tokarev measuring exhaled nitric oxide aboard ISS, October 2005. © ESA

4.2.3 Human centrifuge (Papers II – IV)

The centrifuge at Karolinska Institutet has a radius of 7.25 m and a gondola where the subjects can be studied in either sitting or supine positions. During the hypergravity runs, the gondola swings out so that the resultant gravitational vector is always in the head-foot direction (sitting subjects, **Paper II and III**) or in the anterio-posterior direction (supine subjects, **Paper IV**) (Fig. 12).

All signals from the gondola were transmitted via slip rings to a control room were the main units of the monitoring system were supervised and the data were stored.

Standard monitoring of the subjects included audiovisual communication between the gondola of the centrifuge and the test supervisor in the control room by means of a colour video system and a two-way audio communication. Heart rate (HR) was obtained from precordial ECG electrodes and arterial oxygen (haemoglobin) saturation was monitored using pulse oximetry (SpO_2). The SpO_2 probe was placed either on a finger or an earlobe. When the probe was placed on a finger, the subject wore a warm mitten, and when placed on an earlobe, the lobe was pre-treated with capsaicin ointment to enhance local perfusion. Rohdin *et al.* (2003a) has previously shown an excellent agreement between SpO_2 and oxygen saturation in arterial samples during identical experimental conditions. The G force in the head-foot/anterio-posterior direction was measured continuously with an accelerometer. G level, ECG, heart rate and SpO_2 were acquired and stored using a digital data acquisition system with a sampling frequency of 200 Hz.

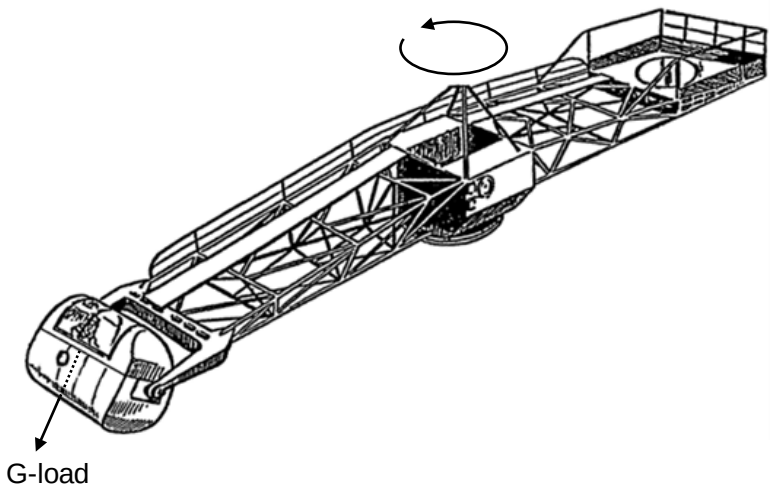


Figure 12. The human centrifuge at Karolinska Institutet.

4.2.3.1 Sitting subjects (Papers II and III)

For the sitting hypergravity experiments, the subjects sat in the gondola with the backrest of the seat in a 28º angle to the direction of the gravitational vector. Since having an upright position in hypergravity is associated with a risk of lowered cerebral arterial blood pressure that can induce a black-out, assessment of brain perfusion was carried out, testing peripheral vision by way of three coloured lamps (arrows in Fig. 13).

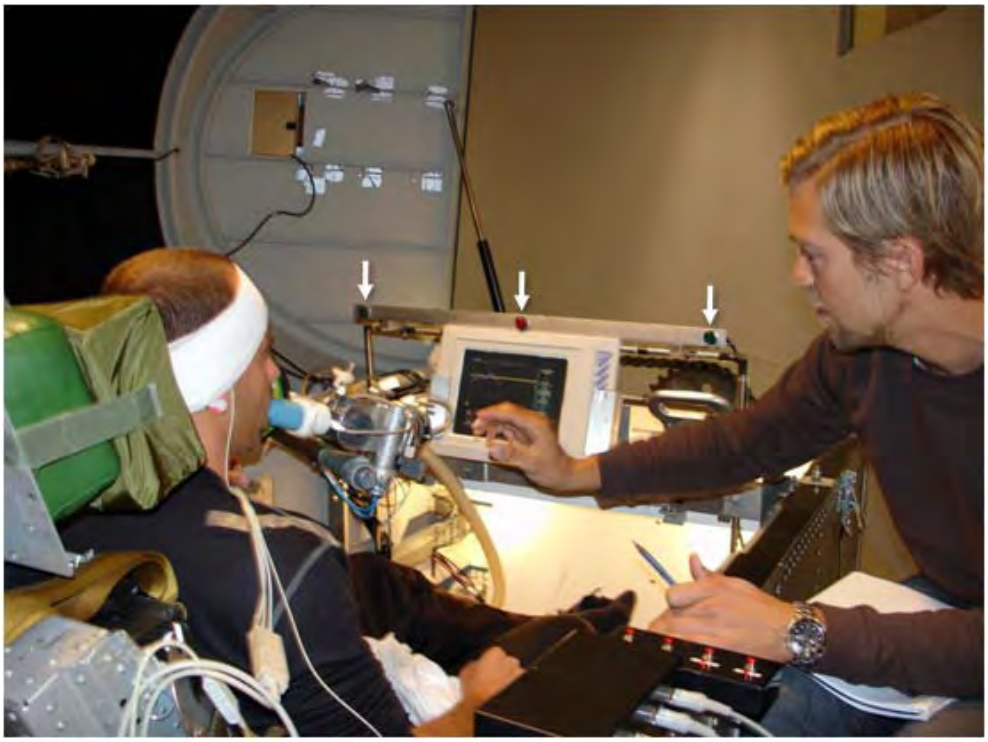


Figure 13. Seated subject in the gondola of the human centrifuge during a 1 G control test. Arrows show lamps for monitoring of peripheral vision.

The subjects breathed through a remote-controlled rotational valve. In one position of the rotary valve, the subjects' airways were connected to the cabin air and in the other, to a non-rebreathing valve. The inspiratory port of the non-rebreathing valve provided an NO-free inspirate. The exhalation port of the non-rebreathing valve was connected to a heated pneumotachograph and an array of four orifices with different resistances connected in series. Vented openings between the series of four orifices were controlled by valves and could be closed or opened in different combinations, so that the expired flow at a preset expired pressure was 50, 100, 200 or 500 $ml \cdot s^{-1}$. One side port in the mouthpiece was connected to a pressure transducer.

The mouthpiece pressure signal was displayed on a LCD screen in front of the subject, together with reference lines for zero pressure and +15 hPa. From a second side port, there was an inlet to a 10 m long capillary tube that forwarded sample gas of near vacuum to a chemiluminescence NO analyzer located at the centre of the centrifuge. Through a third side port, a sample was sent to an infra-red CO_2 analyzer.

4.2.3.2 Supine subjects (Paper IV)

For the experiments with supine subjects (**Paper IV**), the floor of the human centrifuge gondola was covered with a mattress and a head support in order to accommodate the subjects. They were secured to the floor with a 5-point safety belt. Since there was no risk for lowered cerebral arterial pressure, no assessment of brain perfusion was done. Arterial (haemoglobin) oxygen saturation was measured with a pulse oximetry probe on the earlobe. Arterial blood pressure was measured with a finger cuff plethysmograph. A mitten was used to keep the hand warm in order to prevent vasoconstriction in the hand (Fig. 14).

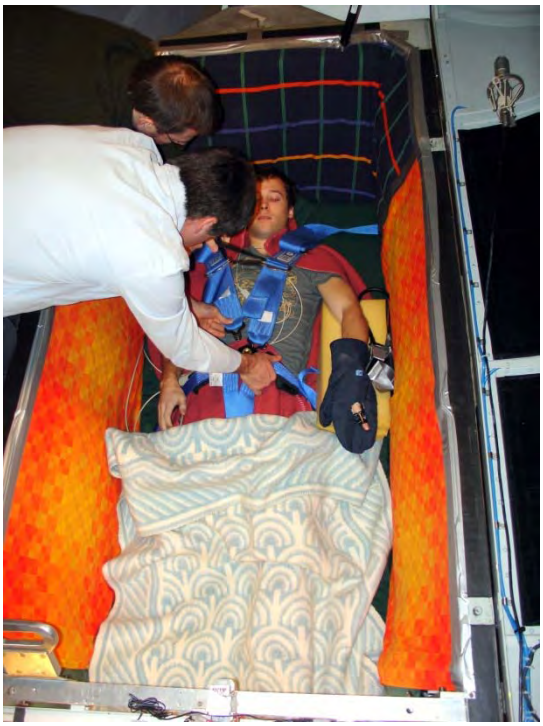


Figure 14. Supine subject in the gondola.

4.3 PROCEDURES

4.3.1 Hypobaric pressure chamber (Paper I)

Due to the risks involved in decompression studies (Webb & Pilmanis, 2005) we chose an incrementing pressure/time profile. Two subjects were exposed to 386 hPa

(equivalent to 7500 m pressure altitude) for one hour (Study A), eight subjects to 386 hPa for two hours (Study B), and ten subjects to 386 hPa for six hours (Study C).

The subjects were studied in pairs with one or two attendants accompanying them inside the chamber. The subjects donned oro-nasal masks and breathed 100 % oxygen during a pre-oxygenation period of 1 h, ascent, exposure and descent. To simulate microgravity, subjects were placed in a supine position on a gurney in the hypobaric pressure chamber. They remained supine on the gurney during the whole experiment including 1 h pre-oxygenation, ascent, exposure to simulated altitude, and descent.

As an additional safety measure against DCI, the operators had 2 h of oxygen pre-breathing and like the subjects they continued to breathe oxygen throughout the altitude exposures. Nevertheless one operator had DCI symptoms (see below) and procedures were subsequently changed so that operators performed 15 min of leg exercise during the oxygen pre-breathing period and never stayed for more than 2 h at altitude.

Arm exercise was performed twice every hour at minutes 0 (except the first and last hour) and 30. The exercise consisted of full biceps curls at 0.5 Hz with 2 × 1.25 or 2.5 kg for 5 min.

At regular intervals, four to six times per hour, subjects performed manoeuvres to measure P_{ENO} and P_{ECO_2}. The measurements of exhaled NO conformed to the internationally established standards (ATS/ERS, 2005). Thereafter ultrasound Doppler recordings were performed with one resting measurement followed by a second measurement after 5 calf contractions. The ultrasound recordings were evaluated in real-time to check if the VGE end-point (a KM score of 3) was reached and once bubbles were heard Doppler recordings were made every 5–15 min. One out of every 2–3 P_{ENO}/P_{ECO_2} measurements occurred immediately after arm exercise.

To be able to maintain the supine posture for the whole exposure, the subjects wore diapers and the male subjects also had the possibility of using a bedpan. In one case, a female subject used the in-chamber toilet next to the gurneys. Test termination criteria/endpoints were reached either upon completion of the exposure time, on measuring two consecutive Doppler scores greater than KM 3 (Kisman *et al.*, 1978) at rest, or on the occurrence of symptoms of DCI. Such symptoms included joint pain, skin manifestations, neurological symptoms, or respiratory problems.

The pressure chamber was ventilated with room air and the temperature in the chamber was similar to the room temperature, which ranged 20–22 °C during the experiments. During the decompression the temperature was lowered temporarily to 17 °C, but returned to 20–22 °C within 10 min. The humidity in the chamber during the altitude exposures was the same as in the room, i.e. 30–70 %.

4.3.2 International Space Station (Papers I and II)

Onboard the International Space Station, the gravitational force of the earth is counterbalanced by the centrifugal force resulting from the circular trajectory of ISS, which results in weightlessness/microgravity.

Training, and pre- and post-flight exhaled nitric oxide measurements (ATS/ERS, 2005) were performed in Russia, United States, or Germany. The microgravity experiments were performed onboard the International Space Station during the period 2005–2008. Pre- and post-flight measurements were performed in a sitting posture, and the in-flight measurements aboard the ISS were performed in a semi-recumbent position. All measurements were performed in duplicate.

Since ingested food and beverages rich in nitrite and nitrate have shown to affect exhaled NO (Vints *et al.*, 2005) the subjects had to refrain from such food and beverages for 24 hours before the tests. They rinsed their mouth with water before each test.

4.3.2.1 Space walk (Paper I)

Russian cosmonauts performed $F_{E_{NO}}$ measurements before and within a few hours after EVA from the International Space Sation (ISS).

4.3.2.2 Long-term monitoring of exhaled NO in microgravity (Paper II)

Astronauts performed at least four control measurements on one to three occasions before the spaceflight and then approximately every sixth week during their 23–28 weeks long stays onboard the International Space Station. After returning to earth, they performed daily measurements during the first week after landing.

4.3.3 Human centrifuge (Papers II – IV)

4.3.3.1 Sitting subjects, experimental study (Paper II)

Subjects performed the experiments at 1, 2 and 3 G. Once seated in the centrifuge, the vital capacity (VC) was determined (Rohdin *et al.*, 2004b) and once complete, the following respiratory manoeuvre was performed in triplicate for each combination of the four gravity conditions (1 G pre, 2 G, 3 G, and 1 G post) and for the four expired flows. Initially the subjects exhaled to residual volume, the rotary valve was then activated and inhalation of NO free air to total lung capacity and controlled full exhalation took place, keeping the airway pressure at +15 hPa by means of visual feed-back.

In every case, the subject initially exhaled half of his vital capacity at a rate of 500 $ml \cdot s^{-1}$. When the time integral of the expired flow signal had reached 50 % of the vital capacity, the test leader then activated the solenoids so that the expired flow rate for +15 hPa airway pressure became either 50, 100, 200 or 500 $ml \cdot s^{-1}$ for the remainder of the exhalation. This procedure allowed an initial rapid elimination of the dead-space gas in order not to prolong the manoeuvre so that subjects experienced "air hunger" while in hypergravity. At 2 and 3 G, the VC values were assumed to be reduced to 92 and 87 % respectively of the 1 G values (Rohdin *et al.*, 2004b). During a typical session, the subject sat first for one minute at the target G level and then repeated the above manoeuvre 12 times; four manoeuvres with different expired flows in random order were performed with a one minute interval in between. Thereafter, the subject rested for three minutes followed by two more sets of four manoeuvres. During the 2 and 3 G sessions, subjects rested at 1.4 G between the sets. The choice of 1.4 G rather than 1 G between the sets of four manoeuvres at 2 and 3 G was made in an effort to avoid the vestibular stimulation caused by accelerating and breaking the centrifuge repeatedly. The subjects rested at 1 G for approximately 30 min between repeated sessions. The order of the 2 and 3 G sessions was randomized. Subjects were instructed to abstain from food and beverages rich in nitrite and nitrate 24 h before the tests. Before each test session they rinsed their mouth with water.

4.3.3.2 Sitting subjects, modelling study (Paper III)

In **Paper II**, exhaled levels of NO (FE_{NO}) were measured at multiple flows in healthy subjects at normal and increased gravity. From these data, alveolar NO concentration ($C_{alv_{NO}}$) and conductive airway NO production ($J'aw_{NO}$) were estimated.

The alveolar NO diffusing capacity (DA_{NO}) is independent of perfusion (see the background section), thus is insensitive to capillary distension, i.e., over-perfusion. Consequently DA_{NO} is essentially determined by the available contact surface between the alveoli and the capillaries. Since DA_{NO} influences both FE_{NO} and $C_{alv_{NO}}$, we believed that by using hypergravity data from **Paper II** in a mathematical model we would get insight into contact surface change due to gravity-induced perfusion redistribution.

A mathematical two-compartment model (see the background section) incorporating convective and diffusive NO transport and NO source terms (Van Muylem *et al.*, 2003) with geometrical boundaries based on Weibel's symmetrical model (Weibel, 1963) was used (Eq. 1 in **Paper III**). The model was tested for both a uniform lung model (one-trumpet model) and a model with different upper and lower lung characteristics (two-trumpet model). Acinar bronchial cross-sectional area changes may influence FE_{NO} by means of altered axial diffusion. By using this mathematical model, estimates of airway cross-sectional area changes also were computed. Experimental FE_{NO}, $C_{alv_{NO}}$ and $J'aw_{NO}$ values were used as parameters in the model to estimate the main output

variable DA_{NO} (i.e., variables were adjusted so that the theoretical outcome matched experimental data). The effects of bronchoconstriction (BC) and differences between the one- and two-trumpet models were also evaluated. Parameters involved in the model that were not achieved from the experimental study were adopted from the literature. The fitting process was simplified by focusing on the 1 and 2 G data only.

4.3.3.3 Supine subjects (Paper IV)

The subjects came to the laboratory on two (study A) or three (study B) occasions separated by at least 48 hours. All subjects had the chance to familiarize themselves with the centrifuge before the tests. All sessions included pre-medication with 100 mg dimenhydrinate to protect against motion sickness. The study was carried out in a single-blinded format during the following conditions in random order.

Study A:

- Sildenafil 50 mg (S_{50}) (Viagra, Pfeizer AB, Sollentuna, Sweden): administered orally 60 min before the start of the centrifuge run.
- Control (C_A): placebo tablets were administered orally with the same timing as above.

Study B:

- Sildenafil 100 mg (S_{100}) (Viagra, Pfeizer AB, Sollentuna, Sweden): administered orally 60 min before the start of the centrifuge run, followed by inhalation of 10 ml nebulized saline 30 min later.
- Iloprosttrometamol (Ilo) (Ilomedin, Schering Nordiska AB, Järfälla, Sweden): placebo tablets were administered orally 60 min before the centrifuge run, followed by inhalation of an aerosol with 10 μg iloprosttrometamol in 10 ml saline 30 min before the start of the centrifuge run.
- Control (C_B): the subjects received placebo tablets orally and inhaled saline aerosol with the same timing as above.

Centrifuge runs were identical for all conditions. The subject was placed supine in the centrifuge gondola and allowed 10 min of quiet rest. Thereafter the centrifuge was started and the G-level (G in the anterio-posterior direction) was increased at a rate of 0.2 $G \cdot s^{-1}$ to 5 G. This G-level was maintained for 7 min, whereupon the centrifuge was slowed at a rate of 0.2 $G \cdot s^{-1}$. The slow onset and offset rates were chosen to minimize the risk of motion sickness. After the centrifuge had stopped, the subject remained supine and quiet for 10 min and was then instructed to take three deep breaths in order to re-open collapsed airways.

4.4 ETHICAL CONSIDERATIONS

The experimental procedures conformed to the Declaration of Helsinki. All subjects received written information concerning the procedure they were to undergo and written consent was obtained. All studies were approved by the Regional Ethics Committee in Stockholm and for the astronaut/cosmonaut studies also by the European Space Agency (ESA) Medical Board. The incident with one of the attendants in **Paper I** was reported to the Regional Ethics Committee in Stockholm.

4.5 STATISTICAL PROCEDURES

When applicable, probability plots were used to indicate if data were normally distributed. Analysis of variance (Statistica 7.1 and earlier versions, Statsoft Inc., Tulsa, OK, USA) with a repeated measures design was used. When the sphericity assumption held, Tukey's HSD post hoc test was performed. In other cases, paired t-tests with Bonferroni correction were used. Results were considered statistically significant if $P < 0.05$ and all tests were 2-sided. Data are given as means ± SD if not stated otherwise.

5 RESULTS

5.1 SIMULATED AND ACTUAL EXTRAVEHICULAR ACTIVITY (PAPER I)

5.1.1 Venous Gas Emboli

5.1.1.1 Simulated spacewalk

Due to the potential risk for DCI associated with the experimental setup, a conservative approach was applied with incrementing altitude durations, thus the altitude exposure to 386 hPa was gradually increased from 1 h (Group A) to 2 h (Group B) and 6 h (Group C). Since there were no observations of VGE in Group A, longer exposures were applied for Group B and C. Out of twenty subjects, only two observations of VGE were made. Additionally, one attendant showed symptoms of DCI:

- In one subject from Group B (Table 1 in **Paper I**), VGE started to appear after 56 min at 386 hPa. They were of KM grade 0 at rest and grade III after calf contractions (the KM grading system runs from grade 0 to grade IV). After 1 h and 26 min the Doppler score endpoint of III was reached at rest and the experiment was aborted. Altogether, six observations of bubble grade III at rest were observed in this subject until recompression was completed. Thirty minutes after recompression no further VGE were detected. During the medical follow-up, the subject recalled that he recently had experienced a trauma to his right lower arm and an X-ray taken 5 days after the experiment showed fractures of the scaphoid bone and of the head of the radial bone. There were no subjective symptoms of DCI.

- Subject 2: This subject was member of the group scheduled for a 6 h exposure (Group C, Table 1 in **Paper I**). He had Doppler scores of 0 until 2 h and 6 min of exposure to 386 hPa, and thereafter had VGE grade I after calf contractions, but not at rest during the remainder of the exposure. The experiment was then aborted due to DCI symptoms in an attendant (see below).

- Attendant: One attendant reported mild subjective DCI symptoms after 2 h 50 min at altitude with fullness of the knees and mild pain in the knees and wrists. This occurred after he attempted to open a malfunctioning medical lock and had performed very forceful arm and leg muscle contractions. At Doppler measurement, he had grade IV KM bubble scores after calf contractions, so the altitude exposure was aborted. He was thereafter treated according to a hyperbaric oxygen standard table, and his DCI symptoms gradually disappeared.

The main observation was that very few VGE were observed and that no subjective symptoms of DCI were reported in the subjects.

5.1.1.2 Actual spacewalk

No reports of DCI or other decompression-related symptoms were reported.

5.1.2 Exhaled Nitric Oxide

5.1.2.1 Simulated spacewalk

Exhaled NO did not differ in any obvious way between the two subjects who had VGE and those that did not. A consistent finding was that $P_{E_{NO}}$ was markedly lower at altitude than during the sea level oxygen prebreathing period. Thus $P_{E_{NO}}$ fell from 1.45 ± 0.12 mPa (mean ± SEM) before to 1.10 ± 0.08 mPa (N = 20, $P < 0.0001$) after 1 h at 386 hPa. There was no trend for $P_{E_{NO}}$ during the altitude period. No differences in exhaled CO_2 were found at altitude compared to the recordings at sea level and there were no changes in exhaled CO_2 immediately after the arm exercise.

5.1.2.2 Actual spacewalk

Exhaled NO values for the cosmonauts during their stay on the ISS was 0.9 ± 0.3 mPa before EVA, and 0.6 ± 0.2 mPa after EVA with no significant difference.

5.2 EXHALED NO IN HYPERGRAVITY AND MICROGRAVITY (PAPER II & III)

5.2.1 Hypergravity

Exhaled NO values at a flow of 50 ml·s^{-1} were 16.0 ± 4.3 (mean ± SD), 19.5 ± 5.1, and 18.6 ± 4.7 ppb at 1, 2, and 3 G, respectively. $F_{E_{NO}}$ values for a given flow were higher at 2 and 3 G than at 1 G pre, but did not differ between 1 G post and 1 G pre. There was a linear relationship between $F_{E_{NO}}$ and the inverse of expired flow in the group mean data (Table 1 and Fig. 5 in **Paper II**).

The estimated $C_{alv_{NO}}$ was 2.3 ± 1.1 ppb in 1 G and increased significantly to 3.9 ± 1.4 and 3.8 ± 0.8 ppb at 2 and 3 G ($P < 0{,}002$). Estimated $J'aw_{NO}$ tended to be elevated at 2 G compared with 1 G pre.

As with $F_{E_{NO}}$, $P_{E_{CO_2}}$ changed significantly with the main factor G ($P < 0.001$), but only for the three lowest flows. When this change was analyzed, it was found that $P_{E_{CO_2}}$ was significantly lower at 3 G compared with 1 G pre at all exhalation flows except

500 ml·s^{-1}. $P_{E_{CO_2}}$ at 2 G did not differ from 1 G pre. $P_{E_{CO_2}}$ tended to be higher at 1 G post than 1 G pre at 50 ml·s^{-1}. Heart rate (HR) increased from 63 ± 11 beats·min^{-1} at 1 G pre to 82 ± 18 and 99 ± 19 beats·min^{-1} at 2 and 3 G, respectively. Post hypergravity HR was 65 ± 10 beats·min^{-1}.

5.2.2 Microgravity

Data from all five subjects were obtained for the first 14 weeks in space. For one subject, the analyzer malfunctioned after week 14, and the analyzer was subsequently exchanged when the next subject arrived at ISS. For another subject, the stay on the ISS lasted only 23 weeks. Pre-flight $F_{E_{NO}}$ was 12.3 ± 4.7 ppb (mean ± SD). There was no clear trend of changes of $F_{E_{NO}}$ over time during the stays on ISS. Thus all in-flight data for each subject were pooled, and in-flight $F_{E_{NO}}$ averaged 6.6 ± 4.4 ppb. This was significantly lower than the pre-flight value (P = 0.016). Similarly pooled data for the first week post-flight was 9.7 ± 2.8 ppb, which did not differ significantly from either pre-flight (P = 0.30) or in-flight (P = 0.28). There was no clear time trend of $F_{E_{NO}}$ after landing.

5.3 EXHALED NO IN HYPERGRAVITY, MODELLING STUDY (PAPER III)

The NO transport equation (Eq. 1 in **Paper III**) was solved for both the uniform lung model (one-trumpet) and the heterogenous lung model (two-trumpet). Experimental values on $F_{E_{NO}}$, $C_{alv_{NO}}$ and $J'aw_{NO}$ were used to calculate $D_{A_{NO}}$ with and without compression/elongation of the acinar airways (bronchoconstriction, BC).

The increased $F_{E_{NO}}$ and $C_{alv_{NO}}$ values at 2 G compared to 1 G, are likely to be caused by a dramatic decrease of the alveolo-capillary contact area in the non-dependent (i.e., the upper) lung zones. A moderate BC in hypergravity may also have contributed to the overall $F_{E_{NO}}$ increase in hypergraviy. Without including BC, $C_{alv_{NO}}$ had to be adjusted (overestimated) to account for experimental $F_{E_{NO}}$ levels.

Calculated $D_{A_{NO}}$ values were 1558 (both lung models) at 1 G and 994 (one-trumpet) and 929 (2-trumpet) pl·s^{-1}·ppb^{-1} at 2 G. The lumen reduction (two-trumpet model) required to match the experimental data was 36 % (BC in both compartments), 55 % (BC in non-dependent part only) and 64 % (BC in dependent part only). To account for the increase in the experimental $J'aw_{NO}$ values, either an imposed 15 % increase in bronchial production, or reduced back-diffusion of NO due to BC, was assumed. The two-trumpet model showed better fit to the experimental data than the one-trumpet model.

5.4 PHARMACOLOGICAL SUPPRESSION OF HYPOXIC PULMONARY VASOCONSTRICTION IN HYPERGRAVITY-INDUCED HYPOXEMIA (PAPER IV)

There was a relatively rapid drop of SpO_2 during the first minutes of hypergravity with a tendency to level off after 4-5 min. After the hypergravity exposure, SpO_2 remained reduced for 15-30 s, then rose gradually but did not recover completely until the subject made a few deep breaths.

SpO_2 dropped by 5 to 25 % in study A and by 8 to 30 % in study B by the end of the hypergravity period during all conditions (study A: control, C_A, Sildenafil 50 mg, S_{50}; study B: control, C_B, Sildenafil 100 mg, S_{100}, Ilomedin, Ilo). Thus, there were no significant differences between C_A and S_{50} (study A), or between C_B, S_{100} and Ilo (study B). The lowest values found generally occurred immediately after the end of hypergravity. Eight subjects participated in both study A and B.

Study A: SpO_2 values were 97 ± 1 (mean ± SD) at 1 G pre (last two minutes of the ten minute rest before hypergravity exposure), 84 ± 6 at 5 G (last minute of the seven minutes of hypergravity exposure) and 95-96 ± 2 (% units) at 1 G post (last two minutes of the ten minutes of rest after hypergravity exposure) for both treatments (C_A and S_{50}).

Study B: SpO_2 values were 97 ± 1 at 1 G pre, 78-79 ± 6-7 at 5 G and 92-93 ± 3 % units at 1 G post for all treatments (C_B, S_{100} and Ilo).

6 DISCUSSION AND PERSPECTIVES

6.1 SIMULATED MICROGRAVITY AND DECOMPRESSION

Decompression illness (DCI) including decompression-induced venous gas emboli (VGE) are serious risk factors, especially when humans are exposed to remote and isolated environments such as during spaceflight.

The decompression profiles used during the simulated microgravity experiments (**Paper I)** mimicked the actual profiles used by cosmonauts performing space walks from the ISS. Earlier studies (Norfleet & Butler, 2001; Balldin *et al.*, 2002; Webb *et al.*, 2005a; Webb & Pilmanis, 2005) showed that similar decompressions performed on earth provoked both VGE evolution and DCI. Therefore there was much care taken in the experimental design and preparations; a conservative protocol with incrementing durations at altitude was used. However, the data collected were in contrast with data from the study performed by Webb *at al*. (2005), that showed 53 % occurrence of DCI and 43 % of significant VGE. In another study by Webb *et al*. (2005a), ambulation vs. non-ambulation during simulated EVA was investigated. In this study a high occurrence of DCI was also noted (49 % overall DCI in the ambulatory group and 53 % overall DCI in the non-ambulatory group). However, the non-ambulatory group consisted of subjects in sitting, supine and recumbent posture during altitude exposure, whereas the ambulatory group walked during exposure.

To quantify the presence of VGE in the simulated EVAs, a Doppler ultrasound monitor was used. Standard VGE monitoring includes Doppler measurements both at rest, and during deep knee-bends (squats). To simulate a more realistic microgravity simulation, an alternative squat routine was adopted, with supine calf-contractions used to better simulate the lower body unloading in space. In this way, subjects may be further protected from VGE and DCI symptoms when exposed to decompression, as the movement made is not as loading as a standing squat.

In the microgravity trials, none of the four participating cosmonauts reported any DCI symptoms, and no changes were seen in exhaled NO levels.

The absence of DCI symptoms and limited occurrence of significant VGE noted in this study is most likely caused by the complete unloading of the lower extremities. This is so since tissue-pressure transients caused by extremity loading and movement has shown to elicit bubble formation (Foster & Butler, 2009). Considering these results the Russian cosmonauts appear to be well protected with their decompression routine. The current US decompression protocol is extensive, with staged decompression and breathing of gas with increasing oxygen content. The preparations take more than

twelve hours. The present results suggest that future studies of the US decompression protocol might lead to shorter pre-EVA routines with preserved safety.

An important but disappointing consequence of the present results is that the hypothesis that exhaled NO can be used to monitor pulmonary VGE during and after decompression could not be substantiated. Future studies with a higher prevalence of VGE than in the present experiments are required for that purpose, but the ethical considerations may render such studies difficult.

6.2 EXHALED NO AT ALTITUDE

During the simulated spacewalks (**Paper I**), consistently lowered $P_{E_{NO}}$ values compared to controls during 100 % oxygen breathing at sea level pressure were found. This might be due to several reasons:

Inhaled oxygen partial pressure

Molecular oxygen is a substrate for NO synthesis (Ricciardolo *et al.*, 2004). However data from Gustafsson *et al*. (1991) and Dweik *et al*. (1998) suggest that one must go to an inspired oxygen level below 10 % of an atmosphere to observe a decrease of the NO synthesis rate. On the other hand, also very high oxygen partial pressures may theoretically influence net pulmonary NO synthesis by formation of reactive oxygen species. However, preliminary data from Hemmingsson (personal communication) indicate that long-term respiration of 100 % oxygen at one atmosphere may decrease $P_{E_{NO}}$ slightly after six hours, but not after one to two hours. Thus, the present oxygen exposure of 1000 hPa for one hour and 386 hPa for up to six hours are not likely to have influenced $P_{E_{NO}}$.

Gas density and diffusivity for NO in oxygen

The decreasing pressure at altitude increases the speed of molecular diffusion: the binary diffusion coefficient for a gas pair such as NO in O_2 increases in inverse proportion to the ambient pressure (Chang, 1985). Thus a lowered pressure could lead to increased molecular diffusion of NO from conductive airways to the alveolar space where it could be rapidly taken up by the haemoglobin in the blood, thereby decreasing the amount of exhaled NO (Van Muylem *et al.*, 2003). Hemmingsson & Linnarsson (2009) showed that for a given inhaled partial pressure of oxygen, $P_{E_{NO}}$ is reduced by 33 % at an ambient pressure of 540 hPa, compared to sea level pressure when inspired oxygen partial pressure is the same at the two ambient pressures.

In the future it is recommended that measurement of exhaled NO be used to monitor lung health at remote locations such as in space, when it is possible that lung health is at risk. This should certainly be the case during future moon flights, since inhalation of moon dust may give rise to toxic and/or inflammatory reactions in the airways (Lam *et al.*, 2002; Darquenne & Prisk, 2008; Latch *et al.*, 2008). The atmospheric pressure in a

future moon habitat is likely to be substantially less than that on earth and the present study shows that normal healthy control values will be lower than on earth in a hypobaric environment. As will be discussed below, the reduced moon gravity (~1/6 of earth gravity) is likely to lower the levels of exhaled NO even further due to a better perfusion and ventilation matching.

6.3 GRAVITY AND EXHALED NO

6.3.1 Exhaled NO in hypergravity

Both FE_{NO} and $C_{alv_{NO}}$ levels were found to be increased in hypergravity (**Paper II**). Current lung models include contributions to exhaled NO from both conducting airways and the alveolar compartment. Thus, a change in FE_{NO} may be caused by either an alteration of airway production, an alteration of the balance between production and blood recapture in the alveolar compartment, or both. By utilizing multiple flow FE_{NO} measurements and using the method proposed by Pietropaoli *et al*. (1999), we could discriminate between these two effects.

Conducting airways

The present values of $J'aw_{NO}$ at 1 G are in line with corresponding data in the literature (Pietropaoli *et al.*, 1999; Condorelli *et al.*, 2007; Kerckx *et al.*, 2008). There was a trend for $J'aw_{NO}$ to be increased at 2 G compared to 1 G (P = 0.054) and under the present experimental conditions, the airway uptake is likely negligible (Jorres, 2000; Puckett & George, 2008). Quantitatively, the $J'aw_{NO}$ increase could account for half of the increase of FE_{NO} (at 50 $ml \cdot s^{-1}$) from 1 to 2 G. There are a number of factors that theoretically can influence $J'aw_{NO}$ in hypergravity. Tissue stretch may induce increased NO synthesis (Bannenberg & Gustafsson, 1997; Artlich *et al.*, 1999) and during hypergravity, lung tissue is certainly both stretched in some parts and compressed in others (Rohdin *et al.*, 2004b). Another aspect of lung stretch and compression is that both may cause reductions of the calibre of peripheral airways. A recent theoretical study by Verbanck *et al*. (2008) showed that such reductions may influence FE_{NO}. A reduction of the cross sectional area of the acinar airways may impair the peripheral effect of molecular diffusion, hence decreasing blood uptake and increasing FE_{NO}. Furthermore, results from the present modelling study (**Paper III**) indicate a reduction in acinar bronchial cross-sectional area in hypergravity compared to normal gravity.

Alveolar compartment

Assuming that there was no change in alveolar production in hypergravity, observed $C_{alv_{NO}}$ changes allow an estimated loss of DL_{NO} of 41 % between 1 and 2 G, and a quasi-steady state between 2 and 3 G (+2.6 %). Rohdin & Linnarsson (2002) measured DL_{CO} at 1, 2 and 3 G. They showed a decrease by 21 % between 1 and 2 G and by another 16.6 % between 2 and 3 G. No previous data on DL_{NO} in hypergravity have been found, instead an interpretation of DL_{NO}/DL_{CO} suggested by Glenet *et al*. (2007)

was used. They showed that this ratio is proportional to the Dm_{CO}/V_c ratio where Dm_{CO} is the membrane component of D_{LCO} and V_c is the capillary volume. If there had been a pure recruitment or de-recruitment at hypergravity, the contact surface of blood could have changed and this would affect both Dm_{CO} and V_c in the same way. Hence, the ratio D_{LNO}/D_{LCO} would remain unchanged. To alter the D_{LNO}/D_{LCO} ratio, either a change in the alveolo-capillary membrane thickness (affecting Dm_{CO}) and/or a change in the thickness component of V_c (i.e. a change in the blood-filling of already recruited capillaries) is needed. If we assume that the alveolo-capillary membrane thickness in hypergravity is unchanged, the estimated 41 % decrease in D_{LNO} suggests a de-recruitment of capillaries from 1 to 2 G and an estimated 26 % decrease in the D_{LNO}/D_{LCO} ratio suggests an increase in the thickness component of V_c, i.e. an over-filling in the still recruited capillaries. This is in line with the increase in tissue volume found by Rohdin & Linnarsson (2002) and is compatible with a blood volume shift from non-dependent to dependent lung zones. From 2 to 3 G, an estimated increase in D_{LNO}/D_{LCO} ratio by +23 % indicates some perfusion decrease, in contradiction with the further increase in tissue volume found by Rohdin & Linnarsson (2002). However, D_{LNO} is in principle perfusion-independent and the quasi-unchanged D_{LNO} strongly suggests no further capillary de-recruitment between 2 and 3 G. However, the above reasoning assumes constancy of alveolar NO production and alveolo-capillary thickness, which has not necessarily been the case. Furthermore, modelling (**Paper III**) supports the notion of a dramatic decrease of the alveolo-capillary contact area (Fig. 15) in the non-dependent lung zones. The theoretical study proposed a reduction in D_{ANO} by 36 % (one-trumpet model) and by 40 % (two-trumpet model) for the 2 G exposures compared to normal gravity.

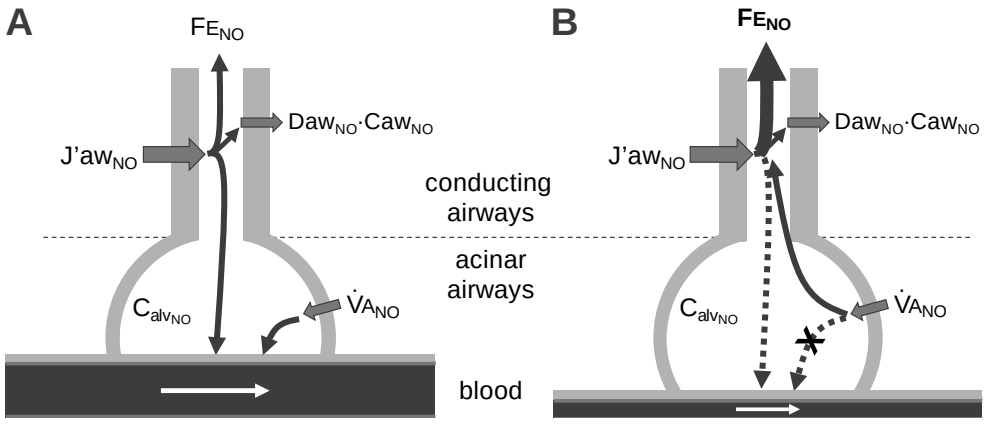


Figure 15. Two-compartment model of the human lung. Panel A: normal gravity. Panel B: hypergravity. Briefly, hypergravity leads to an impaired matching of ventilation and perfusion, which in turn lowers the diffusing capacity (e.g., NO). This leads to decreased molecular diffusion and NO uptake, resulting in increased levels of exhaled NO. $C_{alv_{NO}}$, acinar airway NO concentration, $J'aw_{NO}$, conductive airway NO production, $\dot{V}A_{NO}$, acinar airway NO production, Daw_{NO}, conducting airway compartment diffusing capacity of NO, Caw_{NO}, conducting airway NO concentration, F_{ENO}, fraction exhaled NO.

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Other influences on pulmonary NO formation

Stress could also potentially affect exhaled F_{ENO} levels. Linnarsson & Rosenhamer (1968) showed arterial hypotension in the head and neck and increased heart rate, indicating physiological stress in subjects exposed to hypergravity. Furthermore, increases of plasma catecholamine levels have been found in hypergravity studies (Schneider *et al.*, 2008; Strempel *et al.*, 2008) and adrenaline infusions have been shown to increase exhaled NO in a rabbit model (Adding *et al.*, 1999a). Therefore, had stress been a major factor for the F_{ENO} increase, even higher values should have been found at 3 G than at 2 G (Strempel *et al.*, 2008) and that was not the case. In contrast, Persoons *et al*. (1995) have shown that the NO production from alveolar macrophages was suppressed by stress in a rat model. If true also for humans, such a mechanism may have accounted for the lack of increase of exhaled NO from 2 to 3 G.

Strömberg *et al*. (1997) showed that CO_2 can inhibit NO formation. The results from the hypergravity study with sitting subjects (**Paper II**) does not support the findings of Strömberg *et* al. since the subjects had lower exhaled CO_2 levels at 3 G than in 2 G and would thus have shown higher exhaled F_{ENO} concentrations at 3 G than in 2 G if CO_2 inhibition of NO would have been a major factor.

6.3.2 Exhaled NO in microgravity

The experimental setup aboard the ISS provided the opportunity to measure exhaled NO in microgravity (**Paper II**). All five subjects had lower F_{ENO} values during the first measurement in-flight than before the flight and the decreases ranged between 21–76 % from the pre-flight value. The ISS set-up limited the F_{ENO} measurements to one exhalation flow (50 $ml \cdot s^{-1}$), therefore alveolar and airway contributions could not be estimated separately. However, the F_{ENO} decrease averaged more than 5 ppb, which is a larger value than what is a normal for $Calv_{NO}$. Thus, a reduction of $Calv_{NO}$ could have contributed to, but could not explain the full extent of the F_{ENO} reduction in microgravity. Again, assuming unchanged alveolar NO production in sustained microgravity, the previously documented improvement of the membrane component of D_{LCO} by 28 % in microgravity (Prisk *et al.*, 1993) could only have resulted in a proportional reduction of $C_{alv_{NO}}$.

Without measuring F_{ENO} at multiple flows, we can only speculate about the explanations for the lowered F_{ENO} values in microgravity. Tissue relaxation may lead to a peripheral airway lumen increase, improving peripheral molecular diffusion, and decreasing the net NO production as measured at the mouth. In addition, decreased mechanical stress of lung tissue might have resulted in less induction of NO formation in the lung tissue (Artlich *et al.*, 1999). F_{ENO} in microgravity might also represent a much better estimate of the NO concentrations in the lungs because a more representative sample for all lung units is obtained during exhalation. These potential

effects of tissue relaxation as well as the $D_{L_{CO}}$ increase would be “mirror effects” compared to hypergravity.

Future studies with $F_{E_{NO}}$ measurements at multiple flows would give us the possibility to discriminate the contributions from conductive airways and the alveolar compartment.

6.4 HYPOXIC PULMONARY VASOCONSTRICTION AS A POTENTIAL PROTECTIVE MECHANISM IN HYPERGRAVITY-INDUCED TRANSIENT LUNG INSUFFICIENCY

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are both serious lung disorders (Randolph, 2009) that induce pulmonary oedema, increasing the weight of the lung (Gattinoni *et al.*, 2001). Oedematous lung tissues compress underlying lung tissues that results in poor aeration of the compressed lung parts (Pelosi *et al.*, 1994) and severe hypoxemia. Similarly, subjects exposed to hypergravity will develop a severe, but temporary gas exchange impairment (Rohdin *et al.*, 2003a; Rohdin *et al.*, 2003b) due to the increased lung weight in hypergravity.

It can be reasoned that the lung tissue in dependent compressed and non-aerated lung parts would be severely hypoxic. Thus, if these areas were perfused they would cause shunting of venous blood to the systemic circulation. However, Petersson *et al*. (2006) using SPECT determinations of lung perfusion of supine subjects exposed to hypergravity, showed that these regions were not perfused. This could be seen as a protective mechanism and it was speculated that it could be due either to passive compression of lung blood vessels or an active mechanism such as hypoxic pulmonary vasoconstriction.

Hypoxic pulmonary vasoconstriction is, in most cases, a beneficial and potent local control mechanism which redirects lung blood flow away from poorly to better ventilated alveoli (von Euler & Liljestrand, 1946). When subjected to general hypoxia, e.g., altitude exposure, lung oedema, etc., this mechanism is no longer beneficial; instead there is a marked elevation of pulmonary vascular resistance, increased pulmonary arterial pressure and increased load on the right ventricle of the heart (Ghofrani *et al.*, 2004).

More recently it has been shown that pulmonary vascular tone, including HPV, depends on the balance between several endothelium-derived factors including nitric oxide (NO). A number of animal and clinical studies (Zhao *et al.*, 2001; Kleinsasser & Loeckinger, 2002; Ghofrani *et al.*, 2004; Fesler *et al.*, 2006) have shown that sildenafil, a phosphodiesterase inhibitor causing increase of the intracellular concentration of cyclic GMP, reduces pulmonary hypertension. Similar effects can be obtained in

patients with primary pulmonary hypertension by inhalation of iloprosttrometamol, a long acting prostacycline analogue (Rimeika *et al.*, 2009).

In the present study (**Paper IV**) temporary and reversible lung insufficiency in healthy subjects was induced by exposing them to five time normal gravity. The effects of sildenafil and iloprosttrometamol on arterial oxygen saturation were examined. No effects of sildenafil or iloprosttrometamol on the arterial oxygen saturation in hypergravity-induced ALI/ARDS were found. One explanation for these results may be a hypergravity-induced redistribution of pulmonary blood. In a similar hypergravity setup, Petersson *et al*. (2006) found a reduction of pulmonary blood flow in dependent areas during hypergravity, as compared to exactly the same tissue segments in normal gravity. This finding suggests that this blood-flow reduction acts as a protective mechanism without which a much more severe shunting would take place.

One important limitation of the present 5 G experiments is that the gravity induced compression of dependent lung tissue might have been so severe that other mechanisms were "overwhelmed" by the external forces. Thus, there could have been an element of HPV in the present placebo experiments, that could have been suppressed with sildenafil and/or iloprosttrometamol, but of no consequence for perfusion (here = shunting) due to the dominating tissue compression. Therefore, it was justified to test the same experimental design as above, but at lower degrees of gravity. Fig. 16 shows preliminary data from a recent series of experiments performed at two and three times normal gravity.

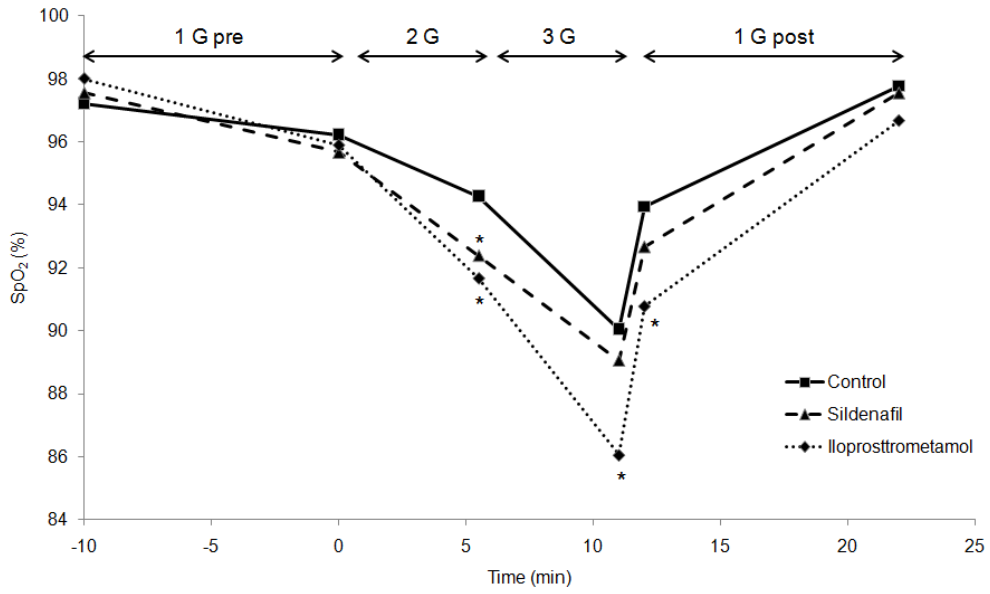

Figure 16. Preliminary data. Arterial oxygen saturation (SpO_2) as a function of time during an exposure to two and three times normal gravity (2 and 3 G respectively) in supine subjects after medication with placebo, sildenafil and iloprosttrometamol. Values are group mean values and interpolated for clarity. * significant difference ($P < 0.05$) compared with control (Placebo).

At both 2 and 3 G, the gravity-induced arterial desaturation became worse after premedication with Ilo. Quantitatively, the difference from placebo was about 5 % units of arterial oxygen saturation, corresponding to a relative worsening of the desaturation by some 70 %. These preliminary results suggest that a process including increased tone of pulmonary vascular resistance vessels provided active protection against shunting and also prevented some of the desaturation in the placebo situation, and that this process was at least in part suppressed with Ilo. In summary, our preliminary data from 2 and 3 G are compatible with the notion that HPV has a protective effect against desaturation in supine subjects exposed to hypergravity.

6.5 PERSPECTIVES

In brief, within the present thesis, new methods for studying lung biology and exhaled nitric oxide in microgravity and hypergravity have been developed. These methods are of interest for studying human activities in extreme conditions and also in helping to provide insight into respiratory disease. Studies investigating exhalation at different flow rates in microgravity would give valuable information on NO and lung biology, as would further studies on VGE and exhaled NO. Hypergravity studies investigating moderate increments of gravity level would be especially of benefit for knowledge on pulmonary vascular regulation and acute lung injury; they should involve analysis of inhaled vasodilators such as those used in the present study and imaging methods that could be used to determine the topographical distribution of ventilation and perfusion.

7 CONCLUSIONS

- The current EVA procedures used by astronauts and cosmonauts with unloading of the lower body, appear protective against venous gas emboli and decompression illness symptoms

- None, or a very small number of venous gas emboli were observed in the simulated spacewalk, which precludes any conclusions regarding the use of elevated levels of exhaled nitric oxide to detect venous gas emboli in the lungs

- Reduced ambient pressure reduces exhaled levels of nitric oxide, presumably by means of lowered gas density leading to the enhancement of axial back-diffusion and blood uptake of lung nitric oxide

- Gravity-induced alterations of the distribution of gas, blood and tissue in the lungs do influence exhaled nitric oxide; microgravity decreases and hyper-gravity increases exhaled nitric oxide levels

- A reduced uptake of pulmonary NO to the blood in hypergravity could be due to slowed back-diffusion, in turn caused by compression and/or elongation of the small conductive airways

- A reduced uptake of pulmonary NO to the blood in hypergravity could be caused by a reduced contact area between the blood and the alveolar gas

- Hypoxic pulmonary vasoconstriction is probably not protective against hypergravity-induced desaturation at five times normal gravity. However when pulmonary tissue distortion is less marked at two and three times normal gravity, HPV could be protective

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Kvävemonoxid och lungan: effekter av rymdfärd och förhöjd gravitation

Lungans huvudsakliga och livsviktiga funktion är att transportera gaser mellan luften i lungblåsorna (alveolerna) och blodet. Syre transporteras från den inandade luften via alveolerna till blodet, och koldioxid transporteras från kroppens vävnader via blodet ut till alveolerna för att sedan vädras ut. Lungan med sina 450 miljoner alveoler har en anatomi som möjliggör för detta gasutbyte genom en stor yta (130 kvadratmeter) där gas och blod möts, samt en kort transportväg för gaserna mellan blod och alveol (1 mikrometer). Lungorna är genom sin speciella anatomi mycket känsliga för gravitationens riktning och storlek. Gravitationen styr på så sätt normalt det mesta av blodflödet i lungorna till dess nedre delar. Även fördelningen av den inandade luften påverkas av gravitationen så att ventilationen i de övre lungdelarna är lägre än i de nedre.

Effekter av tyngdlöshet och ökad gravitation

Molekylen kvävemonoxid (NO) har flera viktiga funktioner i kroppen. En av de viktigare är i hjärt-kärlsystemet där NO kan reglera blodflöden. NO finns även i gasform i utandningsluften och kan användas som en markör för luftvägsinflammation. Vid astma och andra luftvägsinflammationer ses ökade nivåer av utandat NO. Kliniskt används idag utandad NO för att följa och utvärdera behandlingen av luftvägsinflammationer. NO bildas i hela lungan, det vill säga både i luftrören (där inget gasutbyte för syrgas sker med blodet) och i alveolerna (där utbytet av syrgas med blodet sker). En del av den bildade NO-gasen andas man ut medan den andra delen tas upp av blodet. För att öka förståelsen för utandad NO utfördes två parallella studier; fem försökspersoner undersöktes ombord den internationella rymdstationen (tyngdlöshet), och tio försökspersoner undersöktes i en humancentrifug (under två och tre gånger normal tyngdkraft). Resultaten från dessa studier användes sedan i en matematisk modell. I tyngdlöshet blir gasutbytet bättre på grund av att lungvävnaden och blodet fördelas mer jämt i brösthålan. Detta leder till att mer NO tas upp av blodet vilket gör att den utandade nivån av NO samtidigt minskar. Motsvarande mätningar gjordes även vid ökad gravitation och följdriktigt befanns utandade NO-värden vara förhöjda vid ökad gravitation på grund av en mer ojämn fördelning av vävnad och blod i lungorna, därav minskat upptag av NO till blodet. En ökad kunskap om utandat NO skulle kunna bidra till exaktare behandlingar av luftvägsinflammation samt nya användningsområden.

Effekt av lågt omgivningstryck vid rymdpromenader

Under kirurgiska ingrepp, vid stora trauman och vid dykrelaterade sjukdomar är gasbubblor i kärlsystemet en mycket allvarlig komplikation som är svår att diagnostisera. Gasbubblor i blodet kan blockera blodtillförseln till vävnader vilket är mycket farligt. I rymden råder vakuum och astronauter och kosmonauter som gör rymdpromenader måste använda sig av rymddräkter. På rymdstationen och i rymdfarkosterna har man ett normalt lufttryck som motsvarar trycket på havsnivå på jorden. Trycket i rymddräkterna är kraftigt reducerat (cirka en tredjedel av normalt lufttryck vid havsytan) för att göra dess leder rörliga i rymdens vakuum. Detta medför en oundviklig trycksänknig då en person ska på rymdpromenad. Motsvarande trycksänkning på jorden innebär en mycket stor risk för bubbelbildning i blodet och eventuell dykarsjuka. Djurförsök har visat att bubblor i blodet kan leda till ökade nivåer av NO i utandningsluften. Om motsvarande gällde för människa skulle diagnostiseringen av gasbubblor i kärlsystemet eventuellt kunna underlättas. För att undersöka detta utförde vi två parallella försök; en tryckkammarstudie där tjugo friska försökspersoner utsattes för en simulerad sex timmar lång rymdpromenad, samt en studie där fyra kosmonauter undersöktes ombord internationella rymdstationen strax före och efter rymdpromenader. Under de simulerade rymdpromenaderna låg försökspersonerna på britsar för att simulera den tyngdlöshet och avlastning av kroppen som kosmonauterna upplever under de riktiga rymdpromenaderna. I simuleringsstudien upptäcktes nästan inga bubblor alls och inga förhöjda nivåer av utandat NO sågs vare sig under simuleringsstudien eller i rymden. Sammanfattningsvis verkar avlastningen av kroppen under rymdpromenader skyddande mot bildandet av gasbubblor i blodet och dykarsjuka. Med fortsatta studier kan eventuellt en ny diagnosmetod för gasbubblor i blodet tas fram vilket skulle vara viktigt då symptomen för gasbubblor i blodet är diffusa och svårtolkade. Dessutom skulle denna relativt enkla metod kunna användas på avlägsna och svårtillgängliga platser som till exempel under en Mars-färd.

Ökad gravitation och lungsvikt

Patienter med svåra lungsjukdomar får ofta problem med syresättningen av blodet. Detta vanligen till följd av svullnad av lungvävnaden vilket försvårar gasutbytet genom att öka transportsträckan för gasen mellan blod och alveol, samt genom hopklämning av de nedre delarna av lungan till följd av den svullna vävnaden. Ett av kroppens försvar mot detta är att blodflödet i lungan styrs om till delar som är bättre syresatta och potentiellt har NO en betydande roll i denna reglering. Genom att utsätta friska försökspersoner för förhöjd tyngdkraft uppnås en temporär och övergående akut lungsvikt. Genom att sedan medicinera dessa försökspersoner på tre olika sätt undersöktes NO:s roll i lungsvikten. Denna information ger oss ökad förståelse för regleringen av blodflödet i lungan och kan i framtiden förhoppningsvis leda till förbättrade behandlingsmetoder för patienter med akut lungsvikt.

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